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POSTER ABSTRACTS

The program number and the abstract/poster board number are one and the same. It appears in bold print followed by the day of presentation, title and author names. **The first author listed is the presenting author.**

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Knockdown of the ATR/CHEK1 pathway overcomes resistance to radiation therapy resulting from loss of distal 11q. *S. Gollin[1,2], M. Sankunny[1].* 1) Dept Human Genetics, Univ Pittsburgh GSPH, Pittsburgh, PA; 2) Univ Pittsburgh Cancer Institute, Pittsburgh PA.

One of the most common chromosomal alterations in carcinomas is distal 11q loss, which results from breakage at FRA11F (11q14.2) and/or rearrangement involving segmental duplications on distal 11q. We showed that breakage and rearrangement initiates breakage-fusion-bridge (BFB) cycles that result in 11q13 amplification and overexpression of most of the dozen or so genes in the amplicon, including *CCND1*. Although the literature indicates that 11q13 amplification leads to a poor prognosis, we demonstrated that the first step in the amplification process, distal 11q loss, results in a statistically significantly (p=0.0401) poor prognosis in a series of 42 oral cancer patients with a median followup 6.3 years. The poor outcome may be related to the laboratory observation that head and neck, non-small cell lung, and ovarian carcinoma cell lines with distal 11q loss measured by fluorescence *in situ* hybridization express loss of sensitivity to ionizing radiation (IR) at therapeutic or higher doses as assessed by clonogenic survival assay. Further, we showed that carcinoma cell lines with distal 11q loss have loss or haploinsufficiency for critical DNA damage response genes, including *ATM*, *MRE11A*, and *H2AFX*, a diminished DNA damage response, and overexpression of the ATR/CHEK1 pathway. Knockdown of CHEK1 by siRNA or a targeted CHEK1 small molecule inhibitor (Pfizer PF-00477736) in carcinoma cell lines with distal 11q loss substantially decreased colony formation in response to a single 2.5 Gy fraction of IR. Cell lines without distal 11q loss did not show differential radiosensitivity. These findings led to development of a biomarker for loss of sensitivity to IR (patent pending), to ongoing studies of additional types of carcinomas and investigation of whether these same therapeutically resistant cell lines show decreased sensitivity to other DNA damaging agents, including chemotherapeutic drugs. This biomarker may be useful as a prognostic marker that could be added to predictive personalized cancer genomic assays, some of which are already available, and as a companion diagnostic for CHEK1 small molecule inhibitors under development or in clinical trials.

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COPY NUMBER ALTERATIONS IN MYELODYSPLASTIC SYNDROME. *X. Hu[1], D.J. Wolff[5], C. Verghese[2], H. Safah[2], M.M. Li[1,2,3,4].* 1) Louisiana Cancer Research Consortium; 2) Tulane Cancer Center; 3) Hayward Genetics Center; 4) Dept. of Pediatrics; Tulane Univ. Sch. Med, New Orleans, LA; 5) Medical University of South Carolina, Charleston, SC.

Myelodysplastic syndromes (MDS) are a group of heterogeneous myeloid neoplasms that range from indolent conditions with a near-normal life expectancy to acute myeloid leukemia (AML). A few chromosomal rearrangements are known to be associated with disease prognosis. However, 50 to 60% of the cases show normal cytogenetic results but considerably variable outcomes. We hypothesize that cryptic genomic copy number alterations (CNAs) may underlie the prognosis of MDS. We studied 36 patients with newly diagnosed MDS to assay cryptic genomic alterations using a custom designed cancer specific CGH microarray that targets over 400 cancer genes and over 100 cancer-associated genomic regions. We also studied 7 of the 36 cases using a SNP array to evaluate copy neutral loss of heterozygosity (CN-LOH) and cross platform variations. A total of 713 CNAs were detected in the 36 patients, including 413 deletions and 300 duplications. Cryptic deletions (156 kb - 5.9 Mb) were identified at one or both translocation breakpoints in 5 of the 6 cases with apparently balanced rearrangements. Although the CNAs were distributed throughout the whole genome, they were enriched in a few genes: partial or full deletion of the BLM (27.8%, 10/36) and REL (33.3%, 12/36) genes, and partial or full duplication of the PTCH1 (30.6%, 11/36), CSF1R (27.8%, 10/36), and AKT1 (25%, 9/36) genes. The SNP array identified all major cytogenetic alterations but missed some small CNAs. Interestingly, in a patient who showed monosomy 7 at diagnosis and normal karyotype at three-year follow-up, SNP array identified a CN-LOH of 7q at three-year follow-up. Our study demonstrates that cancer-specific microarray analysis in MDS patients reveals unrecognized CNAs that may allow risk stratification of these patients for appropriate therapeutic interventions.

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Known and novel copy number alterations in GBM and their patterns of co-occurrence are revealed by GSVD comparison of array CGH data from patient-matched normal and tumor TCGA samples. *C. Lee[1], O. Alter[1,2,3].* 1) Department of Biomedical Engineering, The University of Texas at Austin, Austin, TX; 2) Institute of Cellular and Molecular Biology, The University of Texas at Austin, Austin, TX; 3) Institute of Computational Engineering and Sciences, The University of Texas at Austin, Austin, TX.

Glioblastoma multiforme (GBM) is the most common primary brain tumor in adults and is characterized by rapid growth, treatment resistance, and poor prognosis. Like other cancers, GBM tumors exhibit a range of copy number alterations (CNAs), many of which play roles in the pathogenesis of this disease. Here, we use the generalized singular value decomposition (GSVD) [1,2] to analyze array CGH data from 251 patient-matched normal and tumor samples from The Cancer Genome Atlas (TCGA). The GSVD simultaneously separates the two (normal and tumor) genome-wide copy number datasets into one set of “probelets” and two corresponding sets of “arraylets”. Each probelet represents a pattern of CNAs across the patients, and the two corresponding arraylets describe the corresponding patterns of CNAs in the normal and tumor genomes, respectively. We find that arraylets of significance in the normal datasets identify normal copy number variations (CNVs) in the human genome, and the corresponding probelets describe the distribution of these CNVs in the patients. One of these arraylets captures differences in X chromosome copy number, and the corresponding probelet separates male from female patients. More notably, we find that arraylets of almost exclusive significance in the tumor dataset identify known and novel CNAs prevalent only in tumors and describe their patterns of co-occurrence. The second most tumor-exclusive arraylet identifies most of the important, known CNAs that occur in at least 2% of GBM cases [3], including amplifications of MDM4, AKT3, PDGFRA, EGFR, CDK4, and MDM2, and deletions of CDKN2A/B and PTEN. This arraylet also identifies a frequent pattern of chromosome 7 gain associated with chromosome 9p and 10 loss, as well as several rare (observed in <5% of patients), previously unreported CNAs, including regions of 1p36.21 (containing the tumor suppressor PRDM2), 17q23.2 (containing the histone-associated protein TLK2), and 12p13.33 (containing the Rb-interacting protein KDM5A). Analysis of other tumor-exclusive arraylets reveal common but previously unreported amplifications of regions of 19q12 containing cyclin E1 (CCNE1) and 17q24.1 containing DDX5, which has been implicated in the pathogenesis of other human cancers. [1] Golub and Van Loan, Matrix Computations, 3rd ed. (Johns Hopkins University Press, Baltimore, MD, 1996). [2] Alter, Brown and Botstein, PNAS 100, 3351 (2003). [3] The Cancer Genome Atlas Research Network, Nature 455, 1061 (2008).

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A novel complex chromosomal change in a chronic myelomonocytic leukemia with marked ring sideroblasts and normoblastic dysmorphism. *H.O. Shah[1,3], W. Xu[1], A. Yuil[1], A. Pigal[1], M-L. Desormeaux[1], L. Hanna[2], J. Lin[1,3,4,5].* 1) Dept of Pathology, Cytogenetics, Nassau Univ Med Ctr, East Meadow, NY 11554; 2) Dept of Oncology, Nassau Univ Med Ctr, East Meadow, NY 11554; 3) Dept of Pathology, Health Sciences Center, State University of New York at Stony Brook, NY; 4) New York College of Osteopathic Medicine, NY; 5) American University of Caribbean School of Medicine, Netherlands Antilles/Coral Gables, FL.

Chronic myelomonocytic leukemia (CMML) is a clonal hematopoietic malignancy that is characterized by features of both a myeloproliferative neoplasm (MPN) and a myelodysplastic syndrome (MDS). Clonal cytogenetic abnormalities are found in 20-40% CMML, but none is specific. The most frequent recurring abnormalities include +8, -7/del(7q) and structural abnormalities of 12p. Here we report of a case of CMML with novel complex chromosomal changes. A 77 years old female had history of anemia. Peripheral blood (PB) analysis showed WBC 10.2 x 10^9/L with 22%; monocytes (absolute 2.2x10^9/L), 3% myeloblasts, 2.5% promyelocytes, 8% myelocytes, 7% metamyelocytes, 5% bands, 25% neutrophils, 3% eosinophils, 0.5% basophils and 24% lymphocytes, RBC 2.7 x 10^{12}/L, Hb 7.4 g/dL, Hct 22.7%, MCV 84.7 fL, RDW 33.5%, 8 nucleated RBC/100 WBC, and platelet 180x10^9/L. RBC showed marked anisopoikilocytosis and basophilic stippling. Other relevant laboratory assays showed iron 96 ug/dL, total iron binding capacity 268 ug/dL, ferritin 280 ng/mL, vitamin B12 1327 pg/mL and folate 6.7 pg/mL. Bone marrow was hyperplastic with an average of 95% cellularity and the differential revealed 40.0% normoblasts, 3.8% myeloblasts, 0.2% promyelocytes, 32.0% granulocytes, 4% monocytic cells, 18% lymphocytes and 2.0% eosinophils. Iron stain revealed ring sideroblasts in 60% of normoblasts. Flow cytometry analysis of bone marrow aspirates showed approximately 3% blasts expressing CD13, CD 33, CD34, CD117, HLA-DR and CD56. Theses features were consistent with CMML-1 (blasts <5% in PB). The patient died of intracranial hemorrhage one month later after the diagnosis. Cytogenetic study showed a novel complex genotype: 44-45,XX,der(2)t(2;10)(q33;q11.2),del(5)(q13q33),-7,add(10)(q11.2),-12,-13,dic(14;18)(p11.2;p11.2),+mar1[5],+mar2[5],mar3[cp20]. Chromosomal aberrations involving chromosome 2, 5, 7, 10, 12 and 13 have been described in MDS and/or acute myeloid leukemia (AML). The novel complex karyotype in this case might underlie a progression of refractory anemia with marked ring sideroblasts (RARS) to CMML and the poor prognosis.

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Frequency, Molecular Pathology and Clinical Significance of Partial Chromosome 3 Alterations in Uveal Melanoma. *M.H. Abdel-Rahman[1, 2], B. Christopher[1], M. Faramawi[3], K. Said-Ahmed[1, 4], C. Cole[5], A. McFaddin[5], A. Ray-Chaudhury[6], N. Heerema[5, 6], F. Davidorf[1].* 1) Ophthalmology, The Ohio State University, Columbus, OH; 2) Clinical Cancer Genetics Program, Department of Internal Medicine, The Ohio State University, Columbus, OH; 3) Department of Epidemiology, School of Public Health, University of North Texas Health Science Center, Fort Worth, Texas; 4) Ophthalmology, Faculty of Medicine, Menoufiya University, Egypt; 5) James Cancer Hospital Cancer Cytogenetics Laboratory, The Ohio State University, Columbus, OH; 6) Pathology. The Ohio State University, Columbus, OH.

Purpose: Uveal melanoma (UM) is the most common primary intraocular tumors in adults. Monosomy of chromosome 3 (M3) is a common somatic alteration in UM. M3 is associated with aggressive disease and is currently used as a prognostic clinical marker. The clinical significance of partial chromosome 3 alterations (P3) is not clear. Also, the frequency of P3 in UM varies considerably in the published literature from 0 to 47%. The aims of the following study were to identify the frequency, molecular pathology and potential clinical significance of P3 in UM. Methods: 47 UMs with an average follow up of 35.3 months were included in the study. Fourteen patients had confirmed metastasis. Allelic imbalance/loss of heterozygosity of chromosome 3 markers was studied using microsatellite genotyping with 16 markers on chromosome 3, eight on each chromosome arm. Chromosomal alterations were assessed by conventional cytogenetics in 11 tumors and by comparative genomic hybridization (CGH) in an additional 13 cases. Results: M3 was detected in 26 (55.3%), P3 in 14 (29.8%) and heterodisomy of chromosome 3 in 7 (14.9%). In the 24 tumors with available cytogenetic/CGH, P3 was detected in 9/24 (37.5%) confirming the high frequency of P3 detected by genotyping. In these 24 tumors P3 was caused by both gains (5/9) and losses (4/9) of chromosome 3. Cytogenetics showed high frequency of complex chromosome 3 aberrations in tumors with P3. Out of the 14 tumors with confirmed metastasis 13 had M3 and only one showed P3. The P3 tumor with confirmed metastasis showed a bi-lobed tumor and a heterogenous genotype profile with part of the tumor showing M3 and other parts showing P3. Genotyping with limiting the assay to M3 was highly sensitive (92.9%) and specific (68.4%) for detection of aggressive UMs. Inclusion of P3 cases increased the sensitivity of the assay to 100% but considerably diminished its specificity. Conclusions: P3 alterations are rather common in UM and are commonly caused by complex chromosome 3 alterations leading to partial gains and/or partial losses of chromosome 3. P3, whether caused by partial gains or losses, is not likely to be associated with aggressive disease. Microsatellite based chromosome 3 genotyping with limiting the aggressive phenotype to M3 tumors is highly sensitive for detection of aggressive UM.

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Cytogenetics in diagnosing and prognosing in 114 patients with multiple myeloma. *O. Altiok Clark[1], CF. Sargin Ozkaya[2], A. Timuragaoglu[3], B. Akkaya[4], L. Undar[3], I. Karadogan[3], F. Unal[2], F. Asik[2], G. Luleci[2], S. Berker Karauzum[2].* 1) Department of Medical Genetics, Akdeniz University Medical Faculty; 2) Department of Mediacal Biology and Genetics; 3) Department of Hematology; 4) Department of Pathology, Antalya, Turkey.

Recently, there has been a significant increase in patients diagnosed with Multiple Myeloma (MM) in hematology clinics. This increase is due largely to the use of cytogenetic and molecular cytogenetic methods as diagnostic tools which allow us for prognoses to be made for each patient. Between the years 2000-2010, bone marrow samples were collected at the Akdeniz University Medical Faculty Hospital from patients who were suspected of having MM. The patient's karyotypes were assessed using conventional cytogenetic and molecular cytogenetic methods. Of the karyotypes analyzed in 114 patients, 65% were found to be normal, while 32% of the analyzed karyotypes showed either numerical or structural changes, or a combination of the two. Some of these showed expected cytogenetic changes while others were found to contain complex chromosomal rearrangements. A small number of the patients (3%) showed novel, as-yet-unpublished karyotypes. Among the patients for which sufficient metaphases were not obtained, molecular cytogenetic methods allowed for the detection of certain abnormalities which were used to diagnose of MM. The relationship between the cytogenetic results and patient clinical appearance will be discussed in terms of how they relate to prognoses for patients with MM.

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A Primate Specific Segmental Duplicated Superstructure and its Assocation with a Cluster of Genetic Diseases and Polymorphisms on Human Chromosome 17q. *D. Chen[1, 2], T. Miettinen[2], V. Leppä[2], O. Choi[3], A. Palotie[2,4, 5, 6], L. Peltonen[2,4, 5, 6], J. Saarela[2].* 1) University of California Irvine, Irvine, CA; 2) Institute for Molecular Medicine, Finland FIMM, Univ of Helsinki, Helsinki, Finland; 3) Dept of Human Genetics, Univ California, Los Angeles, Los Angeles, CA; 4) Dept of Clinical Chemistry, University of Helsinki, Helsinki; 5) Program in Medical and Population Genetics, Broad Instititute of Harvard and Massachusetts Insttitue of Technology, Cambridge, MA; 6) Wellcome Trust Sanger Institue, Wellcome Trust Genome Campus, Cambridge, UK.

Human Chromosome 17 is enriched for a variety of genetic diseases with underlying structural variations and chromosomal abnormalities. We have previously identified a segemental duplicated superstructure on human 17q. This structure appeared to primate specific. The human segmental duplicated superstructure on chromosome 17q contains 13 discrete domains. Corresponding segemntal duplicated superstructures found in genomes of chimpanzee, orangatun and rhesus monkey contain 19, 18 and 13 domains respectively. Each of domains are significantly enriched for homologous sequence fragments. Over 300 fragments pairs with homology greater than 90% and longer than 200bps are found exclusively within human superstructure. These homologous sequence pairs can serve as template sequences for non-allelic homologous recombination (NAHR) thereby contribute to chromosomal structural instability observed in human chromosome 17q. The longest homologous sequence pair is 49.3Kb in lenght with 97.4% homology. Several studies have implicated this particular homologous sequence pair are the template for the partial deletion of NF1 gene. This contribute to the high de novo mutation rate observed in neurofibromatosis type 1 patients. In addition, homologous fragments pairs contributing to the deletions of exone 1&2 of BRCA1 in familial breast cancer, deletion of 17q21.31 in Williams syndrome and inversion of 1Mb sequences in structural polymorphism in 17q are also part this segmental duplicated superstructure. Furthermore, we have also found two homologous and actively expressed retrotransposable elements (AK125814 and AK125932) to be highly associated with segmental duplicated superstructures found in all 4 primate genomes examined. In human chromosome 17, the two retrotransposable element are found to duplicated 34 times--all within the duplicated superstructure. Such association suggest a potential evolutionary relationship between retrotransposable elements and the segmental duplicated superstructure. In addition, we have conducted pair-wise sequence analysis on each of the possible domain pair in all four primate genomes. The results suggest the segmental duplicated superstructures are duplicated in tandem through evolutionary history. In conclusion, a cluster of genetic diseases found on human chromosome 17q appear to have a common evolutionary origin initiated from the transposition of retroelements resulting in the segmental duplicated superstructure.

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Monosomy 10 mosaicism as an incidental finding in acute lymphocytic leukemia. *D. Curi[1], C. Stein[2], R. Hurley[1], R. Lebel[3], G. Kennedy[1].* 1) Pediatrics Department, SUNY Upstate Medical University, Syracuse, NY; 2) Pathology Department, SUNY Upstate Medical University, Syracuse, NY; 3) Pediatrics Department, Genetics Section, SUNY Upstate Medical University, Syracuse, NY.

A 3.5 year old previously healthy Caucasian male presented with bone pain, intermittent fever and decreasing appetite. Initial impression of the CBC was pancytopenia due to viral suppression. Bone marrow biopsy revealed a background karyotype of 46,XY with a cell line typical of acute lymphocytic leukemia (translocation 12;21), and another cell line 45,XY,-10 (monosomy 10) in 14% of cells. After treatment to clinical remission, repeat bone marrow study showed persistence of the monosomy 10 cell line (4%). Peripheral lymphocytes showed monosomy 10 in 3% of cells, and a skin biopsy confirmed constitutional mosaicism at a level of 5%. This child was the product of an uncomplicated pregnancy in a 42 year old G6P6->7 Caucasian woman who had one previous set of twins. On evaluation at age 4 years 2 months, the patient was not dysmorphic; he had a Vineland composite score of 96 (components ranging from 88 to 109). We have reviewed the literature and will present a discussion of the significance of these findings. Low-grade constitutional monosomy 10 mosaicism is apparently consistent with normal development, and may be encountered as an incidental finding.

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Secondary Chromosome Aberrations in Non Hodgkin Lymphoma with IG/MYC Rearrangement Defined Burkitt Lymphoma from Diffuse Large B-cell Lymphoma: A Meta-analysis of Cytogenetic Data. *O. Davila, R. Garcia, N. Garay, M. Koller, Z. Lou, J. Liu, B. Elias, F. Valdez, M. Duc, P. Koduru, C.A. Tirado.* The UT Southwestern Medical Center-Department of Pathology Clinical Cytogenetics.

Burkitt lymphoma and diffuse large B-cell lymphoma are categorized as aggressive B-cell non-Hodgkin lymphomas in the WHO classification. The characteristic t(8;14)(q24;q32) of Burkitt lymphoma (BL), which fuses the MYC gene on 8q24 to the IgH loci on 14q32 or its variants, is also detected in up to 10% of diffuse large B-cell lymphoma (DLBCL) cases. Distinction between these two aggressive B-cell lymphomas is critical since they require different treatments. In the present study, we examined the Mitelman database (from 1982-2009) to determine whether additional recurrent chromosome aberrations (RCA) could reliably predict Burkitt lymphoma versus DLBCL. To differentiate BL from DLBCL, we searched for IGH/MYC rearrangement (R) having the following criteria: a defined morphological pattern (i.e., BL or DLBCL) in lymph node tissues with a B-lineage immunophenotype and no previous tumors. Each B-cell non-Hodgkin lymphoma group was assessed for RCA (gains, losses and rearrangements for all band regions). The Fisher Exact Test was used to compare differences in RCA between the two morphological groups, and a p value (2-tail) of less than .05 was considered significant. We identified a total of 151 and 56 unselected cases for BL and DLBCL respectively. Of 151 BL cases, a total of 116, 25 and 10 carried the t(8;14), t(8;22) and t(2;8) respectively. The most frequent events and frequencies in BL included: +7, 4.5% and -X, 3.9%. Likewise, of 56 samples for DLBCL, a total of 45, 8 and 3 cases were detected with t(8;14), t(8;22) and t(2;8) respectively. The most frequent events and frequencies in DLBCL were t(14;18), 26%; +12, 22%; +7 and +18, 18% respectively; +X, 16%; del(6)(q21) and +21, 10% respectively. When comparing between morphological groups, we found the following RCA significantly associated with DLBCL: +X, p = .006; 1p36 L, p = .015; -4, p = .006; 4q L, p = .001; 6q L, p = .0001; 6q21-27 L, p = .00007; +7, p = .001; 7q G, p = .000004; 7p G, p = .001; -9, p = .043; 9p L, p = .015; 9q L, p = .015; 12q G, p = .005; -15, p = .019; 15q15-q22 L, p = .008; 17p L, p = .042; +18, p = .0005; +21, p = .02 and 22q L, p = .007. In summary, our results demonstrate unique RCA in IGH/MYC positive non Hodgkin lymphoma that can be used to clearly defined BL from DLBCL.

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"Comparison of Secondary Chromosome Aberrations between Follicular Lymphoma and Diffuse Large B-cell Lymphoma with t(14;18): REVIEW FROM THE MITELMAN DATA BASE". *N. Garay, R. Garcia, P. Koduru, C. Tirado*. UT Southwestern Medical Center, Department of Pathology.

The characteristic t(14;18)(q32;q21) present in up to 90% of follicular lymphoma (FL), also occurs in 20-30% of diffuse large B-cell lymphoma (DLBCL) cases. The translocation juxtaposes the BCL2 gene on 18q21 to the immunoglobulin heavy chain (IGH) on 14q32. The t(14;18) is not commonly present as a sole abnormality and secondary recurrent cytogenetic abnormalities (RCA) are commonly identified, suggesting RCA may play an important role in disease biology. In the present study, we have try to identify differences in RCA between follicular lymphoma and DLBCL with t(14;18). To differentiate FL from DLBCL, we searched the Mitelman database from 1987-2007 for t(14;18) with a defined morphological pattern (i.e., FL or DLBCL). All case karyotypes retrieved from the Mitelman database were previously published. Karyotypes from each morphological group were evaluated for RCA (chromosome gains, losses and rearrangements for all band regions). Results were then compared with the Fisher Exact Test to determine any significant differences in RCA between the two morphological groups. All p values were 2 sided and a p value less than 0.05 was considered significant. We have identified a total of 118 and 30 cases for FL and DLBCL respectively. Among the most frequent events and frequencies in FL included the following: +X (30.5%), +7 (32.2%), loss (L) of chromosome band region 1p36 (24.6%) and i(6)(p10), 8.4%. Whereas gain (G) of chromosome 7 (60%), -15 (26.7%), -4, +8, +11, +5 (20%) respectively, -Y (16.7%), loss of the long arm of chromosome 6 (16.7%) and +12 (13.3%) were more frequent in DLBCL. When comparing between the two morphological groups, the following RCA were significantly associated with DLBCL when compared to FL: 1p12-1p35 L, p = .0005; 1q12-q44 L, p = .017; -3, p = 0.021; 3q27 L, p = .032; -4, p = .0006; 9p22-24 L, .0006; -9, p = .001; +11, p = 0.028; 9q L, 0.027; 11q G, p = .0047; 13q14-34 G, p = .044; 15q L, p = .0068 and 22q13 L, p = .032. In conclusion, taken together, our findings indicate that DLBCL with t(14;18) carried a significantly different RCA profile compared to FL, and may be used to clearly distinguish it from FL with t(14;18). Keywords: FL, DLBCL, t(14;18), recurrent cytogenetic aberrations, RCA profile.

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"Recurrent Aberrations in High Grade B-Cell Lymphomas with Concurrent 8q24/ MYC Rearrangements and t(14;18): REVIEW FROM THE MITELMAN DATA BASE". *R. Garcia, N. Uddin, W. Chen, I. Villalobos, K. Mark, O. Davila, P. Koduru, C. A. Tirado.* UT Southwestern, Dallas, TX 75235.

Concurrent 8q24/MYC rearrangements and t(14;18) occur rarely in high grade B-cell lymphomas, and are associated with an aggressive clinical outcome. These dual translocations or double-hit event (DH) in high grade lymphomas have been reported in transformed follicular lymphoma (FL), Burkitt lymphoma (BL), acute lymphoblastic leukemia (ALL), diffuse large B-cell lymphoma (DLBCL) and plasmablastic myeloma. The aim of this study was to determine frequent chromosome alterations with concurrent 8q24/ MYC and t(14;18) rearrangements. Simultaneous 8q24 alterations and t(14;18) with specific morphological patterns (BL, FL and DLBCL) were searched from the Mitelman database (1981-2007). We identified a total of 25, 35 and 39 cases for FL, BL and DLBCL, respectively. Each morphologic entity was first evaluated for frequent alterations. According to the 2008 WHO classification, double-hit BL cases are considered unclassifiable B-cell lymphoma with features intermediate with BL and DLBCL (referred to as INT hereafter). Results were then compared with the Fisher Exact Test and chi square to determine differences in different morphologic groups: In FL, the 9 most frequent alterations were +7, +12, t(2;8), +X, t(8;14), +21, t(8;22),-13 and -X. In DLBCL, the 9 most recurrent alterations included +7, t(8;22), t(8;14), +12, -15, +11, +21, +X and -X. Similarly, t(8;22), +7, +20, t(8;14),+8, +X, +12, t(2;8), +11 were the 9 most frequent aberrations in the INT group. Comparison between morphologic groups showed a higher frequency for t(8;22) in both INT and DLBCL than FL (18 and 15 vs. 4 events respectively); however, only INT showed a statistically significant association ($p < .05$). Other significant findings included the following: a higher frequency for the pair rearrangement +8/+20 in INT compared to both DLBCL and FL (5, 0, 0 respectively; $p < .05$); loss of 10q24-25 in DLBCL compared to FL (8 vs. 0 events respectively; p = .044); loss of Xp10-q28 in DLBCL compared to the INT group (6-7 vs. 0 events respectively; p = .032); loss of 9p11-24 in DLBCL compared to INT (6-11 vs. 0 respectively; p = .032 - .002) and gain of +20 in the INT group compared to DLBCL (6 vs. 0 respectively; p = .032). Taken together, findings in this study help to outline frequent alterations and statistically significant aberrations within specific morphologic groups between double-hit high grade B-cell lymphomas.

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Myeloproliferative disease with cytogenetic biclonality and JAK2 mutation: molecular-cytogenetic characterization of an unusual case. *K. Geiersbach[1], N. Uddin[1], E.C. Kingsley[3], M. Salama[2], S. Shetty[1].* 1) Department of Clinical Cytogenetics, ARUP Laboratories, University of Utah, Salt Lake City, UT; 2) Department of Hematopathology, ARUP Laboratories, University of Utah, Salt Lake City, UT; 3) Comprehensive Cancer Centers of Nevada.

Biclonality in hematopoietic neoplasms is rare. SNP microarray can help to clarify cytogenetic findings and can also unmask genetic abnormalities not detected by traditional molecular cytogenetics. We present a case of essential thrombocytosis (ET) that transformed into a myeloproliferative disorder with myelodysplasia (MPD/MDS). The patient was a 74 year old male with a history of ET with JAK2 V617F mutation, previously treated with hydroxyurea, anagrelide and uracil mustard. He presented with transfusion dependent anemia and progressive splenomegaly. Due to his declining medical condition, aggressive treatment options were avoided and he was discharged on supportive therapy. His bone marrow was hypocellular with dysmyelopoiesis, ringed sideroblasts and 1 percent blasts; the core biopsy showed moderate fibrosis. Flow cytometry showed a left shifted myeloid lineage without increased blasts. Bone marrow and peripheral blood were submitted for cytogenetic evaluation. Both samples showed two separate clones, one with a 20q deletion and one with complex chromosomal rearrangements and clonal evolution. The karyotype was 46,XY,t(4;13;5)(q21;q14;q33)[13]/46,sl,t(2;7)(p13;q36)[2]/46,XY,del(20)(q11.2)[4]/46,XY[1]. FISH with PDGFRB (Veridex and Cytocell), D7S486, and D20S108 (Abbott Molecular) probes confirmed the cytogenetic findings. Deletions at each of the breakpoints of the three way 4;13;5 translocation were confirmed by high resolution CNV/SNP genomic microarray (Illumina OMNI1 Quad). The 5q deletion spanned the PDGFRB gene but did not involve the RPS14 gene. Array also showed copy neutral loss of heterozygosity of 22q12-qter. The chronology and exact significance of these abnormalities are difficult to decipher, but the combination of abnormalities is consistent with this patient's unfavorable outcome. The JAK2 V617F mutation has been shown to promote homologous recombination and mitotic instability, and in this case, JAK2 was likely an influential factor in generating the cytogenetically unrelated clones. Thorough characterization of unusual cases such as this may help elucidate the pathogenesis and natural history of MPD.

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A novel t(9;22)(p24;p11.2) involving the *JAK2* locus in a pediatric case of acute lymphoblastic leukemia. *M. Hiemenz[1], W. Chen[2], L. Huang[4], C. Laborde[3], Z. Lou[1], F. Valdez[1], N. Winick[3], S. Patel[1], R. Smart[1], R. Garcia[1], P. Koduru[1], C. Tirado[1].* 1) Clinical Cytogenetics, Department of Pathology, UT Southwestern; 2) Hematopathology Division, Department of Pathology, UT Southwestern; 3) Hematology/Oncology, Department of Pediatrics, UT Southwestern; 4) Department of Cell Biology, UT Southwestern.

Rearrangements of *JAK2* are rare and have been described in a variety of hematological neoplasms including chronic myelogenous leukemia, acute megakaryoblastic leukemia, CD10+ B-cell acute lymphoblastic leukemia, T-cell ALL and chronic myeloproliferative disorders. Herein, we report a 14-year-old boy who presented to clinic with complaints of increasing weakness over the course of 3 weeks, decreased energy and decreased appetite. His parents also noted 1-2 weeks of pallor. Peripheral blood smear and bone marrow aspirate revealed a predominant population of medium-sized lymphoblasts that had variably irregular nuclei, moderately dispersed chromatin, inconspicuous nucleoli and scant cytoplasm. The marrow core biopsy was populated mainly by lymphoblasts with little normal trilineage hematopoiesis. Immunophenotypic analysis on peripheral blood revealed 80% lymphoblasts that were CD10(bright +), CD13(partial +), CD19(+), CD20(partial +), CD22(+), CD33(partial +), CD34(+), CD36(partial +), CD38(partial +), CD45(partial +), CD79a(+), HLA-DR(+), surface Ig(-), MPO(-), TdT(+). This immunophenotype was consistent with B lymphoblastic leukemia/lymphoma. He was treated with Children's Oncology Group's protocol (AALL0232) but without remission on day 29, and in remission after re-induction (day 36). Conventional cytogenetic studies revealed a t(9;22)(p24;q11.2) balanced translocation. Fluorescence in situ hybridization studies (FISH) using a 172 Kb bacterial artificial chromosome probe RTP1-927116 encompassing the whole Janus kinase 2 (*JAK2*) gene at 9p24, showed a split signal suggesting involvement of the *JAK2* gene with a partner on 22q11.2. To the best of our knowledge, this is one of the few cases with *JAK2* rearrangement in acute lymphoblastic leukemia. The clinical course in this case suggests that this *JAK2* rearrangement may portend an unfavorable prognosis. Ultimately, this patient received a bone marrow transplant as he had a 10/10 HLA matched sibling.

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Genetic imbalances detected by Array CGH in a patient with undifferentiated embryonal sarcoma of the liver. *X. Hu[1,2], H. Chen[1,2], M. Jin[2], X. Wang[1], R. Zhang[1,3], S. Li[1], J. Niu[2].* 1) Department of Pediatrics, the University of Oklahoma Health Sciences Center, OK 73104; 2) Department of Internal Medicine, the First Hospital of Jilin University, Jilin, China 130021; 3) Department of Heamatology, the First Hospital of China Medical University, Shenyang, China 110001.

Undifferentiated embryonal sarcoma (UES) of the liver is an unusual malignancy of childhood. It represents a heterogeneous group of tumors. This group of tumors arises from mesenchymal tissues. In this report, we present a 19-year-old girl with UES of the liver. This patient had an abdominal distension and increasing abdominal girth. Computed tomography showed a massive lesion in the right lobe of the liver. The incision biopsy was performed and pathological findings were consistent with UES. Array comparative genomic hybridization (CGH) analysis was performed in this case to study genetic imbalances. The genomic profiles of this tumor showed significant losses and gains on multiple chromosomal regions. Within these regions, we found that several genes were tumor-related genes, including NIR (1p36.33), PITSLRE (1p36.33), HEN2 (1p13.1), CD9P1 (1p13.1), TRIM45 (1p13.1), B7H4 (1p13.1), HEN1 (1p13.1), PRG2 (2p25.3), TSSC1 (2p25.3), SPAK (2p24.3), AHRR (5p15.3), TCF7 (5q31.1), WNT8A (5q31.2), EGR1 (5q31.2), FGFR4 (5q35.2), HIN1 (5q35.3), MYC (8q24.21), NAIF1 (9q34.11), CIZ1 (9q34.11), IgH (14q32.33) and TP53 (17p13.1). To our knowledge, this is the first array CGH data of UES of the liver. It is unclear whether these regions play roles in tumor initiation or progression. Additional tumor samples have similar pathological features are required to validate our findings, which may provide diagnostic or prognostic values in UES.

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Double hit lymphoma with both a MYC/IgL translocation and an IgH/BCL2 translocation. *J.M. Kogan[1,3], P.A. Kampmeier[2], A.A. Mandernach[1], A.M. Garcia[1], A.H. Sophian[1], D.A. Rita[1,3].* 1) Cytogenetics, ACL Laboratories, Rosemont, IL; 2) Pathology, Condell Medical Center, Libertyville, IL; 3) Clinical Genetics, Lutheran General Hospital, Park Ridge, IL.

"Double hit" lymphomas are rare and aggressive B-cell lymphomas with features of both Burkitt lymphoma and diffuse large B-cell lymphoma. Although these lymphomas often have complex cytogenetic abnormalities, they harbor characteristic chromosome rearrangements that may be readily identified in the cytogenetics laboratory, thus assisting in achieving a pathologic diagnosis. Specifically, they carry rearrangements involving the MYC gene on chromosome 8 as well as translocations between the IgH gene on chromosome 14 and the BCL2 gene on chromosome 18.

We present a patient with a near tetraploid complex karyotype containing both the t(8;22) and t(14;18). A 55 year old male presented with jaundice, weight loss, abdominal pain, and right testicular swelling. Imaging studies revealed a large abdominal and retroperitoneal mass with associated retroperitoneal and iliac chain adenopathy and a right testicular mass. Orchidectomy and biopsies of the bone marrow demonstrated a lymphoid neoplasm comprised of medium to large sized cells with round to oval nuclei, finely dispersed chromatin, prominent nucleoli, and numerous mitotic figures and apoptotic bodies. Immunophenotyping demonstrated a B lineage neoplasm positive for CD19, PAX5, CD10, MUM1 and BCL2 with partial expression of CD20 and TdT and lambda light chain restriction. Final bone marrow karyotype was 86,XXYY,-2,-3,-4,i(6)(p10)x2,der(8)t(8;22)(q24;q11.2),t(8;22)(q24;q11.2)x2,-10,der(13)t(1;13)(q25;q14)x2,t(14;18)(q32;q21.3)x2,-15,-16,-17,+20[4]/86,sl,+7,-20[6]/85,sdl1,-9[9]/46,XY[1]. FISH analysis results for MYC rearrangements and for the IgH/BCL2 rearrangement supported the chromosome findings.

Although morphology and immunophenotyping were indeterminate, chromosome analysis revealed the diagnosis. The near-tetraploid complex karyotype with the classic 8;22 and 14;18 translocations pointed to a diagnosis consistent with the WHO classification of B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma. This entity, also known as a "double hit" lymphoma, is an underrecognized, often puzzling neoplasm with a very poor prognosis. Chromosome and FISH analysis play a very important role in the diagnosis of this rare neoplasm.

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Copy number changes in the patients with the Philadelphia chromosome (Ph+) by using oligoarray CGH. *J. Lee, X. Lu, X. Wang, Y. Kim.* Dept Pediatrics, OUHSC, Oklahoma City, OK.

Chronic myeloid leukemia (CML) is characterized by BCR/ABL fusion gene, usually a consequence of the Philadelphia (Ph+) chromosome due to the t(9;22)(q34;q11.2). However, there is diversity in the clinical course of the disease that may be frequently associated with genetic heterogeneity. Effort has been made to find prognostic genetic marker with limited success. Array CGH technology has been proved to be a powerful tool to identify subtle genomic segmental alterations, either loss or gain of genetic material. These changes may disclose presence of gene or genes that may play very important roles in disease initiation, progression, or treatment outcome. To investigate whether subtle chromosomal changes is commonly present or not in CML patients, a pilot study of 20 Philadelphia chromosome positive (Ph+) patients was carried out using high density whole genome oligoarray CGH analysis. Five out of these 20 cases had copy number changes, including a loss of 9q34, 15q25.3 and 15q13, and a gain of 7p21.1-p15.3, and 22q11.2. The findings demonstrated that the subtle chromosomal changes are relatively common in CML patients with Ph+ chromosome and the clinical significance of these findings need to be determined in the large patient population studies, especially the newly discovered regions.

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Gene Amplification in Acute Myeloid Leukemia - Two Case Reports. *J. Liu[1], K. Vij[1], C.N. Abboud[2,3], G.L. Uy[2,3], D.M. Lenmann[1], J.Y. Bauer[1], S. Kulkarni[1,3]*. 1) Cytoengomics and Molecular Pathology, Department of Pathology and Immunology, Washington University School of Medicine in St. Louis, MO 63108; 2) Division of Oncology, Department of Internal Medicine, Washington University School of Medicine in St. Louis, MO 63108; 3) Siteman Cancer Center at Barnes Jewish Hospital and Washington University School of Medicine.

Gene amplification allows over-expression of oncogenes that presumably confers a growth advantage to the neoplastic cells and is known to be associated with rapid progression and poor prognosis. It is seen frequently in solid tumors but in only 1% of acute myeloid leukemia (AML) with abnormal cytogenetic findings. The most commonly involved genes in AML are *c-MYC* followed by *MLL* and *AML1*. Cytogenetically, gene amplification is manifested as extrachromosomal double minutes (dmin), intrachromosomal homogeneously staining regions (hsr), or marker chromosomes. Here we report the clinical, hematopathologic, molecular genetic, and cytogenetic findings in two cases of newly diagnosed AML with the presence of gene amplification. Patient 1 is a 57-year-old male with blasts in peripheral blood and low hemoglobin, bone marrow biopsy was hypercellular consistent with AML with maturation (FAB M2). Chromosome analysis exhibited complex aberrant karyotype with double minutes. Fluorescence in situ hybridization (FISH) showed amplification of the *c-MYC*. Mutation testing for *JAK2, FLT3, and NPM1* were all negative, qRT-PCR showed no evidence of *BCR/ABL1* or *PML/RARA* fusion. This patient was treated with induction therapy consisting of idarubicin and cytarabin, a follow-up showed a normocellular bone marrow with no increase in blasts, chromosomal abnormalities persisted but without double minutes. Patient 2 is a 47-year old male with pancytopenia, low hemoglobin and platelets, bone marrow biopsy was consistent with AML-M2 based on cell morphology. Chromosome analysis revealed multiple numerical and structural aberrations involving chromosome 11q, and FISH showed amplification of the *MLL* as well as multiple copies of *MYC, BCR, ABL1, PML, RARA*, and *IGH*, indicative of low-level hyperdiploidy. This patient's primary AML never achieved remission after many attempts of various therapies, and bone marrow transplant was finally performed. We propose that gene amplification in hematological malignancies may not be a rare event, identification of target genes, especially unknown genes, of amplification is important for understanding the tumorigenesis and can be useful for selection of individual cancer therapy; however, the frequency and clinical correlations of gene amplification in leukemia require careful investigation, since the cryptic amplification may not generally provoke confirmatory FISH studies.

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Identification of chromosome 3 aneuploidy in a rare primary yolk sac tumor of the lung using FISH analysis. *M.J. Macera[1,2], S. Arora[2], W. Thelmo[3], A. Afaf[3], P. Chandra[2], V. Tak[4], A. Babu[1,2]*. 1) Division of Molecular Medicine & Genetics; 2) Department of Medicine; 3) Department of Pathology; 4) Department of Surgery, Wyckoff Heights Medical Center, Brooklyn, NY.

A 48-year-old women with a dry cough and occasional hemoptysis for 3 months arrived at our hospital. She had a 10-pack a year smoking history with no alcohol or drug use. Chest Radiograph and CT with contrast confirmed the presence of a large 10 x 12 cm soft tissue mass in the lower left lobe and a 4 x 6 cm mass in the upper right lobe originally believed to be a cyst. The larger left mass was mostly necrotic and only a few areas demonstrated solid patterns and focal papillary features were seen. One of three lymph nodes showed focus of metastasis. The tumor was negative for TTF-1, Cdx-2, Ck7 and CK20. It was focally positive for Oct3/4, strongly positive for Ki-67 and negative for CD34; thus identifying a primordial germ cell origin. Extragonadal germ cell tumors are rare and it is even more unusual to have a primary tumor in the lung with no evidence of gonadal involvement. The patient was started on platinum based chemotherapy, after which the mass in the right lung began to grow and was removed 8 months later. The more recent tumor showed an organoid to solid and glandular pattern and one fortuitous area demonstrating a Schiller-Duval body, thus re-establishing the yolk sac tumor origin. FISH analysis was done on sections from the initial tumor using the probe set (Abbott Molecular) containing probes for cep 3, cep 7, 9p16, and cep 17. In all cells analyzed, cep 3 was present in at least three copies, with a maxium number of 10 copies and a mean of 4.5 copies. Copies of the other three probes were represented at the same copy number, suggesting probable polyploidy or present in fewer copies, mean of three other probes combined 2.7, suggesting a chromosome 3 aneuploidy. In all cells, chromosome 3 was over represented. It is interesting to note that the Oct3/4 protein, a marker for primordial germ cells, reacts with Sox-2, among others, to form a regulatory complex needed for the pleuripotency of primitive embryonic cells. Sox-2 is located at 3q26.3-q27. A repeat FISH analysis of the second tumor gave similar results that were suggestive of a metastatic origin. The patient is scheduled for additional chemotherapy and is doing remarkably well.

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An unusual presentation of an extraskeletal Ewing sarcoma with atypical cytogenetic findings. *J.J.D. Morrissette[1], G.E. Halligan[2], A. McKenzie[2], E. Geller[3], J.P. de Chadarévian[4]*. 1) Dept Pathology, University of Pennsylvania, School of Medicine, Philadelphia, PA; 2) Department of Pediatrics, Section of Oncology, St Christopher's Hospital for Children, Philadelphia, PA; 3) Department of Radiology, St Christopher's Hospital for Children, Philadelphia, PA; 4) Department of Pathology and Laboratory Medicine, St Christopher's Hospital for Children, Philadelphia, PA.

Ewing sarcoma is a soft tissue tumor derived from neural crest cells and is classified as a primative neuroectodermal tumor of the soft tissue. Although most Ewing sarcomas have skelatal involvement, rare presentations of extra-skeletal/extraocceous Ewing sarcoma have been described. Genetically all types of EWS are similar, in that they involve translocations between the EWS gene on 22q12 and various partners. We describe a child with a progressively enlarging scalp lesion that upon resection was identified as an extraosseous Ewing sarcoma by histomorphology. Conventional cytogenetics revealed a deletion of the long arm of chromosome 10 and an apparent deletion of 22q12. FISH studied using the EWSR1 break-apart probe revealed that the 3' region of the EWSR1 locus was deleted in 95% of cells, and that the entire EWSR1 locus was missing in 5% of cells. Array comparative genomic hybridization (aCGH) revealed the deletion on 10q to be interstitial and a mosaic pattern of 22q12 deletion.

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Detection of chromosome abnormalities in Multiple Myeloma using arrayCGH. *A. Patel[1], P. Eng[1], D. Vo[1], J. Taylor[2], J. Chang[2], SW. Cheung[1]*. 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Methodist Hospital, Houston, TX.

Multiple myeloma is presently an incurable disease characterized by a proliferation of malignant plasma cells (PC) and a subsequent overabundance of monoclonal paraprotein. Malignant PCs are characterized by complex genetic aberrations suggesting genomic instability. Several recurrent genetic abnormalities have been identified and a number of these, including t(4;14), 17p13 deletion, 1q amplification, and hypodiploid karyotype, However chromosome abnormalities are only detected in 30-40% of malignant PCs due to a low mitotic index in culture. Recently FISH analysis has allowed for identification of additional submicroscopic abnormalities but there still exists limitations in it use. Array-comparative genomic hybridization (array-CGH) negates many of the limitations of conventional chromosome and FISH analysis. It allows for a genome wide analysis of copy number changes at a high resolution. In this study we compared the standard chromosome and FISH analysis of malignant PC cells to array-CGH using a custom designed 44,000K Agilent array. In a preliminary study, 5 malignant PCs with normal cytogenetics showed copy number abnormalities by array-CGH. Additional data on more samples will be presented.

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Genomic characterization of a recurrent translocation that is associated with myeloproliferative neoplasm (MPN) blast phase progression in a mouse model. *Z. Qi[1], C. Smith[2], M. LeBeau[3], E. Passegue[2], R. Barbeau[4], C. Eisley[4], A. Barczak[4], N. Shah[2], J. Yu[1]*. 1) Department of Laboratory Medicine, University of California San Francisco, San Francisco, CA; 2) Department of Medicine, University of California San Francisco, San Francisco, CA; 3) Section of Hematology/Oncology and the Cancer Research Center, University of Chicago, Chicago, IL; 4) Department of Medicine, Lung Biology Center, University of California San Francisco, San Francisco, CA.

MPN progression from chronic phase to blast phase is believed to involve the accumulation of genomic/genetic alteration(s). The murine MPN associated with JunB-deficiency morphologically resembles chronic phase chronic myeloid leukemia (CML). In contrast to most murine models of CML, blastic phase transformation has been observed in 18% of JunB-deficient mice. Interestingly, leukemic cells from 2/5 JunB-deficient mice that had spectral karyotype analysis performed at the time of blastic phase progression were found to have evolved a clonal translocation between chromosomes 1 and 17. In an effort to characterize the genes located at the sites of the chromosome breakpoints, we first isolated the breakpoint regions of the translocation using chromosome microdissection technology. We further characterized these regions with FISH and mouse chromosomes 1- and 17-specific high-resolution CGH arrays (Roche NimbleGen). We found that the translocation was unbalanced, involving a 1.94Mb and a 1.06Mb deletions on mouse translocation derivative chromosomes 1 and 17, respectively. A total of 16 predicted genes and 5 known genes are involved in the deletion/translocation breakpoint regions. We further identified human homologues of the mouse genes and the human homologous genomic regions of the mouse deletion/translocation breakpoint regions. These findings provide valuable clues for identification of gene(s) and genomic/genetic alteration(s) that are involved in MPN progression in this murine model, and may lead to a better understanding of the pathogenesis of human myeloproliferative neoplasms.

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Investigating genetic abnormalities associated with Y chromosome loss in hematological malignancies by high resolution oligo array CGH. *G. Raca[1,2], X. Xu[1], A. Arthur[1], E. Johnson[1], L. Leverton[1], J. Laffin[1,3].* 1) UW Cytogenetic Services, State Lab Hygiene, Madison, WI; 2) Department of Pathology and Laboratory Medicine, University of Wisconsin-Madison; 3) Department of Pediatrics, University of Wisconsin-Madison.

The goal of our study is to investigate genetic abnormalities associated with Y chromosome loss in hematological malignancies using high resolution oligo array comparative genomic hybridization (aCGH). Y chromosome loss is a common age-related phenomenon in bone marrow of healthy, chromosomally normal males. However, when observed in male patients with hematological malignancies, Y loss can either represent an aging phenomenon or a disease related genetic event in a cell line that has undergone other genetic changes. Previous studies suggest that the loss of Y in a high percentage (>75%) of bone marrow cells rarely occurs in healthy controls, and is more likely to indicate an abnormal clone. We hypothesize that high resolution oligo aCGH could detect disease related genetic abnormalities associated with Y chromosome loss, not detectable by conventional cytogenetics and FISH testing. To test this hypothesis we perform aCGH analysis on genomic DNA isolated from residual bone marrow or peripheral blood specimens from patients with hematological malignancies, where the loss of chromosome Y was the sole abnormality in >75% of bone marrow cells. To date data from ten samples have not detected significant copy number abnormalities and testing of additional specimens is in progress. Discovery of genetic abnormalities associated with loss of the Y chromosome would contribute to better understanding of the biological and clinical significance of this cytogenetic change.

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Validation of home-brew probes for Fluorescence in situ Hybridyzation (FISH) in breast cancer samples. *R.M. Rodrigues-Peres, J.K. Heinrich, K.P. Serra, J. Vassallo, L.O.Z. Sarian.* State Univ Campinas, Campinas, Brazil.

Introduction: The Fluorescence in situ Hybridization (FISH) has become popular in the last decades as a rapid and sensitive technique for evaluation of chromosomes and genes, especially in the detection of amplifications, deletions and aneuploidy in tumors. A variety of commercial probes for FISH is currently available in the market although home-brew probes constitute a much more affordable alternative for laboratories pursuing to establish novel tumor marker targets. Objective: To develop and validate a protocol for the establishment of home-brew probes in the context of a cytogenetics laboratory. Materials and Methods: Sequences corresponding to the ERBB2, AURKB and CCND1 genes were selected from the UCSC Genome Bioinformatics Site. After microbiological cloning and purification, the sequences were amplified, labeled and hybridized onto 10 paraffin-embedded sections of selected ductal or lobular breast tumors. The same samples were also hybridized with commercial gene-specific probes for the same targets to validate the hybridization efficiency. Clinicopathological data were obtained from the clinical records. The study protocol has been fully approved by the institution's ethics review board (CEP #705/2007). Results: The hybridization profile of all the studied genes were found to be similar considering both types of probes, in paired breast tumors samples. Discussion: The use of home-brew probes for FISH may bring many advantages as costs reduction, hybridization of multiple targets using different colors when labeling probes and availability of probes for specific markers that have not been produced commercially. The applicability of these probes in tumor diagnosis should bring benefits not only for the FISH technique itself, but mainly for decisions in the disease management and treatment.

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Sequential retrospective microarray analysis of CLL patients over a period of 14 years. *S. Shetty[1], R. Toydemir[1], N. Uddin[1], Y. Zhang[2], L. Rowe[3], A. Brothman[1,4], J. Johnston[2,5].* 1) University of Utah, Department of Pathology ,School of Medicine and Cytogenetics/Molecular Cytogenetics, ARUP Laboratories, Salt Lake City, UT; 2) Manitoba Institute of Cell Biology, and The Genomic Centre for Cancer Research and Diagnosis (GCCRD)[Clinical Research] Winnipeg, ManitobaWinnipeg, Manitoba, Canada; 3) ARUP Institute for Clinical and Experimental Pathology, ARUP Laboratories, Salt Lake City, UT; 4) University of Utah, Department of Pediatrics, Salt Lake City, UT; 5) Internal Medicine, University of Manitoba, Winnipeg, Manitoba, Canada.

INTRODUCTION AND GOALS: Chronic lymphocytic leukemia (CLL) is a heterogeneous disease with variable clinical course. Genetic characterization has played a pivotal role in the clinical classification and treatment of this disease. The aim of this study was to ascertain whether genetic markers play an important role during the course of the disease and to analyze its possible correlation with disease progression. Thirty sequential peripheral blood samples (taken at initial diagnosis and then again at the latest interval post therapy) from patients with CLL over a period of 14 years are being evaluated retrospectively using the high resolution CNV/SNP microarray platform (Illumina OMNI1 Quad). All results are correlated with other clinical and laboratory information including IgVH mutation status, ZAP-70, CD38, telomeric length, lymphocyte doubling time, response to therapy and survival time. RESULTS: Examples of some of the findings in the first five patient's sequential samples include three patients with at least two genetic abnormalities. In the two samples from each patient, expansion of the clonal population was seen and correlated with disease progression. Genetic abnormalities such as 6q del, 11q del and 13q del with established prognostic clinical value were found in three patients. Other abnormalities detected were loss or gain of regions on 4p, 7p, 7q, 8p, 8q, 10q, 11p. One patient with an unmutated IgVH status (poor prognostic marker) showed no copy number changes (CNC) or loss of heterozygosity (LOH) in sequential samples and is doing well without any treatment for the past nine years since diagnosis. LOH was seen in one patient without any copy number changes and interestingly, this patient partially responded to therapy and showed a decrease in the abnormal clone followed by progression of the disease. INTERPRETATION AND CONCLUSIONS: Preliminary results indicate that sequential samples provide insight into the cytogenetic clonal evolution and clinical implications associated with CNC and LOH information in CLL patients. Additional sequential samples with well characterized genetic abnormalities including 6q, 11q, 13q, 17p and normal karyotype are being evaluated and correlation of findings with clinical outcome will be discussed.

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Extensive whole-chromosome aberrations detected by SNP array in a neurofibromatosis type 1-associated glomus tumor. *D. Stewart[1], A. Pemov[1], E. Beert[2], H. Brems[2], E. Legius[2].* 1) Genetic Disease Research Branch, NHGRI/NIH, Bethesda, MD; 2) Department of Human Genetics, Catholic University Leuven, Leuven, Belgium.

Introduction. Glomus tumors are painful benign tumors of the glomus body, a thermoregulatory shunt located in the fingertips. We recently reported that NF1-associated glomus tumors arise from bi-allelic inactivation of the gene *NF1*. We performed analysis of the genomic architecture in two sporadic and four NF1-associated glomus neoplasms using high-resolution Illumina SNP arrays. **Methods**. DNA was collected from primary cell cultures (except for one case, when DNA was also extracted directly from tumor tissue) that were established from dissected glomus tumors. Comprehensive *NF1* sequencing was performed on DNA from NF1-associated tumors. DNA samples were processed and hybridized to the Illumina HumanOmni1-Quad microarrays. White blood cell DNA was used as germline control in NF1-associated glomus tumors (germline DNA for the patients with sporadic glomus tumors was unavailable). Analyses of copy number variants (CNV) and loss of heterozygosity (LOH) regions was performed using GenomeStudio (Illumina). Every putative CNV or LOH region from NF1-associated tumors was compared to its matching germline DNA sample. For the sporadic tumors, where control germline DNA was unavailable, normal copy number for each region was assumed to be 2 and deviation from that was considered as copy number loss or gain. **Results**. We hybridized DNA from the genomes of four NF1-associated and two sporadic glomus tumors. We found germline mutations of *NF1* in all 4 tumors and a somatic mutation in *NF1* in three of the four NF1-associated tumors. In the fourth tumor there were multiple large-scale chromosomal aberrations, including evidence of likely mitotic recombination of chromosome 17q. Across the entirety of chromosomes 2, 3, 4, 5, 6, 8, 9, 13, 18, 19 and 21, we observed B allele frequencies of 0%, ~33%, ~67% and 100% in conjunction with a log R ratio of ~0. We also found evidence of a large number of smaller CNVs shared by multiple tumors. **Conclusions**. This is the first report of bi-allelic inactivation of *NF1* arising from mitotic recombination of chromosome 17q in an NF1-associated glomus tumor. The multiple large-scale chromosomal aberrations in 1/4 NF1-associated glomus tumors may be due to chromosomal trisomy or, less likely, mosaic whole-chromosome uniparental disomy (UPD). Somatic aneuploidy (and/or UPD) may be more common than expected in NF1-associated glomus tumors.

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Cryptic BCR/ABL1 fusion in a Philadelphia chromosome negative patient, resulting in a 9;22 insertion. *P.D. Storto, G.R. Diggans, D.R. Halinka.* Pathology, The Western Pennsylvania Hospital, Pittsburgh, PA.

A 48 year-old female patient presented with an increased WBC count and nose-bleeding, and cytogenetic testing was ordered. A routine cytogenetic analysis detected mosaicism for both trisomy 8 and trisomy 9, and the karyotype was described as 47,+8,+9[2]/ 46,XX[18]. Subsequently, FISH analysis for the 9;22 translocation was requested. The Extra Signal Dual-Color (ES-DC) BCR/ABL1 probe developed by Abbott Molecular, Inc. was used. This probe is designed to differentiate between the major and minor breakpoints in the BCR region. We examined 218 interphase nuclei and 215 (98.6%) had abnormal hybridization patterns. The majority of cells (200) contained one orange, one large green, one small green, and one fusion signal, indicating an intact ABL1 copy, an intact BCR copy, a deleted BCR copy, and a single BCR/ABL1 fusion. Fifteen interphase cells contained this abnormal pattern plus an additional fusion signal. In the case of the common 9;22 translocation involving the major breakpoint, the expected pattern with this probe would be one large orange, one small orange, one green and one fusion signal. A translocation involving the minor breakpoint would result in one orange, one green, and two fusion signals. Analysis of metaphase cells treated with the FISH probe showed that the fusion signal was located on one copy of chromosome 9. This probe was specifically designed to detect the 5'BCR/3'ABL1 fusion gene, (which is typically located on the derivative chromosome 22). We interpret these results to indicate that the abnormal karyotype is most likely the result of a direct insertion involving chromosome 22 material, with breaks proximal to the 5' BCR region and at the minor BCR breakpoint. The material is inserted into a break within the ABL region, resulting in the 5'BCR/3'ABL1 fusion gene located within the derivative chromosome 9, and no material from chromosome 9 translocated onto the deleted chromosome 22. Cells with an additional fusion signal reflect the trisomy 8 and trisomy 9 clone; as FISH analysis of metaphase cells shows that the additional chromosome 9 contains the fusion gene. Note that the homologs of chromosomes 9 and 22 appeared normal in the G-banded karyotypes. The final karyotype was reported as 47,+8,+9[2]/ 46,XX[18].ish ins(9;22) (q34;q11.2q11.2)(ABL1+,BCR+;BCR+,ABL1-).nuc ish (ABL1x2,BCRx3) (ABL1 con BCRx1) [200/218]/(ABL1x3,BCRx4)(ABL1 con BCRx2)[15/218].

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Recurrent Cytogenetic Abnormalities in Fanconi Anemia Patients with MDS/AML. *L. Wang[1], F. Boulad[2], A. Auerbach[3], S. Jhanwar[1].* 1) Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, NY; 2) Department of Pediatrics, Memorial Sloan-Kettering Cancer Center, New York, NY; 3) The Rockefeller University, New York, NY.

Fanconi Anemia (FA) is a rare inherited disease characterized by physical abnormalities, bone marrow failure, and increased risk of MDS/AML, head/neck squamous carcinomas and some other malignancies. Genetically, FA is associated with high spontaneous mutation rate and chromosomal instability. Interestingly, the non-random cytogenetic abnormalities seen in FA with MDS/AML are similar to that identified in secondary MDS/AML. We reviewed cytogenetics findings in bone marrow samples from 55 Fanconi Anemia patients who had either Aplastic Anemia, or MDS/AML. The main objective of such an analysis was to identify chromosomal sites of tumor suppressor genes associated with malignant transformation in cells with underlining germline mutation related to genomic instability. Clonal abnormalities were detected in 32 patients, majority of which were characterized by unbalanced chromosome rearrangements (only three balanced chromosome translocations were identified). In addition, clonal evolution was evident in 12 patients. Recurrent abnormalities identified in this study included gain of 1q (entire 1q or partial segments of 1q) [23/32]; -7/del(7q) [10/32]; gain of 3q specifically 3q26qter [6/32]; loss of 6p with 6p21pter as the commonly involved region[7/32]; and del(11)(q23) [4/32]. Clonal abnormalities, such as gain of 1q, -7/del(7q), gain of 3q26qter, and del(11)(q23) in FA with AA/MDS-AML are well demonstrated by other studies. We present here a significant number of cases with loss of 6p21pter. Moreover, in most of the cases [5/7], the loss of 6p21pter was due to unbalanced translocations between 6p and either chromosome 1 or chromosome 3, which also resulted in partial trisomy of 1q or 3q, respectively. In our study, the loss of 6p21pter was highly associated with clonal evolution in FA patients with AA/MDS-AML, which indicated the presence of tumor suppressor gene(s) and the loss of which may play an important role in the development/progression of hematological malignancies in FA patients. While the nature of genes located at 11q23 (ATM, MLL) and 3q26(EVI1) has been well recognized, the nature of genes located on 1q and 6p21 remain unknown. It is interesting to note that one of the FA genes, FANCE, is mapped to 6p22-21. Further molecular studies are likely to provide information related to nature of genes at these sites.

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Acute leukemia of ambiguous lineage associated with t(4;11). *S.L. Wenger[1], R. Livengood[1], S. Paul[2], J.A. Vos[1].* 1) Dept Pathology, West Virginia Univ, Morgantown, WV; 2) Dept Pediatrics, West Virginia Univ, Morgantown, WV.

We report on a 16 year old girl with acute leukemia of ambiguous lineage. The patient presented with pallor, petechiae, dyspnea and menorrhagia of approximately 10 days. Initial CBC revealed a white blood cell count of 43,000/mm3, hemoglobin of 6.4 g/dl and platelet count of 17,000/mm3. The bone marrow aspirate showed 80% blasts comprised of two distinct populations. Approximately 75% of blasts had an immunophenotype positive for CD19, HLA-DR, CD38 and CD79a with block positivity for PAS, consistent with B-lymphoblasts with a primitive phenotype. The second population, representing 25% of blasts, was positive for CD33, HLA-DR, CD38, CD11b as well as nonspecific esterase and lysozyme, consistent with myeloblasts with possible monocytic differentiation. Overall, the diagnosis was consistent with acute leukemia of ambiguous lineage. FISH for ALL panel was negative for trisomy 4, 10 and 17, t(8;21) and t(9;22), but was positive for MLL translocation in 88% of interphase cells. The karyotype showed a t(4;11)(q21;q23) in 50% of metaphase cells. The patient initially received myeloid induction chemotherapy, became pancytopenic and recovered counts by day 30 of treatment. Peripheral blasts were present at this time and a bone marrow biopsy revealed persistent bi-lineal acute leukemia. She was re-induced with lymphoid therapy with remission pattern seen on day 28. Five months from diagnosis, the patient received an allogenic matched related donor transplant, resulting in 100% donor cells one month post-transplant consistent with engraftment. She was admitted to the hospital 2 weeks later with an RSV infection. She received treatment and was released. Despite a relatively uncomplicated post-transplant course, the patient relapsed and died of her disease 5 months following her transplant. Acute leukemia of ambiguous lineage is rare, occurring in less than 4% of all acute leukemia cases, with poor prognosis. The most common rearrangement associated with 11q23 is a translocation involving 4q21.

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A rare case having t(8;22) and t(14;18) double hit with up to 3 copies of t(8;22) and morphologic and immunophenotypic features of large B cell lymphoma of possible follicular origin. *J. Xu[1], C. Hamm[2], P. Allevato[3].* 1) Cytogenetics, London Health Sciences Centre and University of Western Ontario, London, ON, Canada; 2) Oncology, University of Western Ontario, Windsor, ON, Canada; 3) Pathology, University of Western Ontario, Windsor, ON, Canada.

A 43 year old female had 1 week long history of fatigue, easy bruising and persistent nosebleed. WBC was 22.7x109/L (neutrophils 9.4; lymphocytes 8.5; monocytes 2.5; eosinophils 1.1; basophils 0.3), platelets, 22x109/L. LDH was > 2400u/L(177-217). CT scan showed moderate splenomegaly (14.8cm), 3.4cm axillary and retroperitoneal adenopathy. Bone marrow (BM) biopsy showed a diffuse infiltration of large atypical blast-like cells with moderate amounts of basophilic cytoplasm and irregular, somewhat coarse nuclear chromatin with several nucleoli. These atypical cells strongly expressed CD45 and were positive for CD10 and bcl-2 with weak positivist CD79a and were negative for CD20, Tat, CD99, CD34, CD117, CD30, CD3, CD5, CD23, bcl-6 and cyclin D1. several lymphoid nodules had B cells (CD20, CD79a), which were bcl-2 and focally bcl-6 positive. Ki67 staining showed a high proliferate rate of ~90%. Megakaryocytogenesis was only slightly decreased. Flow cytometry showed 2 monoclonal B cell populations: the 1st represented ~33% of the leukocyte population and was CD19/CD10, dim to negative CD20, kappa light chain restricted. The 2nd represented 6% of the leucocyte population and was CD19, CD 20, and CD23 positive and CD 10 and CD 5 negative with kappa light chain restriction. G-banding of cultured BM showed a complex karyotype: 68-83,XXXX,+X,+X,+X,+X, del(1)(p36)x2,-4,-5, del(6)(q15q23)x2,der(6)t(6;17)(q13;q11.2)x2,-7,-8, t(8;22)(q24;q11),t(8;22)x2,t(8;22)x3,-9,add(9)(p22),-10,del(10)(q24),-11,+12,-13, -14, t(14;18)(q32;q21),-15,-16,-17,-18, add(18)(q11.2),-19, +r(cp22)/46,XY[3]. FISH showed fusion of IGH/BCL2 and split signal of CMYC in 69% and 42% of 200 cells, respectively, confirming the presence of t(14;18) and t(8;22). Both t(8;22) and t(14;18), "a double-hit" were found in all 21 G-banded cells. Notably t(8;22) was seen in 1 copy in 1/21 cells, 2 copies in 18/21 cells, and 3 copies of t(8;22) in 2/21 cells. In contrast, only 1 copy of t(14;18) was detected in all 21 cells. t(8;22) is commonly associated with Burkitt lymphoma (BL) whereas t(14;18) and deletions in 1p36, 6q, 9p and 10q24, with follicular or diffuse large B-cell lymphomas (DLBCL). This is likely the 1st reported case with a double hit with up to 3 copies of t(8:22) and probably is best classified as a B cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL (2008 WHO classification). This cytogenetic finding indicates an aggressive disease and poor prognosis.

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Utility of High Resolution Oligo Array CGH in Clinical Testing for Hematological Malignancies. *X. Xu[1], A. Arthur[1], E. Johnson[1], L. Leverton[1], J. Laffin[1,3], G. Raca[1,2].* 1) UW Cytogenetics Services, State Laboratory of Hygiene, Madison, WI; 2) Department of Pathology and Laboratory Medicine, University of Wisconsin-Madison; 3) Department of Pediatrics, University of Wisconsin-Madison.

Our study compares the ability of array Comparative Genomic Hybridization (aCGH) and conventional cytogenetic methods -G-banded analysis and Fluorescence In Situ Hybridization (FISH)- to detect chromosomal abnormalities in patients with hematological malignancies. Our goal is to investigate the suitability of using aCGH as a routine diagnostic test for hematological disorders. DNA samples are isolated from residual bone marrow or peripheral blood specimens from clinical testing. aCGH analysis is performed using a custom 2x105k whole genome oligo array designed by the Cancer Cytogenetics Microarray Consortium (CCMC) and manufactured by Agilent (Agilent Technologies, Inc., Santa Clara, CA). This array has over 105,000 probes targeting 500 cancer genes and more than 130 cancer-associated genomic regions. Prior to aCGH testing the samples are analyzed by conventional cytogenetics and FISH, as a part of routine diagnostic evaluation. Comparison of the aCGH results with the results of the conventional testing methods allows to determine whether: 1) aCGH can detect all the gains and losses of chromosome regions which were detected by cytogenetic and FISH studies, and 2) aCGH can detect additional chromosome abnormalities which were not detected by traditional testing. Our results to date illustrate the specific advantage of aCGH methodology to aid in characterization of complex karyotypes. We tested a subset of specimens where multiple numerical and structural abnormalities were observed by G-banded analysis, but could not be fully characterized by conventional methods. We show two cases (one case of acute lymphoblastic leukemia and one case of multiple myeloma) where aCGH successfully determined the origins of marker chromosomes and characterized complex derivative chromosomes. In both cases aCGH helped to clearly identify recurrent disease associated abnormalities with diagnostic and prognostic significance. Although the inability to detect balanced rearrangements remains a serious disadvantage for application of aCGH, this study shows how aCGH provides useful clinical information both by detecting submicroscopic deletions and duplications as well as by allowing accurate characterization of gross unbalanced rearrangements.

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New genomic profile signatures revealed in Western World adult T-cell leukemia/lymphoma patients by 244K array CGH. *S. Xu, R. Lima, J. Ramos, Y. Fan.* University of Miami Miller School of Medicine.

Adult T-cell leukemia/lymphoma (ATLL) is a peripheral T-lymphocytic malignant disease caused by human T-lymphotropic virus type 1 (HTLV-1). ATLL is endemic in several regions of the world, including Japan and the Caribbean region. We performed aCGH analysis in 50 ATLL patients from Brazil and the USA, which were most of Caribbean origin, using a high density oligo array 244K platform (Agilent). All patients presented copy number changes (CNCs) with variable sizes and complexity. The common CNCs were gains at 1q21-q44, 3, 3p, 7q22-q36, 8, 18, 19p13.1-p13.3, 21q21.1-q22.3, 22q12-q13 and losses at 5q13.2-q32, 6q11-q15, 9q13-q21. Gains in the 14 q32 (IGH) regions and losses in the 7p14.1 (TCRG), 7q34 (TCRB) and 14q11.2 (TCRA) regions involving small DNA segments were frequently observed. We compared the CNCs observed in two subgroups of patients, one with acute T-cell leukemia (n=34) and the other with T-cell lymphoma (n=8). We found that the acute leukemia group showed a high frequency of loss of tumor suppressor gene CDKN2a/2b (50% in leukemia group vs. 12.5% in lymphoma group). On the other hand, the lymphoma group showed high frequency of TP53 loss in comparison with the leukemia group (75% vs. 11.8%). As expected, patients with chronic leukemia (n=8) showed a lower frequency of CNCs. Two genes, LATS2, a tumor suppressor gene, and BAGE which encodes a tumor antigen recognized by autologous cytolytic lymphocytes were frequently deleted in lymphoma type. We also observed frequent gain of CCNL2 in acute leukemia type (41.2%) and gain of PDCD6IP in lymphoma type (62.5%) while gain of IFRD2 and RASSF commonly occurred in all types (avg. 40%). Our studies have revealed genomic profile signatures in the two subtypes of ATLL. Our results have shown that CNCs of specific genes may have contributed to tumorigenesis and progression of ATLL and these CNCs may be used as biomarkers to predict patient survival and treatment outcome.

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Myeloid malignancies with acquired trisomy 21 exhibit a heterogeneous pattern of mutations and copy number alterations. *N. Larsson, H. Lilljebjörn, C. Lassen, B. Johansson, T. Fioretos.* Department of Clinical Genetics, Univeristy and Regional Laboratories, Skåne University Hospital, Lund University, Lund, Sweden.

Acquired trisomy 21 is a recurring cytogenetic aberration in myeloid malignancies that is present in about 5% of all cytogenetically abnormal acute myeloid leukemias (AML) and in 3-4% of myelodysplastic syndromes (MDS). Clinically, the prognostic value of this cytogenetic change is debatable, although most often it has been associated with intermediate prognosis. To investigate the role of trisomy 21 in myeloid malignancies, we studied a cohort of 11 such cases with trisomy 21 as the sole acquired cytogenetic aberration. We looked for a possible association between trisomy 21 and mutations in eight genes known to be important in differentiation, proliferation or survival of early hematopoietic cells (NPM1, TET2, FGFR1, FLT3, NRAS, KRAS, KIT, JAK2).Our results showed that the highest frequency (3/11, 27%) of mutations were found in the RUNX1 transcription factor, frequently known to be mutated or rearranged in both AML and MDS. The mutations included two missense mutations and one frameshift mutation, all located in the Runt domain of the gene. Apart from RUNX1, single mutations were detected in the TET2 and NPM1 genes. We also performed SNP array analysis using Affymetrix 500 K arrays on all cases to look for copy number alterations (CNA) and patterns of uniparental disomy (UPD) that could be common to these patients. Although CNAs and areas of UDP were observed, no recurrent genetic alterations were detected.

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Prognostic and predictive impact of cytogenetic changes in relapsed multiple myeloma patients treated with bortezomib or thalidomide based regiments: a Czech group experience. *J. Smetana[1,2], P. Kuglik[2], H. Greslikova[1], R. Kupska[1], P. Nemec[1,2], R. Zaoralova[1,2], E. Braggio[3], L. Pour[1,4], L. Zahradova[1,4], M. Holanek[4], Z. Adam[4], R. Hajek[1,4].* 1) Babak Research Institute, Brno, Czech Republic; 2) Masaryk University, Faculty Of Science, Brno, Czech Republic; 3) Mayo Clinic Scottsdale, Collaborative Research Building, Scottsdale, Arizona, USA; 4) Department of Internal Medicine - Hemato-oncology, Brno University Hospital, Brno, Czech Republic.

Multiple myeloma (MM) is a hematological disease characterized by high clinical and genetic heterogeneity. There are two major genetic entities of MM: a hypediploid MM (H-MM), associated with the incidence of trisomies of chromosomes 3,5,7,9,11,15,19 and 21, and a non-hyperdiploid variant (NH-MM) associated with high incidence of IgH translocations (t(4;14)(p16.3;q32); t(14;16)(q32;q23)) and thus usually worse prognosis of disease. In this paper, we present pilot results of our study focuisng on utilization of modern cytogenetic and genomic techniques for finding new prognostic and predictive markers in MM. The main aim of this work was to evaluate predictive and prognostic significance of hyperdiploidy / non-hyperdiploidy in relapsed MM patients treated with bortezomib (median follow-up 18,3 months) or thalidomide-based regiments (median follow-up 21.5 months) by interphase FISH. 57 samples were investigated for H-MM / NH-MM by using LSI D5S23 /D5S721, CEP 9, CEP 15 Multi-Color Probe Panel and for other chromosomal abnormalities with prognostic value in MM as t(4;14)(p16.3;q32), del(13)(q14), del(17)(p13) and gain(1)(q21). At least 100 cells per slide were scored, 20% cut-off level was used. Prognostic impact of hyperdiploidy / non-hyperdiploidy was evaluated using Kaplan-Mayer surviving curves and treatment intervals. Hyperdiploidy was found in 25 of 53 patients (47%), non-hyperdiploidy in 28 of 53 patients (53%). Although no significant correlation between incidence of structural aberrations and hyperdiploid / non-hyperdiploid groups was found, our data suggest that t(4;14)(p16.3;q32) and del(13)(q14) are associated with NH-MM group (P=0.065 and P=0.10), similarly as described in literature in studies with newly diagnosed patients. Neither for bortezomib, nor for thalidomide treated patients, any statistically significant difference was observed between treatment responses and treatment intervals between hyperdiploid (median OS 87.1 vs. 72.2 months, P=0.27) and non-hyperdiploid group (113.6 vs. 84.6 months, P=0.49) According to our hypothesis, bortezomib or thalidomide-based regiments are able to overcome negative prognostic impact of non-hyperdiploidy in relapsed multiple myeloma patients. However, confirmation will require analysis of a larger cohort of patients with a longer monitoring period. Supported by VZ LC06027, MšMT ČR (MSM0021622415, MSM0021622434) and IGA NS10207.

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Simultaneous measurement of copy number and copy-neutral genome alterations in cancer on CGH+SNP microarrays. *P. Anderson, B. Curry, B. Peter, N. Sampas, P. Costa, L. Bruhn.* Agilent Technologies, Inc., Santa Clara, CA.

For the characterization of genomic copy number changes that occur in the development and progression of cancer, oligonucleotide array comparative genomic hybridization (aCGH) offers high-resolution and precise determination of chromosomal copy number and genome-wide aberrations. We have developed a new method for making simultaneous measurements of Single Nucleotide Polymorphism (SNP) genotypes and copy number alterations in the same microarray assay to provide detection of copy-number neutral events, such as acquired loss of heterozygosity (LOH), as well as allelic imbalance in and around amplified regions. We profiled genomic DNA isolated from cancer cell lines, blood samples, and solid tumors on CGH+SNP arrays to measure chromosomal copy number at >200,000 loci and genotypes for >50,000 SNPs. DNA was digested with AluI and RsaI restriction enzymes and labeled with a standard enzymatic labeling protocol. In a two color assay, the sample was hybridized against a known genotyped reference to the CGH+SNP arrays. Copy number, SNP and aberration calls, including copy-neutral LOH, were generated using novel algorithms implemented in Agilent's Genomic Workbench software and Matlab. We detected copy number alterations and copy neutral LOH in many samples. For example, we found several focal amplifications contained within larger regions of copy-neutral LOH in a model Burkitt's lymphoma cell line (Raji). In a chronic lymphocytic leukemia sample, we measured a single copy deletion of 18p as well as copy-neutral LOH at 18q22.3-qter. Tumor heterogeneity and variable sample quality create significant challenges for accurate determination of SNP genotypes and copy number in both solid and liquid tumors. In paired tumor/normal tissues, we determined SNP genotypes and were able to calculate the fractional clonality of the tumor and detected copy number aberrations in samples with as little as 20% tumor cell content. In regards to sample variability we found that inaccurate quantitation of DNA input to the labeling reaction is a significant contributing factor to noise in the CGH and SNP measurements. To further decrease assay noise, a GC correction algorithm was used to improve the overall data quality for some cancer samples. Low noise assays with accurate measurements of both copy number and SNP genotypes will enable a deeper understanding of the alterations related to tumor development and progression.

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Cell Line Identification by SNP: an Emerging Need in Cell Biology. *R. Fang, J. Shewale, M. Furtado.* Life Technologies, 850 Lincoln Centre Drive, Foster City CA 94404, USA.

Cancer cell lines are used worldwide in biological research for applications like cancer studies, genetics, and drug screening. Data misinterpretation due to incorrectly identified cell lines is a growing concern. Identification of human cell lines, therefore, has become a focus area. NIH has issued a guideline for mandatory identification of human cell lines used for drug screening. Short tandem repeat (STR) profiling is being used for cell line authentication. It is observed that the STR profile of some cell lines drift over the passages. In this study, we proposed to use the single nucleotide polymorphism (SNP) markers for this application because of its high genetic stability. To prove the concept, a PCR-OLA based SNP profiling system developed for human identification was evaluated for this application. This system enables to genotype 48 SNPs simultaneously in single reaction and provides accurate results. In this study, we profiled DNA preparations from 60 cell lines from National Cancer Institute (NCI) and 50 cell lines from American Type Culture Collection (ATCC) using the 48-Plex SNP detection system. Our results show that each human cell line typed has its own unique SNP profile. Three mislabeled cell lines were identified during the study. Our study demonstrates that the SNP based DNA profiling can be used for human cell authentication. This approach is more robust than the method using the genomic length polymorphism which may get altered under various cell culture conditions and the number of cell line passes. In addition, our study would enable researchers to authenticate the human cell line and monitor the contamination of cell lines in the laboratory.

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Hemihypertrophy, Bilateral Pheochromocytomas, and Subtle Somatic Mosaicism: the Utility of SNP Array Analysis. *J.M. Kalish[1], L.K. Conlin[2], A.B. Wilkens[1], S. Mulchandani[2], M. Kowalski[3], K.E. Nichols[3], J.A. Biegel[1,2], N.B. Spinner[2], M.A. Deardorff[1].* 1) Division of Human Genetics, Children's Hospital of Philadelphia, Philadelphia, PA; 2) Department of Pathology, Children's Hospital of Philadelphia, Philadelphia, PA; 3) Division of Oncology, Children's Hospital of Philadelphia, Philadelphia, PA.

We report a case of a girl with hemihypertrophy, bilateral pheochromocytomas, and 11p15 mosaic uniparental disomy (UPD). Hemihypertrophy of the upper arms was diagnosed at 4 months with a 7.5% difference in girth. Clinical methylation testing for 11p15 in the blood was normal with a reported detection threshold of 20%. SNP array analysis (610K) performed on the blood secondary to additional minor facial dysmorphia was normal. She was subsequently diagnosed at 18 months with bilateral, non-secreting, non-metastatic adrenal masses. Following surgical resection, her tumor histology demonstrated eccentric round nuclei, eosinophilic cytoplasm that was diffusely positive for neuron-specific enolase, with some nodules positive for synaptophysin, all findings consistent with pheochromocytoma. Mutation analysis of genes known to cause pheochromocytoma (*VHL*, *SDHB/SDHD*, and *RET*) was negative. SNP array analysis of the pheochromocytoma tissue demonstrated mosaic deletions of chromosome regions common to pheochromocytomas (8p12pter, 21q21.1qter, and 22q11.23qter) and a novel finding of homozygosity for 11p15.3pter. The presence of 11p15 homozygosity suggested that it could be the primary event. To pursue a definitive diagnosis, SNP array analysis of skin fibroblasts from the hypertrophied side was performed and demonstrated <5% mosaic paternal uniparental isodisomy for this same region. Two cases of isolated hemihypertrophy and two cases of Beckwith-Wiedemann syndrome (BWS) have been reported with pheochromocytoma. Molecular testing was reported in only one case. This patient had a normal karyotype, and no detectable mutations in *RET* and *NF* in peripheral blood, or *VHL* and *RET* in the tumor, and normal methylation studies of *LIT1* and *H19* genes. We feel our case is unique, given the young age of this patient, the use of SNP array analysis, and the demonstration of a definitive molecular diagnosis. Given our experience, we feel that SNP array analysis is more sensitive in detecting UPD than MLPA due to the larger number of data points available for evaluation. This case underscores the utility of SNP analysis in rapidly identifying low levels of mosaicism and also suggests that 11p15 paternal UPD may be a general mechanism of pheochromocytoma in the pediatric population. Furthermore, we suspect that silencing of *CDKN1C* and segmental loss of growth inhibition likely led to further chromosomal changes that developed in the pheochromocytoma.

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The role of Flt3 in a murine model of B-precursor Acute Lymphoblastic Leukemia. *R.M. Johnson[1, 2], E. Papp[3, 4], I. Grandal[2], J. Danska[3, 4], C. Guidos[1, 2].* 1) Developmental & Stem Cell, The Hospital for Sick Children, Toronto, ON, Canada; 2) Department of Immunology, University of Toronto, ON, Canada; 3) Genetics & Genome Biology, The Hospital for Sick Children Research Institute, Toronto, ON, Canada; 4) Department of Medical Biophysics, University of Toronto, ON, Canada.

Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy characterized by abnormal survival and proliferation of precursor-B lymphocytes. To identify dysregulated developmental pathways in ALL, our laboratory has generated Rag-2$^{-/-}$p53$^{-/-}$Prkdc$^{scid/scid}$ triple mutant (TM) mice, which develop B-precursor ALL. Using a genome-wide approach to uncover genetic alterations, we showed that TM leukemic blasts over-express FMS-like tyrosine kinase 3 (Flt3) relative to normal early B cell progenitors. FLT3 is a class III receptor tyrosine kinase (RTK), which is expressed on early hematopoietic progenitors and on B cell and macrophage precursors. Activation of Flt3 results in autophosphorylation of specific tyrosine residues and activation of signaling intermediates to promote cell survival and proliferation. FLT3 is normally expressed in two forms: a 160kDa membrane-bound fully glycosylated form and a 130kDa immature form. Two types of FLT3 mutations have been characterized in acute leukemias: (1) internal tandem duplications (ITD) in the juxtamembrane domain, and (2) kinase domain (KD) point mutations. Although FLT3 is expressed in most human B-lineage ALLs, ITD and KD mutations occur in only 3% of cases. We examined FLT3 expression on CD19^{+} leukemic blasts from TM mice and found variable surface expression of FLT3 despite high levels of Flt3 transcript. Culturing TM leukemic cells in the presence of class III RTK inhibitor, AGL 2043, revealed that FLT3, and potentially other class III RTKs, are necessary for the proliferation of these cells. Moreover, TM leukemic cells were able to proliferate in vitro in the absence of FLT3 ligand suggesting that ligand-independent activation is responsible for the growth of TM leukemic cells. Immunoblotting studies revealed that all TM ALLs over-expressed a truncated form of FLT3. Further biochemical studies revealed that this truncated FLT3 is constitutively phosphorylated in TM ALLs. Taken together, our studies have identified a novel type of FLT3 alteration in a murine model of B-precursor ALL resulting in a constitutively activated truncated form of the protein. We hypothesize that this aberrant FLT3 activation is responsible for the abnormal proliferation of TM leukemic cells. These findings suggest that aberrant activation of FLT3 plays an important role in the pathogenesis of B-precursor ALL. Future studies are needed to determine whether similar FLT3 alterations are present in human ALL.

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Mapping Transitional Cell Carcinoma of the Bladder in the Dog. *H.G. Parker[1], E.M. Kwon[1,4], D.W. Knapp[2], P. Bonney[2], E. McNiel[3], E.A. Ostrander[1].* 1) Comparative Genomics Section, Cancer Genetics Branch, NHGRI, NIH, Bethesda, MD; 2) Purdue Comparative Oncology Program, Dept. of Veterinary Clinical Sciences, Purdue University, West Lafayette, IN; 3) Dept. of Veterinary Clinical Sciences, University of Minnesota, Minneapolis, MN; 4) Program in Human Genetics and Molecular Biology, Johns Hopkins University School of Medicine, Baltimore, MD.

Transitional cell carcinoma (TCC) of the bladder in the domestic dog is an ideal model of invasive bladder cancer in humans, which takes the lives of more than 14,000 people each year. Both diseases develop spontaneously, respond similarly to drug treatments, and have nearly identical histopathology. TCC is the most common cancer of the urinary bladder in pet dogs. It is an aggressive disease resulting in metastasis in approximately 50% of all dogs diagnosed. Currently little is known about the genetic basis of human bladder cancer. Recent GWAS studies identified two risk loci in both European and Asian populations, however, they account for only a small fraction of the overall risk of developing the disease. In comparison, our group has identified a small number of dog breeds that show a greatly increased risk of bladder cancer, as much as 20 fold higher than average, indicating a strong inherited component to the disease. In order to find mutations that increase susceptibility to TCC of the bladder, we have conducted a genome-wide association study using the Affymetrix canine v2 SNP chip on 122 Scottish terriers (ST), West Highland White terriers (WHWT), and Shetland Sheepdogs (SSD) with confirmed diagnoses of TCC compared to 135 dogs of the same breeds that are nine years or older and have never been diagnosed with cancer of any form. After correcting for both population structure and kinship, we have identified two primary susceptibility loci within the three breeds with $p<1x10^{-6}$. The association at locus1 is driven primarily by the ST and is increased with the addition of WHWT but not with SSD. Locus2 is associated with the TCC in the WHWT and SSD when analyzed apart from the ST and does not show association with the disease in the ST. We hypothesize based on allele frequencies within the region that the ST are nearing fixation at locus2, explaining the 20-fold increased risk in this breed. We have designed and run a Goldengate assay of 768 SNPs covering both loci at ~15kb density in order to fine map the region and assess gross copy number within the germline and within tumor DNA at both loci. We expect to find overlapping regions between the three breeds that contain causal mutations that increase overall risk of bladder cancer. The identification of genetic risk factors for TCC will allow selection of molecular targets for early detection and treatment in both humans and dogs.

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Canine Gastric Cancer GWAS Identifies a Single Locus in the Chow Chow. *D.M. Karyadi[1], E.A. McNiel[2], E. Karlins[1], N. Madrill[2], E.A. Ostrander[1].* 1) Cancer Genetics Branch, NHGRI/NIH, Bethesda, MD; 2) Dept. of Small Animal Clinical Services, Michigan State University, East Lancing, MI.

The role of predisposition genes in human gastric cancer (GC) is not well understood. However, strong associations between specific dog breeds and GC risk have been reported, suggesting a mechanism for identifying genes important in GC susceptibility. Breeds at increased risk of GC include the Chow Chow, Belgian Sheepdog, Belgian Tervuren, and Keeshond. Of note, Chow Chows have striking 10-20-fold increased risk of developing GC compared to other breeds. GC in Chow Chows is clinically and morphologically similar to familial forms of the disease described in humans and, like human GC, has a grave prognosis. Diagnosis is usually made late in the course of the disease when effective treatment is rarely feasible. In humans mutations in limited number of genes have been suggested to increase risk, such as the *CDH1* gene and genes in the DNA mismatch repair pathway. However, these genes explain only a portion of familial human disease, little if any sporadic disease, and none of the canine disease. We hypothesize that a more global evaluation of the canine genome is likely to reveal genes of interest for both human and canine GC susceptibility. We conducted a genome-wide association study with Chow Chow cases, and unrelated and related controls using the Illumina CanineHD BeadChip with 170,000 SNPs. The final data set consisted of 125,713 SNPs informative in Chow Chows. Using the single-locus chi-squared test of significance, we calculated the allelic association of each SNP with the disease phenotype. In the analysis of cases and unrelated controls, the top 4 most significant SNPs were all at the same genomic locus ($P_{raw} = 6.83x10^{-5}$ - $1.09x10^{-5}$), creating a double peak spaced 20 Mb apart. Chromosome-wide permutations (n=100,000) were performed to test for significance of this locus. For the SNP with the best disease association, the chromosome-wide empirical P value is significant at P = 0.021. Analysis of the Chow Chow cases and entire set of controls (unrelated plus related) identified the same locus. Chromosome-wide permutations (n=100,000) were also significant for the top SNP (P_{emp} = 0.033). None of the peaks in other genomic regions are significant at the chromosome-wide level in either analysis. We are in the process of fine-mapping this region and using haplotype analysis in order to identify the causal variant. Our findings would likely highlight genes and biological pathways important for future studies of both human and canine GC.

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Comprehensive resequence analysis of a 123kb region of chromosome 11q13 associated with prostate cancer. *C. Chung[1,2], Z. Deng[2,3], J. Boland[2,3], C. Matthews[2,3], M. Yeager[2,3], K. Jacobs[2,3,4], A. Hutchinson[2,3], R. Hoover[2], J. Fraumeni[2], D. Hunter[2,5,6,7], G. Thomas[2], S. Chanock[1,2].* 1) Laboratory of Translational Genomics/DCEG, NCI/NIH, Bethesda, MD; 2) Division of Cancer Epidemiology and Genetics, NCI/NIH, Department of Health and Human Services, Bethesda, MD; 3) Core Genotyping Facility, Advanced Technology Program, SAIC-Frederick Inc., NCI-Frederick, Frederick, MD; 4) Bioinformed Consulting Services, Gaithersburg, MD; 5) Program in Molecular and Genetic Epidemiology, Department of Epidemiology, Harvard School of Public Health, Boston, MA; 6) Department of Nutrition, Harvard School of Public Health, Boston, MA; 7) Channing Laboratory, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

Recent genome-wide association studies of prostate cancer have identified single nucleotide polymorphism (SNP) markers in a region of chromosome 11q13. A subsequent fine-mapping in the Cancer Genetic Markers of Susceptibility (CGEMS) initiative identified at least three independent SNPs (rs10896438, rs12793759, and rs10896449) that contribute to prostate cancer risk. A comprehensive cataloging of variants by resequencing and refinement of linkage disequilibrium (LD) structure is essential steps to prioritize SNPs for further fine-mapping and functional studies. Therefore, we conducted a resequence analysis of 123kb region of chromosome 11q13 (68,642,755-68,765,690), which encompasses all three independent signals, in 78 individuals of European ancestry using next-generation sequencing technology. We identified a total of 549 polymorphic loci, of which 243 variants (187 SNPs and 56 insertion-deletion polymorphisms (indels)) were novel. We identified 18, 26, and 6 variants strongly correlated ($r^2 > 0.8$) with rs10896438, rs10896449, and rs12793759, respectively. The HapMap genotyped SNPs could interrogate 47%, 59%, and 71% of SNPs from each rs10896438, rs10896449, and rs12793759 bin, respectively. Our comprehensive resequencing results extended list of high correlation variants of priority for follow-up functional studies.

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Mutations in Fanconi Anemia Genes and the Risk of Esophageal Cancer. *M.R. Akbari[1,2], R. Malekzadeh[2], P. Lepage[3], D. Roquis[3], A.R. Sadjadi[2], K. Aghcheli[2], A. Yazdanbod[4], R. Shakeri[2], J. Bashiri[2], M. Sotoudeh[2], A. Pourshams[2], P. Ghadirian[5], S.A. Narod[1].* 1) Women's College Research Institute, University of Toronto, Toronto, Canada; 2) Digestive Disease Research Center, Tehran University of Medical Sciences, Tehran, Iran; 3) Genome Quebec Innovation Centre, McGill University, Montreal, Canada; 4) Department of Internal Medicine, Ardabil University of Medical Science, Ardabil, Iran; 5) Epidemiology Research Unit Research Centre, CHUM-Hôtel-Dieu, University of Montreal, Montreal, Canada.

The incidence of esophageal squamous cell carcinoma (ESCC) is very high in northeastern Iran. Previously, we reported a strong familial component of ESCC among Turkmens, who constitute approximately one-half of the population of this region. Based on different lines of evidence, we hypothesized that the genes for Fanconi anemia may be candidate genes for ESCC. We sequenced the entire coding regions of 12 Fanconi anemia genes in the germline DNA of 190 Turkmen ESCC cases. We identified three heterozygote insertion/deletions; one in FANCD2 (p.Val1233del), one in FANCE (p.Val311SerfsX2) and one in FANCL (p.Thr367AsnfsX13). All three patients had a strong family history of ESCC. None of 811 Turkmen controls carried any of these three insertion/deletions. In addition, we found two homozygote patients for the deleterious FANCA p.858Ser>Arg mutation. We found two more homozygotes in another set of 556 ESCC patients. Four FANCA p.858Ser>Arg homozygotes were identified in total number of 746 ESCC patients, but in none of 1373 matched controls (P = 0.01). The p.3326Lys>X mutation in BRCA2 (Fanconi anemia gene FANCD1) was present in 27 of 746 ESCC cases and 16 of 1373 controls (OR = 3.38; 95% CI: 1.97 - 6.91; P=0.0002). In summary, both heterozygote and homozygote mutations in a number of Fanconi anemia genes are associated with an increased risk of ESCC.

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Association of *CDKN1B* gene (polymorphism c.326T>G) with sporadic prostate cancer in Southern Brazilian Population. *R.B. Alexandre[1], A. Horvath[2], G.P.C. Silva[1], F.R. Faucz[1,2].* 1) Laboratory of Molecular Genetics, Core for Advanced Molecular Investigation (NIMA), Center for Health and Biological Sciences (CCBS), Pontificia Universidade Católica do Paraná (PUCPR), Curitiba - PR - Brazil; 2) Section of Endocrinology and Genetics, Program on Developmental Endocrinology & Genetics (PDEGEN), Eunice Kennedy Shriver National Institute of Child Health & Human Development (NICHD), National Institutes of Health (NIH), Bethesda, MD, USA.

A multigenic model of prostate cancer susceptibility has been proposed, in which common polymorphic variants of genes, such as the androgen and vitamin D receptor, contribute to tumorigenesis. The discovery of additional genetic factors involved in the prostate cancer predisposition is essential for the development of new diagnostic and therapeutic molecular tools. We examined a single nucleotide polymorphic variants in codon 109 of *CDKN1B* [c.326T>G (p.V109G) rs2066827] for association with sporadic prostate cancer in a Southern Brazilian population. Fifty-two cases and 110 controls were analyzed using PCR amplification and Single Strand Conformation Polimorphysm (SSCP) analysis; to characterize the patterns initially, a direct sequencing was applied on 15%; of the samples. The *CDKN1B* genotype was scored as T/T, T/G, or G/G. The T allele showed association with an increased risk of sporadic prostate carcinoma (p=0.015, OR 1.84, 95%; confidence interval, 1.09-3.09). An association between *CDKN1B* T/T genotype and advanced prostate carcinoma has been reported in the context of androgen-independent disease, suggesting that a restricted group of advanced cases might be informative to explore the above association (ongoing research).

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Molecular screening of CDKN2A and CDK4 genes in Spanish Melanoma prone families. *C. Badenas[1,2], J.A. Puig-Butille[1,2], Z. Ogbah[3], C. Carrera[2,3], P. Aguilera[2,3], J. Malvehy[2,3], M. Mila[1,2], S. Puig[2,3].* 1) Biochemistry and Molecular Genetics Service, Hospital Clinic, Barcelona, Spain; 2) Centro Investigacion Biomedica en Enfermedades Raras (CIBERER), ISCIII. Barcelona, Spain; 3) Dermatology Department. Melanoma Unit. Hospital Clinic and IDIBAPS (Institut d'Investigacions Biomediques August Pi i Sunyer), Barcelona, Spain.

Introduction: Melanoma is a multifactorial and polygenic disease. The main risk factors are number of nevi, familial predisposition and skin phototype related to ultraviolet radiation exposition. Ten percent of cases are detected in a familial setting, being then inherited as an autosomal dominant trait. Two high susceptibility genes have been implicated: CDKN2A/p14ARF and CDK4. Mutations are detected in up to 40% of families. Objectives: To describe the prevalence of germ line mutations in CDKN2A and CDK4 in Spanish melanoma families and evaluate differences in clinical variables between carriers and non carriers. Subjects and Methods: we included 255 Melanoma affected patients from 201 Spanish families with at least two melanoma and 100 controls from Spanish population. CKN2A and CDK4 mutational screening was performed by PCR-SSCP and sequencing. Results: Mutations in CDKN2A were present in 12.4% of families (25/201). No mutation was detected in CDK4. We detected 16 different mutations, most of them being located in CDKN2A exon 2 (13/16). Mutation G101W accounts for 40% of mutated families (10 cases). CDKN2A alteration in the family was correlated with: the number of cases in the pedigree (p=0.001) and presence of patients with more than one primary tumor (OR: 3.77 IC 95%: 1.32-10.72). Regarding melanoma patients, mean age of onset for CDKN2A mutation carriers was 35 years while it was 43.5 years for non carriers. Dysplasic nevi were present in 30.6% of cases, although no association between dysplasic nevi and presence of CDKN2A mutation or number of patient melanomas was detected. Furthermore, a non statistically significant trend was observed between presence of A148T polymorhism in non mutation carriers and age of onset. Polymorhism A148T was found more frequently in melanoma cases than Spanish control population (9% vs 4.6%) being higher in subgroup of cases with multiple primary melanomas (17.4%). Conclusion: the frequency of CDKN2A Spanish melanoma families is similar to currently reported frequencies in other populations. Mutations in CDKN2A are implicated in age of onset and in the risk to develop more than one melanoma. The presence of CDKN2A mutation and of dysplasic nevi decreases the age of onset. Our results support the hypothesis that A148T variant is associated with an increased risk to develop melanoma in the Spanish population. More studies should be carried out to elucidate the effect of A148T variant on the age of onset.

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Genetic defects in FOXD3 in Gastrointestinal Stromal Tumors. *E.R. Ball[1], F.R. Faucz[1], G. Assie[3,4], A. Horvath[1], M.Q. Almeida[1], S.Y. Kim[5], M. Matsuda[6], M. Lodish[1], E. Bornstein[1], I. Levy[1], K. Nadella[1], E. Bimpaki[1], K.M. Tsang[1], A. Chitnis[6], M. Raygada[2], C.R. Antonescu[7], N.I.H. Pediatric GIST Clinic[8], K.A. Janeway[9], R. Claus[10], J. Bertherat[3], L.J. Helman[5], C. Plass[10], C. Eng[4], J.A. Carney[11], C.A. Stratakis[1,2].* 1) DEB/SEGEN/PDGEN, NICHD, NIH, Bethesda, MD; 2) Pediatric Genetics Clinic and Section on Developmental and Clinical Genomics, Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), National Institutes of Health (NIH), Bethesda, MD; 3) Département d' Endocrinologie, Métabolisme & Cancer, Institut Cochin, INSERM U567 and CNRS UMR 8104, and Centre de Référence des Maladies Rares de la Surrénale, Service d'Endocrinologie, Hôpital Cochin, Université Paris V-René Descartes, 75014 Paris; 4) Genomic Medicine Institute, Lerner Research Institute, and Taussig Cancer Institute, Cleveland Clinic, Cleveland, OH 44195, USA; 5) Pediatric Oncology Branch, National Cancer Institute (NCI), NIH, Bethesda, MD 20892, USA; 6) Laboratory of Molecular Genetics, Section on Neural Developmental Dynamics, NICHD, NIH, Bethesda, MD 20892, USA; 7) Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, New York 10021, USA; 8) Warren G. Magnuson NIH Clinical Center, NICHD, and NCI, Bethesda, MD20892, USA; 9) Department of Pediatric Hematology-Oncology, Dana-Farber Cancer Institute and Children's Hospital, Boston, MA; 10) Division of Epigenomics and Cancer Risk Factors, German Cancer Research Center, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany; 11) Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota 55905, USA.

Gastrointestinal stromal tumors (GISTs) are a rare mesenchymal neoplasm originating from the pacemaker cells of the GI tract known as the interstitial cells of Cajal. The identification of activating mutations in the tyrosine kinase receptors KIT and PDGFRA in approximately 80 % of GISTs led to the classification of GISTs that do not harbor these mutations, termed "wild-type" (WT) GISTs. WT GISTs can occur (1) sporadically, or (2) as a component of the familial GIST and paraganglioma (PGL) syndrome, (Carney-Stratakis syndrome (CSS) OMIM #606864) or (3) the Carney triad (CT) (OMIM #604287). Recently, inherited germ line mutations in SDHB,-C, and -D and mitochondrial oxidation defects have been implicated in approximately 15 %; of WT GISTs; thus additional genes are likely involved in WT GIST pathogenesis. Using a novel genome wide association study (SOMATICS), somatic deletions at the 1p31 locus were detected in 6/22 tumors from patients with the Carney triad, representing the most frequently deleted region. The 1p31 locus harbors the embryonic stem cell factor FOXD3 (genesis). Western blots and immunohistochemistry revealed a decrease in FOXD3 protein expression in tumors harboring deletions compared to PDGFRA mutant GIST and WT GISTs not harboring 1p31 defects. Somatic methylation in the promoter region of FOXD3 was also observed in at least two tumors from CT patients. FOXD3 mutations were not found in either tumor or germ line DNA from CT patients, however two novel variants were found in two unrelated patients affected by sporadic WT GIST (c.161G>A/p.R54H and c.255_266del12). When compared to wild-type FOXD3, R54H and del12 expressing vectors showed decreased DNA binding and decreased activation of a target luciferase promoter. siRNA knockdown of FOXD3 in embryonic cell lines resulted in an increase in c-KIT expression, and conversely, ectopic expression of FOXD3 downregulated c-KIT. Foxd3 mutant animal models including mice and zebrafish showed abnormalities in the Cajal cell phenotype and GI tract neural networks. Foxd3 mutant zebrafish also showed a hyperpigmented phenotype. Both melanocytes and Cajal cells require c-KIT for normal development and increased c-KIT activity can lead to both hyperpigmentation and GISTs. FOXD3 is expressed in neural crest cells and represses melanocyte development. These data indicate FOXD3 may act as a contributing factor in WT GISTs, possibly through upregulation of c-KIT.

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Association analysis of candidate genes in childhood acute lymphoblastic leukemia. *J. Beuten[1,2], D. Piwkham[1,4], J.A.L Gelfond[3], A. Collier III[5,6], S. Pakakasama[4], B.H. Pollock[3], G.E. Tomlinson[1,2].* 1) Greehey Children's Cancer Research Institute; 2) Department of Pediatrics; 3) Department of Epidemiology and Biostatistics, UTHSCSA, San Antonio, TX; 4) Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; 5) University of Texas Southwestern Medical Center, Dallas, TX; 6) Lehigh Valley Health Network, Bethlehem, PA.

Acute lymphoblastic leukemia (ALL), the most common childhood malignancy, remains a major cause of cancer-related morbidity and mortality in children. Substantial variability across ethnic groups in prevalence and prognosis of ALL exists; Hispanic children have a higher incidence of ALL compared to Caucasian children, and also have a less favorable outcome. We genotyped 29 highly potential candidate genes with 672 tagged single nucleotide polymorphisms (SNPs) in a case-control sample of Caucasian and Hispanic children to identify biomarkers that play a role in the risk for childhood ALL and to determine whether differences in allele frequency of SNPs in the candidate genes are associated with the observed differences in ALL rates between Caucasians and Hispanics. We found 39 SNPs within 13 genes that showed a significant risk effect for ALL in Caucasians under the additive model after correction for multiple testing. The major finding was obtained for LMO1 for which not only highest significance (OR=1.90, P=0.00003) but also multiple (N=13) SNPs were significantly associated with ALL. This gene was not previously reported in genome wide association studies (GWAS) of ALL susceptibility. Of note is that SNP rs6959427 within IKZF1, a gene for which a potential role in ALL has been previously indicated from a GWAS study, was highly significant in Caucasians but not in Hispanics. In Hispanics 24 SNPs within 9 genes showed significant association under the additive model after correction for multiple testing with the most significant finding for SNPs within FBXW7 and MLL (OR=0.55, P=0.002 and OR=1.87, P=0.002, respectively). SNP rs941997 in BLNK was a common SNP significantly associated with ALL in both ethnicities (OR=1.40, P= 0.035 and OR=1.76, P=0.005 in Caucasians and Hispanics, respectively). In addition SNPs within ETV6, RUNX1 and CHEK2 were significantly associated in both ethnic groups. A substantial difference (>20%) in minor allele frequency between the controls of both ethnicities was observed for 37 (6%) SNPs analyzed. However, no significant correlation was found between the ethnic specific allele frequency and association with ALL. Our findings support the hypothesis of a polygenic model in which ALL develops through a variety of interrelated genes. We also show that the genetic associations with ALL susceptibility are population specific.

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Genetic variants in *ITPKC* gene and susceptibility to cervical carcinoma. *T. Chang[1], Y. Yang[2,4], Y. Lee[1,3,5], T. Chen[2], S. Chang[1], W. Lin[1].* 1) Med Res Dept, Mackay Memorial Hosp, Taipei, Taiwan; 2) Gynecology and Obstetrics, Mackay Memorial Hosp, Taipei, Taiwan; 3) Pediatrics, Mackay Memorial Hosp, Taipei, Taiwan; 4) Gynecology and Obstetrics, Taipei Medical University, Taipei, Taiwan; 5) Pediatrics, Taipei Medical University, Taipei, Taiwan.

Cervical cancer is strongly associated with infection by oncogenic forms of human papillomavirus (HPV). However, HPV infection alone is not sufficient for progression to cervical cancer. It is now recognized that host immunogenetic background play an important role in the control of HPV infection and the development of cervical cancer. Inositol 1,4,5-trisphosphate 3-kinase C (ITPKC) is a kinase of inositol 1,4,5-trisphosphate (IP3), which is a second messenger molecule in various types of immune cells including T cells, macrophages, and neutrophils. It negatively regulates T-cell receptor signal transduction by decreasing the amount of IP3 in the cytoplasm, which consequently downregulate the Ca^{2+}/ nuclear factor of activated T-cells signaling pathway. The aim of this study is to investigate the association between *ITPKC* gene polymorphisms and the risk of cervical cancer in the Taiwanese population. We selected 7 tag SNPs (rs2561531, rs2561528, rs890934, rs2303723, rs10420685, rs2561536, and rs2290693) and 1 putative functional SNP (rs28493229) to be genotyped in 128 cervical squamous cell carcinoma (CSCC) patients and 211 age/sex matched healthy controls by using the Pre-Developed TaqMan Allelic Discrimination Assay. The presence and genotypes of HPV in CSCC patients were determined by PCR. We found no significant association between any polymorphisms or haplotypes examined and overall CSCC risk. In addition, no significant association was observed between HPV-16 positive CSCC patients and controls. Our results suggest that specific variations in the *ITPKC* gene did not confer susceptibility to CSCC in the Taiwanese population.

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Pleiotropic effects of diabetes risk loci on colorectal cancer risk: The Multiethnic Cohort (MEC) and Population Architecture using Genomics and Epidemiology Studies (PAGE). *I. Cheng[1], C. Caberto[1], M. Tiirikainen[1], L. Wilkens[1], F. Schumacher[2], B. Henderson[2], L. Kolonel[1], C. Haiman[2], L. Le Marchand[1].* 1) University of Hawaii, Honolulu, HI; 2) University of Southern California, Los Angeles, CA.

Colorectal cancer and diabetes are common chronic diseases that share several risk factors. Previous epidemiologic studies have demonstrated a 30% increased risk of colorectal cancer associated with Type-II diabetes, which is likely attributed to the interplay between obesity, hyperinsulinemia, and hyperglycemia. We investigated whether genetic risk loci for diabetes have pleiotropic effects on the risk of colorectal cancer. Nineteen risk SNPs for Type-II diabetes were selected from genome-wide association studies of this disease (up to September 2009) and genotyped in a case-control study of 1,599 colorectal cancer cases and 6,045 controls nested in the MEC under the auspices of the PAGE project. Odds ratios (OR) and 95% Confidence Intervals (CI) were estimated by unconditional logistic regression to evaluate the association between SNPs and colorectal cancer risk. We identified four diabetes SNPs associated with the risk of colorectal cancer: rs7961581 (TSPAN8, LGR5), rs4402960 (IGF2BP2), rs5219 (KCNJ11), rs7578597 (THADA). The strongest association was for rs7578597 (THADA) such that individuals carrying the CC genotype had a 2.1-fold increased risk of colorectal cancer in comparison to those with the TT genotype (95% CI: 1.54-2.99; P<0.0001). Consistent patterns of association were seen across the African American, Japanese American, and Latino populations. This SNP in the THADA gene results in a missense mutation, Thr1187Ala, and aberrant gene expression in THADA has been observed in pancreatic islets of individuals with and without Type-II diabetes. On-going analyses are evaluating the associations between these SNPs and circulating levels of insulin, insulin-like growth factor-I, insulin-like growth factor binding proteins, and C-reactive protein in the MEC. In summary, our findings suggest that diabetes risk loci have significant pleiotropic effects on the risk of colorectal cancer.

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Obesity-related genetic variants are associated with endometrial cancer risk. *R.J. Delahanty[1], J.R. Long[1], Y.B. Xiang[2], Q. Cai[1], Y.T. Gao[2], A. Beeghly-Fadiel[1], W.H. Xu[2], W. Zheng[1], X.O. Shu[1].* 1) Vanderbilt Epidemiology Center, Institute of Medicine & Public Health, Eighth Floor, Suite 800, 2525 West End Ave, Nashville, TN 37212; 2) Shanghai Cancer Institute, Shanghai, China.

Endometrial cancer is the most common gynecologic malignancy. Obesity is a well established risk factor for endometrial cancer. Recent genome-wide association studies (GWAS) have identified multiple genetic markers for obesity. Using data from a recently completed GWAS scan data from a population-based case-control study of endometrial cancer in Shanghai, we investigated the association of obesity-related genetic markers with endometrial cancer. Our sample consisted of 832 cases and 1834 controls. We identified a total of 92 loci previously associated with obesity or BMI by using the GWAS catalog at http://genome.gov/gwastudies. We applied two approaches in the study: 1) to evaluate “exact” obesity-related SNPs via directly genotyped or imputed variants that are common in our study population (≥5%) and 2) to integrate “regional” obesity-related genetic information by examining all common SNPs that are in linkage disequilibrium (LD) with originally reported obesity-related variants. Using logistic regression with adjustment for age and education, we evaluated associations between 86 of the 92 SNPs in the catalog either by direct genotyping or imputation. Of those, 12 of 86 (14%) variants showed an association with endometrial cancer. For 11 of these 12 associations, the “regional” method identified a variant in LD with the originally reported variant that had a more significant P-value in our study than the variant identified in the original report. The “regional” method identified an additional 24 of the 86 (28%) loci for which SNPs in LD with the originally reported variant also reached statistical significance. Thus, through use of the two methods, 36 of 86 (42%) loci associated with obesity and BMI also showed an association with endometrial cancer. We conclude that many of the same alleles associated with obesity and BMI also increase the risk of endometrial cancer and that a “regional” analysis of originally reported GWAS findings may help refine risk alleles between populations and thus improve the transferability of findings between studies.

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Identification of Genetic Risk Factors for Cutaneous Squamous Cell Carcinoma Susceptibility on Chromosome 7. *J. Fleming[1], A. Dworkin[2], A. Balmain[3], A. Toland[1].* 1) Molecular, Virology, Immunology, and Medical Genetics Dept., The Ohio State University, Columbus, OH; 2) National Institute of Health, Bethesda, MD; 3) Cancer Research Dept., University of California San Francisco, San Francisco, CA.

Non-melanoma skin cancer (NMSC) is the most common cancer in the world. A recent report indicates that more than 3.5 million NMSCs were treated in 2006; of those, 700,000 were cutaneous squamous cell carcinomas (SCC). Factors such as ultraviolet light exposure, industrial carcinogens, and tobacco are all predisposing factors to SCC; however little is known about the genetic risk factors driving this disease. If we are able to identify genetic risk factors, we can target people at risk of developing SCC through more extensive screening measures and develop better treatment options, which will prevent cancer and reduce health care costs. Previously, a SCC susceptibility locus, *Skts5*, had been identified on mouse chromosome 12 by linkage analysis on F1 backcrosses between resistant *Mus Spretus (Spret/GS)* and susceptible *Mus Musculus (NIH/Ola)* mice. *Skts5* is a 14-Mb region with 65 coding elements. Other susceptibility loci identified in these crosses show preferential allelic imbalance in skin tumors, indicating that allele-specific somatic genetic alterations in these regions may be driving cancer development and progression. The orthologous locus to *Skts5* in humans maps to 7p21 and 7q31. The question of this study is whether genetic variations at *SKTS5* are playing a role in human SCC susceptibility. We hypothesize that human SCC tumors will show allele-specific somatic genetic changes at *SKTS5* and that these alterations contribute to SCC risk. Low-resolution genotyping was performed in tumor vs matched normal DNA from 50 patients with multiple independent tumors using ABI genotyping of microsatellite markers. Markers mapping to 7p21 and 7q31 showed preferential allelic imbalance with p-values of 0.047 and 0.016 respectively. In order to identify candidate genes at *SKTS5*, we performed high-resolution genotyping using Sequenom MassARRAY. Normal and tumor DNA from 443 patients were analyzed across 70 single-nucleotide polymorphisms (SNPs) mapping to top candidate genes at *SKTS5*. Our results indicate that 6 SNPs at *SKTS5* show statistically significant evidence of preferential allelic imbalance in SCC tumors. We conclude that one or more of these SNPs is driving SCC susceptibility. The identification of functional germline variants that contribute to SCC susceptibility provides an initial step in developing genetic screening for this disease.

456/T

Eighteen Insulin-like Growth Factor (IGF) pathway genes, circulating levels of IGF-1 and its binding protein (IGFBP-3), and risk of prostate and breast cancer. *F. Gu[1,2], F. Schumacher[3], F. Canzian[4], N. Allen[5], R. Kaaks[6], E. Riboli[7], R. Ziegler[8], P. Kraft[1,2] on behalf of Breast and Prostate Cancer Cohort Consortium.* 1) Program in Molecular and Genetic Epidemiology; 2) Department of Epidemiology, Harvard School of Public Health, Boston, MA, USA; 3) Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA; 4) Genomic Epidemiology Group, German Cancer Research Center (DKFZ), Heidelberg, Germany; 5) Cancer Epidemiology Unit, University of Oxford, Oxford, OX3 7LF, UK; 6) Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany; 7) School of Public Health, Imperial College London, London, UK; 8) Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, USA.

Circulating levels of insulin-like growth factor I (IGF-1) and its main binding protein, IGF binding protein 3 (IGFBP-3), have been associated with risk of several types of cancer. Heritable factors explain up to 60% of the variation in IGF-1 and IGFBP-3 in studies of adult twins. We systematically examined common genetic variation in 18 genes in the IGF signaling pathway for associations with circulating levels of IGF-1 and IGFBP-3. A total of 286 single nucleotide polymorphisms (SNPs) were genotyped in over 5500 Caucasian men and 5500 Caucasian women from the Breast and Prostate Cancer Cohort Consortium (BPC3). After adjusting for multiple testing, SNPs in the IGF1 and SSTR5 genes were significantly associated with circulating IGF-1($p<2.1x10-4$); SNPs in the IGFBP3 and IGFALS genes were significantly associated with circulating IGFBP-3. Multi-SNP models constructed using SNPs that were significant in univariate analyses explained $R2=0.62\%$ of the variation in circulating IGF-1 and 3.9% of the variation in circulating IGFBP-3. We saw no significant association between these multi-SNP predictors of circulating IGF-1 or IGFBP-3 and risk of prostate or breast cancers. In conclusion, common genetic variation in the IGF1 and SSTR5 genes appears to influence circulating IGF-1 levels, and variation in IGFBP3 and IGFALS appears to influence circulating IGFBP-3. However, these variants explain only a small percentage of the variation in circulating IGF-1 and IGFBP-3 in Caucasian men and women. Further studies are needed to explore contributions from other genetic factors such as rare variants in these genes and variation outside of these genes.

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Replication of breast cancer risk loci in a Cypriot case-control association study. *A. Hadjisavvas[1], M.A. Loizidou[1], J.P. Ioannidis[2,3,4], K. Kyriacou[1].* 1) Department of EM/Molecular Pathology, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus; 2) Department of Hygiene and Epidemiology, University of Ioannina School of Medicine, Ioannina, Greece; 3) Biomedical Research Institute, Foundation for Research and Technology-Hellas, Ioannina, Greece; 4) Tufts Clinical and Translational Science Institute and Center for Genetic Epidemiology and Modeling, Tufts Medical Center and Tufts University School of Medicine, Boston, MA, USA.

Over the last three years, seven genome-wide association (GWA) studies, which have identified 11 associations with robust statistical support for influencing breast cancer susceptibility, have been published pertaining to susceptibility loci in FGFR2, MAP3K1, LSP1, TOX3 (formerly known as TNRC9), COX11 and at 1p11.2, 2q35, 3p24, 6q25.1, 6q22.23 and 8q24.21. Most GWA studies and replications have been conducted in Northern European populations and to a lesser extent in Asians, and Ashkenazi Jews. It is important to assess whether these variants confer risk across different populations and also to assess the magnitude of risk conferred. To evaluate whether the GWAS derived risk variants contribute to breast cancer in the Cypriot population, we genotyped these 11 variants in 1,109 Cypriot female breast cancer patients and 1,177 healthy female controls. A total of four of the eleven single nucleotide polymorphisms (SNPs) were found to be nominally significantly associated with breast cancer risk in the Cypriot population. Based on estimated power, 5 associations would be expected to be nominally significant. The correlation coefficients of effect sizes (per-allele OR) between the Cypriot population and the GWAS populations was 0.58 (p=0.064), while allele frequencies were very similar (r=0.88, p<0.001). Overall, we show modest concordance for breast cancer GWAS-discovered alleles and their effect sizes in the Cypriot population.

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Promoter variants in genes involved in the cell cycle and DNA repair pathways and the susceptibility to childhood acute lymphoblastic leukemia. *J. Healy[1], C. Richer[1], J. Dionne[1], H. Bélanger[1], M. Larivière[1], M. Ouimet[1], V. Gagné[1], V. Weth[1], K. Benhamza[1], P. Beaulieu[1], H. Massé[1], M. Bourgey[1], M.-H. Roy-Gagnon[1,2], D. Sinnett[1,3].* 1) Division of Hematology-Oncology, Research Center of the Sainte-Justine University Health Center, University of Montreal, QC, Canada; 2) Department of Social and Preventive Medicine, Faculty of Medicine, University of Montreal, QC, Canada; 3) Department of Pediatrics, Faculty of Medicine, University of Montreal, QC, Canada.

Acute lymphoblastic leukemia (ALL) is the most common neoplasm among children and is likely caused by multiple genetic and environmental factors. However the identification of established genetic risk factors for ALL has been impeded by the complex and heterogenic nature of the disease which likely reflects complex etiologic mechanisms. In this study, we postulated that a combination of cis-acting sequence variants in multiple genes sharing functions in the G1/S cell cycle checkpoint and DNA double-strand break repair pathways could influence interindividual variability in the susceptibility to ALL by modulating gene expression and affecting the overall outcome of these core regulatory processes. In a comprehensive pathway-driven case-control approach, we used hierarchical modeling to investigate gene- and pathway-based associations with childhood ALL and to explore putative gene-gene interactions, while integrating prior biological and functional information into the analysis. We identified variants in genes involved in both cell cycle control (*CDKN2A* rs36228834, *CDKN2B* rs2069416, *HDAC1* rs1741981) and DNA repair (*BRCA1* rs3092986, *XRCC4* rs3763063, *XRCC5* rs11685387) that are significantly associated with increased childhood ALL risk, and pathway analysis supported a role for both processes in leukemogenesis (cell cycle OR(95%CI)= 1.21(1.07-1.38); DNA repair OR(95%CI)= 1.28(1.10-1.47)). This study provides a strong rationale for further elucidation of the mechanisms through which aberrant cell cycle control and double-strand break repair are involved in childhood ALL susceptibility.

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Genetic association with Leukocyte Telomere Length and Cancer Risk: the NHLBI's Framingham Heart Study. *S.J. Hwang[1,2], J.M. Murabito[1,3], C.L. Rosenberg[4], B.E. Kreger[1], G.L. Splansky[1], S.L. Neuhausen[6], S.C. Hunt[7], A. Aviv[8], D. Levy[1,2,5].* 1) Framingham Heart Study, Framingham, MA; 2) Center for Population Studies, National Heart, Lung, and Blood Institute, Bethesda, MD; 3) Department of Medicine, Boston University School of Medicine, Boston, MA; 4) Section of General Internal Medicine and the Sections of Hematology/Oncology, Boston University School of Medicine, Boston, MA; 5) Divisions of Cardiology and Epidemiology, Boston University School of Medicine, Boston, MA 02118; 6) Department Population Sciences, The Beckman Research Institute of the City of Hope, Duarte, CA 91010; 7) Cardiovascular Genetics Division, University of Utah School of Medicine, Salt Lake City, UT 84112; 8) Center of Human Development and Aging, New Jersey Medical School, Newark, NJ 07101.

GWAS recently identified significant associations of SNPs near *OBFC1* and *CXCR4* with leukocyte telomere length (LTL), with individuals carrying the minor alleles having longer LTL. Telomere dynamics have been linked to cancer, but it is unknown whether genetic variants regulating LTL are associated with cancer risk. We characterized the association between SNPs near *OBFC1* and *CXCR4* and LTL and tested whether genetic variants determining LTL are associated with cancer risk. The study sample was composed of 1138 Framingham Heart Study offspring participants with data on LTL, cancer incidence, family history of cancer, and genotype data for rs10786774 near *OBFC1* and rs13024450 near *CXCR4*. Cancer incidence through December 2008 was verified with pathology reports for all participants. We characterized the association between the number of minor alleles for both SNPs and LTL using linear regression adjusted for age and sex. We tested for significant association between time to cancer diagnosis and genetic variation using Cox's proportional hazard models. Results: individuals carrying 0 minor alleles had the shortest LTL , the trend of increasing telomere length by number of minor allele was significant with p-value of 2.7×10^{-10}. We tested for cancer risk for 157 incident cases relative to 926 cancer-free controls and observed an association with cancer risk among carriers of 1 or more minor alleles relative to those with 0 minor alleles (Hazard Ratio=1.61, 95%CI,1.05-2.47, p=0.029). The association persisted after adjusting for age, sex, body-mass-index, cigarette smoking, and family history of cancer. Our results warrant further study in independent samples and suggest genetic variants regulating LTL also confer increased risk for incident cancer.

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CDH10 mutation profiles in pancreatic ductal adenocarcinomas (PDAC). *N. Jinawath[1,5], K. Murphy[2], A. Norris[2], A. Klein[3], R. Yonescu[2], C. Iacobuzio-Donahue[2], J. Brody[4], A. Meeker[2], A. Jinawath[6], S. Harada[2], C. Griffin[1,2,3].* 1) Inst Gen Med, Johns Hopkins Hosp, Baltimore, MD; 2) Department of Pathology, Johns Hopkins Hosp, Baltimore, MD; 3) Department of Oncology, Johns Hopkins Hosp, Baltimore, MD; 4) Department of Surgery, Jefferson Center for Pancreatic, Biliary and Related Cancers, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA; 5) Research Center, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; 6) Department of Pathology, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.

Pancreatic cancer has a dismal prognosis. 5-10% of pancreatic cancer patients report a family history of pancreatic cancer. Genes associated with some familial cancer syndromes such as *BRCA2*, *STK11*, and *PALB2* only explain a small portion of the patients with familial pancreatic cancer. Therefore further studies to identify additional susceptibility genes are needed. One of the driver mutator genes proposed in a recent global mutation analysis of sporadic pancreatic cancers by Jones et al (Science 2008 Sep 26;321(5897):1801-6) is *CDH10*, a little known cadherin gene that is expressed in normal prostate and brain tissue. We analyzed 42 familial pancreatic cancer patients (that had at least 2 relatives with PDAC) for germline alterations of the *CDH10* gene. We identified one patient with a balanced chromosomal translocation and a second patient with a non-synonymous alteration. We narrowed the breakpoints of the constitutional t(5;20)(p14.2p11.1) using BAC FISH, somatic cell hybrids and SNP array. The breakpoint on chromosome 5p14.2 was in frontal proximity of *CDH10*, while that on chromosome 20p was in the pericentromeric region. Thus, we hypothesize that this translocation may result in altered *CDH10* expression in the patient, acting like a germline mutation that inactivates one copy of the gene. Subsequent sequencing analysis did not identify a germline mutation in *CDH10*. Analysis of the patient's tumor using 6 STR markers on 5p14.2 demonstrated a region of LOH covering *CDH10*. The second patient had a germline missense mutation in a highly conserved cadherin cytoplasmic domain on exon 12 (p.Arg688Gln). Tumor tissue was not available from this individual. We found no nonsynonymous changes in exon 12 among the 106 control samples nor in public databases (1000 Genomes project March 2010 release and dbSNP build131). We further investigated whether *CDH10* region is prone to LOH in sporadic PDACs by analyzing STR profiles of 29 microdissected PDACs and identified 38% of tumors with LOH. IHC using a CDH10 antibody is in progress and preliminarily shows a difference in staining pattern between normal pancreatic ducts and tumor tissue. Taken together, our data suggest that CDH10 alterations are important in the development of pancreatic cancer. Further functional studies are needed to elucidate the tumor suppressive role of CDH10 in pancreatic carcinogenesis.

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Copy number alterations of BCAS1 in squamous cell carcinomas. *S.Y. Kim[1], Y.I. Kim[1], A. Lee[2], J. Kim[1], B.H. L[1], S.H. Lee[1], S.W. Nam[1], S.H. Lee[1], W.S. Park[1], N.J. Yoo[1], J.Y. Lee[1], S.H. Kim[1].* 1) Dept Pathology, CUMC, Seoul, Seoul, Korea; 2) Dept. Hospital Pathology, CUMC, Seoul, Korea.

Background: BCAS1, located in 20q13, is amplified and overexpressed in breast cancers. Even though BCAS1 is expected to be an oncogene candidate, its contribution to tumorigenesis and copy number status of other malignancies are not reported. To elucidate BCAS1's role in squamous cell carcinomas, we investigated copy number status and expression level of BCAS1 in several squamous cell carcinoma cell lines, normal keratinocytes and primary tumors. Methods: We quantitated BCAS1 gene by real-time PCR. Expression level of BCAS1 was measured by real-time RT-PCR and immunoblot. Resultes: Seven (88%) out of eight squamous cell carcinoma cell lines showed copy number gain of BCAS1 with various degrees. BCAS1 gene in some cervical cancer (60%) also showed copy number gain. However, expression level did not show linear correlation with copy number changes. Conclusions: In this study, we identified common copy number gain of BCAS1 in squamous cell carcinoma cell lines. Due to lack of linear correlation between copy numbers of BCAS1 and its expression level, we could not confirm that overexpression of BCAS1 is common finding in squamous cell carcinoma cell lines.

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Fine mapping of 5p12 locus in Ashkenazi Jewish breast cancer cases and controls. *T. Kirchhoff[1], P. Pal[2], A. Dutra-Clarke[1], V. Joseph[1], M.M. Gaudet[3], V. Devlin[1], Q. Waisfiz[4], C.A. Hudis[1], K. Offit[1].* 1) Dept Medicine, Mem Sloan Kettering Cancer Ctr, New York, NY; 2) John Hopkins Hospital, Baltimore, MD; 3) Albert Einstein School of Medicine, Bronx, NY; 4) Netherlands Cancer Institute, Amsterdam, Netherlands.

Over a dozen low-penetrant breast cancer susceptibility loci have recently been identified through genome wide association studies GWAS). Of the four breast cancer GWAS studies initially reported, association of 5p12 with breast cancer risk was noted in three (Iceland, Breast Cancer Association Consortium [BCAC], and Cancer Genetic Markers of Susceptibility [CGEMs]) and, we report here, this association is also observed in retrospective analysis of data from our GWAS in Ashkenazi Jews (PNAS 105:4340, 2008). In the CGEMS data, the 5p12 SNP showed stronger association with breast cancer risk in postmenopausal women, suggesting a link with estrogen receptor-positive tumors. Our GWAS of the Ashkenazi Jewish (AJ) population showed association with this locus for rs6893211, with weaker effect size (OR=1.12, p=0.002), but with a stronger signal when a sliding window haplotype analysis was used. The association signal described in these studies maps to relatively large ~200kb region on 5p12, near 2 candidate genes: MRPS30 and LOC441070. Here we examined the structure of the association signal in the AJ population in order to fine map the involved region at 5p12. We genotyped 47 SNPs across ~1.3 Mbp region of 5p12 utilizing a Sequenom platform. 1000 breast cancer cases and 1000 controls were analyzed. We confirmed a modest association with rs4391175 (p= 0.07) and rs1392970 (p=0.09) in the MRPS30 locus. In contrast to other reports, the strongest association signal was near the 5'UTR of FGF10 for 4 correlated SNPs across a ~200kb region. Sliding window haplotype analysis revealed the presence of susceptible common haplotype significantly associated with breast cancer risk in these subsets, (OR=1.23, p=0.0003). This analysis, performed in the AJ population, provides the first evidence pointing to FGF10 as a potential candidate contributing to the 5p12 association signal and breast cancer risk. Confirmatory studies will be performed in larger European ancestry replication cohorts. The results of these analyses, and the prior association with FGFR2 in each of the GWAS studies to date, support the role of mitogen activated pathways in genetic susceptibility to breast cancer. These findings also suggest the FGF pathway as a potential future target for preventive as well as therapeutic approaches.

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Exploring breast cancer epistasis in the PRDX genes. *E. Kistner-Griffin[1], B. Wolf[1], E. Hill[1], E. Slate[1], C. Neumann[2].* 1) Division of Biostatistics and Epidemiology, Dept of Medicine, Medical University of South Carolina, Charleston, SC; 2) Dept of Pharmacology, Medical University of South Carolina, Charleston, SC.

Peroxiredoxins (Prdxs) have recently been shown to be upregulated in breast cancer and other malignancies. Overexpression of both Prdx1 and Prdx3 has been associated with breast tumor genesis. As SNPs in the PRDX genes have not been reported in the genome-wide association studies of breast cancer, a novel method called Logic Forest, which allows the discovery of risk variants as well as epistasis, was implemented to thoroughly investigate possible associations. Using the Cancer Genetic Markers of Suspectibility (CGEMS) Breast Cancer cohort available in dbGaP, SNPs within 50kb of each of the six PRDX genes on the Illumina HumanHap550 platform were identified. The cohort consists of 1145 European ancestry postmenopausal women with breast cancer and 1142 European ancestry controls, all from the Nurses Health Study. A Logic Forest model of breast cancer was explored, which allowed for evaluation of interactions between SNPs predictive of breast cancer in the sample through construction of multiple logic regression trees. Testing 85 SNPs in the PRDX genes, we identified an interaction between SNPs upstream of PRDX3, with empirical significance after permutation (p=0.00035). Using a standard logistic regression approach to test epistasis in the PRDX3 gene region would have also allowed for discovery of the same interaction (p=0.00017). Currently, imputation of other SNPs in the genes is being conducted for better coverage of the genetic variation and the Logic Forest model is being refined for improved detection of gene-gene interactions only implicated in a patient subpopulation. In addition, expression quantitative trait loci (eQTLs) of the PRDX genes have been discovered using the publically available SNP and CNV Annotations Network (SCAN) and the Logic Forest approach is being conducted to test for epistasis with both cis- and trans-acting regulators.

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Screening for *BRCA1*, *BRCA2*, *PALB2*, *EMSY*, *CDH1*, *ATM*, *RAD50*, *CHEK2*, and *BRIP1* mutations in high-risk Finnish *BRCA1/2*-founder mutation-negative breast and/or ovarian cancer individuals. *K.M. Kuusisto[1], A. Bebel[1], J. Schleutker[1], S.-L. Sallinen[2].* 1) Institute of Medical Technology, Tampere, Finland; 2) Department of Pediatrics, Genetics Outpatient Clinic, Tampere University Hospital, Tampere, Finland.

Introduction: Two major high-penetrance breast cancer genes, *BRCA1* and *BRCA2*, are responsible for approximately 20% of hereditary breast cancer (HBC) cases in Finland. Additionally, many other genes that interact with *BRCA1/2* in DNA repair pathway increase the risk of HBC. Still, a majority of HBC cases remain unexplained which is challenging for genetic counseling. We aimed to analyze additional mutations in HBC-associated genes and to define the sensitivity of our current *BRCA1/2* mutation analysis protocol used in genetic counseling. **Methods:** Eighty-two well-characterized, high-risk hereditary breast and/or ovarian cancer (HBOC) *BRCA1/2*-founder mutation-negative Finnish individuals, were screened for germline genetic alterations in nine breast cancer susceptibility genes, *BRCA1*, *BRCA2*, *PALB2*, *EMSY*, *CDH1*, *ATM*, *RAD50*, *CHEK2*, and *BRIP1* by using direct sequencing, the high resolution melt (HRM) method and multiplex ligation-dependent probe amplification (MLPA). Carrier frequencies between studied individuals and controls were compared by using Fisher's exact test. **Results:** Four previously reported breast cancer-associated variants, *BRCA1* c.5095C>T, *ATM* c.7570G>C, *CHEK2* c.470T>C, and *CHEK2* c.1100delC, were observed in twelve (14.6%) individuals. Ten of these individuals had *CHEK2* variants, c.470T>C and/or c.1100delC. Seventeen novel sequence alterations and nine amino acid-changing double-variant individuals were identified. No large genomic rearrangements were detected either in *BRCA1* or *BRCA2*. **Conclusions:** In this study, previously known breast cancer susceptibility genes explained 14.6% of the analyzed high-risk *BRCA1/2*-negative HBOC individuals. The role of the *CHEK2* variants in particular should be evaluated in more detail for genetic counseling purposes. Additionally, we identified novel variants that warrant additional studies. Our current genetic testing protocol for 28 Finnish *BRCA1/2*-founder mutations and PTT of the largest exons is sensitive enough for clinical use as a primary screening tool. However, in cases of extremely remarkable cancer history, analysis of the whole coding regions of *BRCA1/2* would be recommended.

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Copy number variation analysis of a pancreatic neuroendocrine tumor in a patient with von Hippel-Lindau disease. *A. Laus, R. Pellegrino, E. Barreto, J. Vidal, J. Casali da Rocha.* National Tumor Bank, National Cancer Inst. Andre Cavalcanti St, 37. Rio de Janeiro, Brazil.

Von Hippel-Lindau disease (VHL) is an autosomal dominant syndrome that results from germiline mutations in gene VHL, a tumor suppressor gene located in 3p25-p26. This syndrome predisposes individuals to develop multiples benign and malign tumors in many organs, including the kidney, the central nervous system, and pancreas. The most pancreas lesions associated with VHL are benign, but same patients can develop pancreatic neuroendocrine tumors (PNETs), that can behave in a malignant fashion developing metastatic disease. Little is known about the molecular anomalies that occurs both in sporadic or hereditary PNETs, and so, the identification of commonly deleted and amplified chromosomal regions may contribute for understanding of molecular pathways involved in tumorigenesis of this type of tumors. The aim of this study was to perform Copy Number Variation (CNV) analysis using microarray approach in a PNET of a patient with VHL disease. The patient is a male, and his clinical manifestations include a haemangioblastoma at age 25, a renal cell carcinoma and a PNET at age 33, and another haemangioblastoma at age 35. The VHL disease was confirmed by sequencing of VHL gene in blood DNA and revealed a frameshift germiline mutation in exon 3 (778delG). The DNA from PNET was extracted using standard methods and CNV analysis was performed using GeneChip Human Mapping 50K Set (Affymetrix, USA). The CNV analysis was performed using Genotyping Console Software (Affymetrix, USA), applying Markov model algorithm. The CNV analysis detected two homozygosis deletions in chromosome 3, one deletion in chromosome 8, and one duplication in chromosome 7. All regions altered were annotated and the genes located there were analyzed according to their biological processes using DAVID Functional Annotat ion Tool. This analysis revealed a deletion of one allele of VHL gene, this being the somatic event that probably initiate the tumorigenic process. Also, was detected a duplication of MET gene, a known oncogene involved in two important pathways of cell proliferation, invasion, resistance to apoptosis and angiogenesis, PTEN/PI3K/AKT/mTOR and RAS/RAF/MAPK pathway. The PPARG gene, deleted in this tumor, is also involved in PTEN/PI3K/AKT/mTOR pathway, and BRAF gene, involved in RAS/RAF/MAPK pathway, was found duplicated. So, these findings strongly suggest the involvement of these two important pathways in tumorigenesis and progression of PNETs in patients with VHL disease.

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Smoothened expression is regulated by DNA methylation in cancer cells. *H. Lou[1], H. Li[2], B. Gold[3], J. Sawitzke[1], P. Wright[2], S. Anderson[2], M. Dean[3].* 1) Cancer and Inflammation Section, SAIC Frederick, Inc., NCI-Frederick, Frederick, MD 21702; 2) Cancer and Inflammation Program, Laboratory of Experimental Immunology, SAIC Frederick, Inc., NCI Frederick, Frederick, MD 21702; 3) Cancer and Inflammation Section, National Cancer Institute, Frederick, MD 21702.

The hedgehog (HH) signaling pathway is one of the key regulators of embryonic patterning, tissue regeneration, stem cell renewal, and cancer growth. Smoothened (SMO) protein is the key positive regulator of the HH signaling pathway. To clarify the role of the HH signaling pathway in tumorigenesis, we investigated the expression of SMO, PTCH1, GLI1, GLI2 and GLI3 in thirty-three cancer cell lines. Different expression patterns of HH pathway genes were identified in these cancer cells. Although SMO showed the highest level of expression among these genes, absence of SMO expression was identified in seven cancer cell lines. Furthermore, we performed computational analysis of the SMO 5'-flanking region and Exon 1 to determine the functional elements of the SMO promoter region. Three potential CpG islands and five putative SMO promoter elements were identified. In addition, we have determined the methylation status of three SMO CpG islands using bisulfite sequencing PCR (BSP) and methylation specific PCR (MSP) methods. Hypermethylation of SMO was only found in the cell lines lacking SMO expression. Furthermore, the expression level of SMO mRNA was negatively correlated with the methylation status of the SMO promoter. The expression of SMO in a cell line with a fully methylated SMO promoter (MCF7) was restored by treatment with the demethylating agent 5-aza-2'-deoxycytidine (5'-AZA-dCyd). Our study reveals that the SMO promoter is hypermethylated in certain cancer cell lines and suggests an essential role for epigenetic silencing in regulating SMO expression in various cancer cells.

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FOXP3 Polymorphism Association with Prostate Cancer in African-American men. *A. McLauchlin, G. Dunston, T. Mason, L. Ricks-Santi.* National Human Genome Center, Howard University, Washington, DC.

Background: Prostate cancer is the most common cancer of men in Europe, North America and parts of Africa, and a leading cause of death among men in the United States and Western Europe. Some studies report a more aggressive cancer in African American men than in white men. The reason for the disparity is not completely known, however there may be several biological factors involved including variation in immune system activity. FOXP3 expression has been shown to be increased in several cancers, mostly as a function of the increased numbers of regulatory T (Treg) cells in peripheral blood and at the site of the tumor. FOXP3 is a member of the forkhead family of transcription factors that are involved in several cellular processes. Within the natural Treg cell, FOXP3 is responsible for up-regulation of several genes involved in suppression of effector T cells. Recently, FOXP3 has been shown to be expressed in cancer cells. The major objective of this study was to investigate the effect of FOXP3 expression changes in prostate tumor cells with a focus on polymorphisms in conserved regulatory regions as the agents of change. Methods: Four single nucleotide polymorphisms (SNPs) located in a conserved region of intron 1 in the FOXP3 gene were found to be variable only in African and African-American populations. Results: The study found that three of the SNPs were significantly associated with Gleason score as an intermediate marker of tumor aggression (p=0.047). The A allele of one of the SNPs (rs6609859) results in retention of an NFAT binding site in intron 1 of FOXP3 which may result in increased expression of FOXP3 expression. No significant association was found between any of the FOXP3 SNPs and development of prostate cancer. Conclusion: This study shows that common variants in candidate genes underlying host adaptive immune mechanisms may be useful in interrogating the biology of health disparities.

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Colon cancer, adenomas and hyperplastic polyps are linked to chromosome 6q27 in a large family with excess colon cancer. *D.W. Neklason[1,2], T.M. Tuohy[1], J. Stevens[3], R.A. Kerber[1,2], W.S. Samowitz[4], B. Otterud[3], L. Baird[3], S.K. Kuwada[5], M.F. Leppert[3], R.W. Burt[5].* 1) Huntsman Cancer Inst, Univ Utah, Salt Lake City, UT; 2) Department of Oncological Sciences, Univ Utah, Salt Lake City, UT; 3) Department of Human Genetics, Univ Utah, Salt Lake City, UT; 4) Department of Pathology, Univ Utah, Salt Lake City, UT; 5) Department of Medicine, Univ Utah, Salt Lake City, UT.

Colon cancer is one of the more familial of cancers with an estimated 20-30% of cases having a hereditary basis. A fraction of this is accounted for by the highly penetrant colon cancer syndromes (5%) and low penetrance genes identified through association studies (6%). Large family studies offer an opportunity to identify moderately penetrant but sufficiently rare genes that would be missed through association studies. The aim of this study was to identify genetic factors responsible for colon cancer using large multigenerational pedigrees with excess colorectal cancer. A 5-generation family with a statistical excess of colon cancer (p=0.0001) was identified through the Utah Population Database, a genealogic resource linked with cancer records. A total of 72 family members were recruited for phenotypic (endoscopic) and genetic study. Adenomas were twice as common in the proximal vs. distal colon. Known colon cancer syndromes were excluded through clinical and molecular evaluation. Family members were genotyped with 554 polymorphic short tandem repeat (STR) markers across the entire genome. Parametric and nonparametric linkage analyses were applied using adenomatous polyps and colon cancer for affection status. The genome-wide scan revealed a maximum LOD score of 3.33 at D6S386 and adjacent markers in agreement. There are 24 RefSeq genes within this region, but none previously implicated in colon cancer risk. One phenotypic feature of the kindred presented an unexpected issue. Many young individuals developed hyperplastic polyps in the colon, however only 23% were located in the proximal colon. These are not typically regarded as precancerous and we do not use them to establish affection status. To test if this observation associated with the genetic predisposition to colon cancer in the kindred, we reran linkage analysis and changed the phenotype of individuals age 45 and younger with hyperplastic polyps or any age with 3 or more hyperplastic polyps from unaffected to unknown. This increased the resulting two-point LOD score from 3.33 to 4.16. (The multipoint analysis using FASTLINK increased from a LOD score of 3.99 to 5.01). This suggests that this particular genetic locus may influence the proliferation of both hyperplastic and adenomatous colonic tissue and may provide clues as to the etiology of the mixed polyposis phenotype which is commonly observed in the clinical setting but poorly understood.

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***EMSY* and prostate cancer.** *R. Nurminen[1], T. Wahlfors[1], T. Tammela[2], J. Schleutker[1].* 1) Laboratory of Cancer Genetics, Institute of Medical Technology, University of Tampere and Tampere University Hospital, Tampere, Finland; 2) Division of Urology, Tampere University Hospital and Medical School, Tampere, Finland.

Prostate cancer is the most frequently diagnosed cancer in men in developed countries but the genetic changes underlying the development and progression of prostate cancer, particularly aggressive in nature, are still poorly known. One of the recently identified chromosomal regions in genome-wide association studies (GWAS) and linkage analyses is 11q13 but no causal factor(s) predisposing to prostate cancer has yet been identified. The *EMSY* gene maps to 11q13.5 and is associated with breast and ovarian cancers. Based on this we screened *EMSY* for sequence variants and evaluated their association with prostate cancer risk. The study material consisted of 1175 Finnish unselected and 184 familial prostate cancer cases and 854 unaffected male controls. Exons and exon-intron boundaries were screened for variation using sequencing methods, and potential variants were validated using Taqman assay and High Resolution Melting analysis. We identified 27 sequence variants of which 10 had been previously reported. One of the novel variants, IVS6-43A>G, associated with both unselected (odds ratio (OR) 5.49; confidence interval (CI) 95 % 1.25-24.1; p = 0.022) and familial prostate cancer (OR 7.04; CI 95 % 1.17-42.4; p = 0.042). Furthermore, clinicopathologic case-control and case-case analyses revealed that IVS6-43A>G particularly increases the risk of aggressive unselected prostate cancer (OR 9.83; 95 % CI 2.17-44.6; p = $5x10^{-4}$ and OR 5.85; 95 % CI 1.29-26.5; p = 0.010, respectively). IVS6-43A>G is situated 43 bases upstream of an exon but no functional elements are predicted to map to this site. We found no strong linkage disequilibrium between IVS6-43A>G and other observed *EMSY* variants, however, the linkage disequilibrium with more distant functional variant is still to be evaluated. Our findings suggest that the *EMSY* variant IVS6-43A>G could be used as a biomarker for aggressive prostate cancer, but these results need to be confirmed and the exact role in aggressive development of cancer has to be further evaluated.

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Caspase-8 gene SNPs in prostate cancer susceptibility: a replication study. *M. Parry[1], G. Elliott[1], R. Abo[2], N.J. Camp[2], D.E Neal[3], J.L. Donovan[4], F.C. Hamdy[5], A. Cox[1].* 1) Institute for Cancer Studies, University of Sheffield, Sheffield, South Yorkshire, United Kingdom; 2) University of Utah, Salt Lake City, Utah, USA; 3) University of Cambridge, Cambridge, United Kingdom; 4) University of Bristol, Bristol, United Kingdom; 5) University of Oxford, Oxford, United Kingdom.

Introduction A single nucleotide polymorphism (SNP) in the caspase-8 gene, (CASP8 D302H), has been reported to be involved in susceptibility to breast and pancreatic cancer, melanoma, and glioma, but not colorectal or ovarian cancer. We previously have used a tagging-SNP approach to test the hypothesis that this SNP, or other variants in CASP8, were associated with prostate cancer, in 1009 cases, 987 men with normal serum prostate specific antigen (PSA) levels and 961 men with low serum PSA (<0.5ng/ml). These subjects were drawn from those participating in the UK ProtecT (Prostate Testing for Cancer and Treatment) trial. Of the 15 tagging SNPs in caspase-8 that were genotyped, 7 showed some evidence of association with prostate cancer (P<0.05). The aim of the present study was to attempt to replicate these results in an independent set of case and control subjects. Methods A further independent sample of men from the ProtecT trial formed the replication cohort for this study. Blood DNA samples were available for 1262 men with prostate cancer, 1258 men with normal serum PSA, and 609 men with low serum PSA. SNP genotyping was carried out by 5-prime nuclease PCR (TaqmanTM, Applied Biosystems). The association with prostate cancer was tested using logistic regression, controlling for sample set. Results Genotype call rates for the 7 SNPs were all greater than 95%, and genotype frequencies in control subjects were consistent with those expected under Hardy-Weinberg equilibrium. None of the 7 SNPs yielded significant evidence of association in the replication cohort, although some odds ratios were consistent between the two data sets. We combined the data from the test and replication cohorts to evaluate the overall evidence for association. The rare alleles of rs3769826, rs6435074 and rs6723097 conferred a reduced risk of prostate cancer with per-allele odds ratios (95% confidence interval) [OR(95%CI)] of 0.90 (0.82-0.98), 0.84 (0.77-0.94) and 0.88 (0.80-0.96), and P=0.013, P=0.001 and P=0.004 respectively. The deletion allele of the promoter indel rs3834129 conferred an increased risk of prostate cancer, with OR (95%CI) of 1.15 (1.05-1.27), P=0.003. While these results await further replication, it is interesting to note that the rare alleles of rs3769826, rs6435074 and rs6723097, associated with reduced risk, are associated with increased risk of breast cancer, suggesting the possibility of a different mode of action of caspase-8 in the two tumour types.

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An Integrated Approach to Uncover Drivers of Cancer. *D. Pe'er[1,2], O. Litvin[1,2], J. Kim[3,4], F. Sanchez-Garcia[1], H.C. Causton[1], P. Pochanard[3,4], E. Mozes[1], D. Kotliar[1], Y. Tzur[1], L.A. Garraway[3,4], U.D. Akavia[1,2].* 1) Biological Sci, Columbia Univ, New York, NY; 2) C2B2, Columbia Univ, New York, NY; 3) Medical Oncology, DFCI, Harvard Medical School, Boston, MA; 4) Broad Institute, Boston, MA.

Systematic characterization of cancer genomes has revealed a staggering complexity of aberrations among individuals, such that the functional importance and physiological impact of most tumor genetic alterations remains poorly defined. A major challenge involves the development of analysis methods to uncover biological insights from the data, including the identification of the key mutations that drive cancer and how these events alter cellular regulation. We have developed Conexic, a novel Bayesian Network-based framework to integrate chromosomal copy number and gene expression data to detect to detect driver genes located in regions that are aberrant in tumors. The underlying assumption is that a driving mutation might be associated with a characteristic gene expression signature representing genes whose expression is modulated by the driver. Thus our score guided approach searches for genes that are both recurrently aberrant and associated with variance of expression patterns across tumor samples. This method not only pinpoints specific regulators within a large aberrant region, but also by associating drivers with gene modules whose expression vary with the driver, provides insight into the physiological roles of drivers and associated genes. We demonstrated the utility of the CONEXIC framework using a melanoma dataset (Lin et al., Cancer Research, 2008) that includes paired measurements of gene expression and copy number from 62 samples. Our analysis correctly identified known drivers in melanoma (such as MITF) and connected these to many of their known targets, as well as the biological processes they regulate. In addition, it predicted multiple tumor dependencies not previously implicated in melanoma. We tested the effect of knockdown for two predicted drivers, TBC1D16 and RAB27A, and showed that tumors highly expressing these genes are dependent on the same gene for growth. Additionally, gene expression in the associated modules is altered following knockdown as predicted by our model. The identity of these drivers suggests that abnormal regulation of protein trafficking is important for cell survival in melanoma and highlights the importance of protein trafficking in this malignancy. Together, these results implicate a new tumorigenic process in cancer and demonstrate the ability of integrative Bayesian approaches to identify novel drivers with biological, and possibly therapeutic, importance in cancer.

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NPAS3 is a late stage acting progression factor in astrocytomas. *M. Rana[1], TR. Kiehl[2], K. So[3], P. Gould[4], D. Kamnasaran[1,5]*. 1) Pediatrics Research Unit - Centre de recherche du CHUL-CHUQ; 2) Department of Pathology, University Health Network, Toronto, ON, Canada; 3) PRP laboratory, Laboratory Medicine Program, University Health Network, Toronto, ON, Canada; 4) Division of Anatomic Pathology, Department of Medical Biology, CHAUQ Hôpital de l'Enfant-Jésus, Québec, PQ, Canada; 5) Department of Pediatrics, Laval University, Québec, PQ, Canada.

Astrocytomas are the most common primary brain brains and are among the top five causes of cancer related deaths. Despite current treatments, the overall survival of patients still remains poor. In our effort to better comprehend the genetics of gliomas, we explored new therapeutic targets. We previously cloned the human NPAS3 gene and initially identified it as an astrocytoma candidate based on the Cancer Genome Project reporting chromosome 14 deletions (with NPAS3) among ~20-80% of astrocytomas, and with >70% of our panel of 433 human surgically resected astrocytoma specimens having aberrant NPAS3 protein expression. Subsequent functional studies concluded that NPAS3 is a late-stage acting progression factor with tumor suppressive roles. This was based on the discovery of preferential loss of NPAS3 expression in surgically resected human high grade astrocytomas, in comparison to low grade astrocytomas. Furthermore, malignant astrocytoma specimens with loss of NPAS3 expression had loss of function mutations which were associated with loss of heterozygosity. A dominant-negative heterozygous loss-of-function mutation was also identified in a lesion with elevated NPAS3 expression. While an over-expressed or re-expressed NPAS3 in malignant glioma cell lines significantly suppressed transformation, the converse reduced expression significantly and induced more aggressive in-vivo intracranial tumor growth. Most remarkable, knock-down of NPAS3 expression in human astrocytes promoted in-vivo intracranial growth of tumors reminiscent of malignant astrocytomas, in concert with other gliomagenesis pre-disposition genes. In conclusion, our data identified crucial evidence of NPAS3 as a novel tumor suppressor gene with a role in the late-stage progression of malignant human astrocytomas.

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Exome sequencing in the identification of breast cancer predisposition genes. *K. Snape[1], P. Tarpey[2], S. Seal[1], A. Renwick[1], G. Bowden[1], D. Hughes[1], M. Ricketts[1], C. Turnbull[1], M.R. Stratton[1,2], N. Rahman[1]*. 1) Section of Cancer Genetics, Institute of Cancer Research, Sutton, United Kingdom; 2) The Wellcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom.

Breast cancer is a common condition affecting approximately 1 in 10 women in the UK. The recognised familial clustering of breast cancer, in combination with the known increased incidence in first degree relatives of women with the disease and evidence from twin studies points to a strong genetic susceptibility underlying this disorder. High, intermediate and low penetrance genetic variants that predispose to breast cancer have been identified but only account for <30% of the genetic risk of the disease. By resequencing candidate genes involved in the DNA repair pathway we previously identified intermediate penetrance breast cancer predisposition genes *CHEK2, ATM, BRIP1* and *PALB2*. These genes are characterized by rare, inactivating, primarily truncating mutations that confer a relative risk of breast cancer of 2-4. It is highly plausible that there exist additional genes acting in a similar fashion. Systematic large-scale resequencing offers the opportunity to search for disease genes with an agnostic rather than a candidate-gene approach, and has already proved successful in simple Mendelian disorders. We are utilising whole-exome capture in combination with next-generation sequencing technology in familial breast cancer cases to identify further intermediate penetrance genes that function in pathways not predictable from current paradigms. We have undertaken next-generation sequencing using Agilent SureSelect Target Enrichment technology to sequence the exomes of 22 individuals with familial breast cancer. We are validating and following up all truncating variants that are not known polymorphisms in larger series of breast cancer cases. The analytical processes and up-to-date data will be presented.

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Association of Chromosome 6p Multiple Regions with Nasopharyngeal Carcinoma and Gene Expression Changes in Cancer Tissue. *Y. Song[1], L. Fu[2], Y.H. Fan[1], A.M.G. Wong[2], M.X. Li[1], X.Y. Guan[2]*. 1) Dept Biochemistry, Univ Hong Kong, Hong Kong, hk, China; 2) Department of Clinical Oncology, Univ Hong Kong, Hong Kong, hk, China.

Nasopharyngeal carcinoma (NPC) is a malignancy that has nearly 100-fold higher in southern Chinese than in most European population. NPC clusters in families, which suggests that both geography and genetics may influence disease risk. The age group is younger than most adult cancer patients. A population study has reported that the risk of suffering from NPC is 9.31 times higher in the first-degree relatives of patient with NPC than in the first-degree relatives of spouse. The heritability is 68.08%. We employed case-control analysis to study the association of Chromosome 6p regions with nasopharyngeal carcinoma. Total 360 subjects and 360 healthy controls were included. Significant associations were found for multiple markers (most significant p= 3.36E-05, rs2076483) and genes (*GABBR1, HLA-A* and *HCG9*). Further investigation of the allele frequencies between cases and controls suggested regions of micro-deletion within *GABBR1* and *NEDD9*. Real-time PCR using 11 pairs of NPC biopsy samples confirmed significant decrease in the cancer tissues (p=0.059 and 0.015 respectively). Our study from both genetics and functional points of view demonstrated Chromosome 6p multiple regions contribute to risk of nasopharyngeal carcinoma.

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mRNA expression profiling and siRNA studies identify the short stature homeobox 2 gene (SHOX2) as a downstream target of JAZF1. *W. Tang[1], Y. Fu[1], M. Tarway[1], L. Liu[1], J. Hall[2], L. Prokunina-Olsson[1]*. 1) LTG, DCEG, NCI, NIH, Bethesda, MD; 2) Developmental Biology Center, University of Minnesota, Minneapolis, MN.

Genome-wide association studies (GWAS) have identified genetic variants within *JAZF1* gene to be associated with height, body stature, risk of type 2 diabetes, systemic lupus erythematous and prostate cancer. However, the molecular phenotypes that link *JAZF1* to these diverse traits are currently unclear. We used Ingenuity Pathway Analysis (IPA) to analyze genome-wide mRNA expression in the NCI-60 cell line set and observed that one of the genes with the strongest correlation with *JAZF1* expression (r=0.64, FDR-adjusted $p=4.3x10^{-4}$) was the short stature homeobox 2 gene (*SHOX2*), associated with delayed chondrocyte differentiation and shortened long bones of the limbs in the *Shox2* deficient mouse model. Expression of *JAZF1* and *SHOX2* was also correlated in a panel of non-cancerous human tissues (n=15, r=0.59, p=0.021) and in normal human pancreas (n=36, r=0.68, $p=2.8x10^{-5}$). siRNA knock-down of *JAZF1* expression in the pancreatic cancer cell line PANC1 resulted in a significant decrease of *SHOX2* expression, suggesting that *SHOX2* is a downstream target of *JAZF1*. Our analysis provides the first insights into possible molecular phenotypes relevant for genetic associations reported for *JAZF1* and identified *SHOX2* as a downstream target of *JAZF1*.

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Down-regulated PLZF is associated with anti-apoptosis in Lung Cancer. *X. Wang[1], J. Wang[1], J. Xu[2], L. Wang[2], Y. Bao[3], Z. Zhao[1], Y. Ma[1], Z. Xu[2], L. Jin[1].* 1) State Key Laboratory of Genetic engineering, MOE Key Laboratory of Comtemporary Anthropology, Fudan University, Shanghai, China; 2) Department of Cardiac and Thoracic Surgery, Changhai Hospital, Shanghai, China; 3) Yangzhou No.1 People's Hospital.

The promyelocytic leukemia zinc finger (PLZF) protein, a transcriptional repressor and tumor suppressor, regulates the expression of genes involved in physiological development, proliferation, differentiation, and apoptosis. Apoptosis-inducer TP53INP1, ID1, ID2 and ID3 were considered as functional mediator of apoptosis induced by PLZF expression. So far there has been no research on PLZF in lung cancer. By comparing gene expression profiles in 6 pairs of lung cancer tissues using microarray analysis of gene expression, it has been found that PLZF was down-regulated in lung cancer tissues and the expression levels of PLZF-related genes were also changed accordingly. We further detected PLZF expression in 111 pairs of lung cancer tissues and corresponding normal lung tissues by Real-Time PCR and found that PLZF was down-regulated in 89% of cancer tissues. Moreover, the expression of PLZF mRNA was decreased by 20-fold in 50% of cancer tissues. It suggested that abnormal PLZF expression contributed to lung tumorigenesis. Further analysis found that PLZF expression was associated with patient gender (P = 0.017). Apoptosis TUNEL Assay of 63 pairs of paraffin-embedded lung cancer tissues and their corresponding normal tissues indicated that abnormal PLZF expression may also contribute to anti-apoptosis in lung cancer. The expressions of TP53INP1, ID1, ID2 , ID3, CASP9 and BIRC5 (survivin) were measured in 40 pairs of lung cancer tissues in which PLZF expression was significantly reduced. As expected, the functional mediators of PLZF, TP53INP1, ID1, ID2 and ID3 were down-regulated in more than 75% of lung cancer tissues. Meanwhile, the inhibitor of apoptosis, BIRC5, was up-regulated in 65% of cancer tissues, whereas the mediator of apoptosis, CASP9, was down-regulated in 65% of cancer tissues. This is the first report that deregulation of PLZF plays an important role in the carcinogenesis of lung cancer, and reduced expression of PLZF may contribute to anti-apoptosis in lung cancer. P53INP1, ID1, ID2 and ID3 could be mediators of growth-inhibition and apoptosis induced by PLZF.

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Systematic Detection of Putative Tumor Suppressor Genes Through the Combined Use of Exome and Transcriptome Sequencing. *Q. Zhao[1], E.F. Kirkness[1], O.L. Caballero[2], P.A. Galante[3], R.B. Parmigiani[3], Z. Ye[5], S. Kuan[5], L. Edshall[5], S. Levy[1], A.R. Vasconcelos[4], B. Ren[5], S.J. de Souza[3], A.A. Camargo[3], A.J.G. Simpson[2], R.L. Strausberg[1,2].* 1) Human Genomics, J Craig Venter Inst, Rockville, US; 2) Ludwig Institute, New York Branch, New York, NY 10021, USA; 3) Ludwig Institute, São Paulo Branch at Hospital Alemão Oswaldo Cruz, São Paulo, SP 01323-903, Brazil; 4) Laboratório Nacional de Computação Científica, Laboratório de Bioinformática, Petrópolis, RJ 25651-070, Brazil; 5) Ludwig Institute, San Diego Branch, San Diego, CA 92093-0660, USA.

Cancer arises from gain of function in oncogenes and loss of function in tumor suppressor genes. To characterize two types of frequent genomic events resulting in loss of function in cancer, we took a genome-wide search from exome and transcriptome sequencing data to look for genes with loss of heterozygosity (LOH) and allele specific expression (ASE). The analysis was conducted on a breast cancer cell line and its corresponding control cell line from lymphoblast from the same individual. By comparing variant allele frequencies using exome sequences between the two cell lines, we identified a significant number of LOH events in the breast cancer cell line, which are either clustered in large chromosomal regions or as isolated somatic event. The combination of exome and transcriptome sequence data within the same cell line also revealed genes with ASE events, which comprise 2% to 5% of genes surveyed. Molecular function and cellular network analysis revealed that many of these genes identified by LOH and ASE are known or putative tumor suppressor genes such as BRCA1, MSH3 and SMARCA4, which participate in DNA replication-coupled DNA repair pathways. Our results demonstrate that the combined application of high throughput sequencing to exome and allele-specific transcriptome analysis can reveal genes with known tumor suppressor characteristics, and a short-list of novel candidates for the study of tumor suppressor activities.

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Genetic modifiers of age of onset in Lynch syndrome (hereditary non-polyposis colorectal cancer) in South Africa. *S. Owens[1, 2], D. Velez Edwards[3], A. Vorster[1], U. Algar[4], R. Ramesar[1].* 1) MRC Human Genetics Research Unit, Division of Human Genetics, Institute for Infectious Diseases and Molecular Medicine, University of Cape Town, Cape Town, South Africa; 2) Fogarty International Clinical Research Fellow, Institute for Global Health, Vanderbilt University Medical Center, Nashville, TN USA; 3) Vanderbilt Epidemiology Center, Institute of Medicine and Public Health, Department of Obstetrics and Gynecology, Vanderbilt University Medical Center, Nashville, TN USA; 4) Familial Colorectal Cancer Coordinator, Groote Schuur Hospital, Cape Town, South Africa.

Colorectal cancer (CRC) is the second leading cause of cancer-related deaths in the U.S. and the third leading cause in South Africa, with both genetic and environmental factors playing a role. Hereditary nonpolyposis colorectal cancer (HNPCC), more recently referred to as Lynch syndrome, is an inherited disorder caused by mutations in DNA mismatch repair (MMR) genes. Individuals with HNPCC have an 80% lifetime risk of CRC but the age of onset is highly variable due to additional genetic and environmental factors. We hypothesized that in individuals predisposed to HNPCC with a primary mutation in a MMR gene, one or more secondary mutations act to influence the age of CRC onset. This study aimed to identify such genetic modifiers.

A South African cohort of 232 HNPCC individuals harboring primary mutations in MMR genes *MLH1* and *MSH2* were genotyped for selected polymorphisms in six candidate loci (*GCLC*, *CYP1A1*, *EPHX1*, *IGF1*, *OGG1* and 8q24). Modifier effects for each SNP were tested individually for association to age of onset. Epistatic interactions between potential modifiers were also assessed, including gender-specific effects.

Preliminary Kaplan-Meier analysis for a variant in *CYP1A1* demonstrated a significant difference in age of onset (p=0.04). Analysis for the remaining variants revealed no statistically significant difference in gender, MMR gene mutation or ethnicity.

These results, very likely in addition to other environmental and genetic modifiers currently being investigated, will directly translate into improved clinical management of CRC. This is particularly important in South Africa and other resource-poor countries, where risk stratification based on genetic testing for modifier polymorphisms has the potential to improve surveillance schedules for individuals identified to be at high risk due to a predisposing primary mutation in a MMR gene.

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A large genomic rearrangement in the 17q21 chromosomal region disrupts the *BRCA1* gene. *K. Pylkäs[1], M. Haanpää[1], J. Nikkilä[1], J. Ignatius[1,2], R. Winqvist[1].* 1) Laboratory of Cancer Genetics, Department of Clinical Genetics, University of Oulu, Oulu, Finland; 2) Department of Genetic Counseling, Turku University Hospital, Turku, Finland.

BRCA1 is one of the two major genes associated with familial breast and ovarian cancer susceptibility. Most of the alterations identified in *BRCA1* are point mutations and small insertions/deletions, but an increasing number of large genomic rearrangements have also been identified in different populations. Here we have analyzed 61 index patients of Northern Finnish breast and/or ovarian cancer families for large genomic rearrangements in *BRCA1*. One large rearrangement, deleting most of the gene including the promoter region, was observed in one family with multiple cases of ovarian cancer. The breakpoints of the observed deletion have now been characterized further by MLPA and high-resolution array comparative genomic hybridization (aCGH) analyses for the involved 17q21 chromosomal region. Our results demonstrate that the deletion extends over 260 Kbs in size and leads to the loss of several neighboring genes.

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Carney complex phenotype in patients carrying *PRKAR1A* mutations and *PDE11A* variations. *R. Libe[1], A. Horvath[2], A. Fratticci[1], D. Vezzosi[1], J. Coste[1], K. Perlemoine[1], B. Ragazzon[1], M. Guillaud-Bataille[1], L. Groussin[1], E. Clauser[2], M. Raffin-Sanson[1], X. Bertagna[1], C. Stratakis[2], J. Bertherat[1].* 1) Institut Cochin, INSERM U56, Paris, France; 2) NICHD, NIH, Bethesda, MD, USA.

CNC is an autosomal dominant disorder featuring skin lesions, cardiac and other myxomas and different types of endocrine tumors, among the most common of which primary pigmented nodular adrenocortical disease (PPNAD), and testicular lesions (Large Cell Calcifying Sertoli Cell Tumor, LCCSCT). Mutations in regulatory subunit type 1 alpha (*PRKAR1A*) of Protein Kinase A (PKA) are responsible for the disease in at least 65% of the CNC patients. *PDE11A* inactivating mutations have been described in patients with isolated PPNAD, as well as in patients with bilateral or familial testicular germ cell tumors. We evaluated the impact of *PDE11A* variations on the phenotype in CNC patients who were carriers of a pathogenic *PRKAR1A* mutation. We analyzed the coding sequence of *PDE11A* in 150 patients with CNC and mutations in *PRKAR1A* and in 279 controls free of endocrine tumors and family history. A significantly higher prevalence of *PDE11A* mutations was found among CNC patients compared to controls (25.3% vs 6.8% in the controls, $\chi 2=29$, $p<0.00001$). Within the CNC patient group, the PDE11A mutations were significantly more frequent in the patients with PPNAD versus the ones without PPNAD (30% vs 13%, p=0.025). Similarly, CNC patients with LCCSCT had higher prevalence of PDE11A variations compared to those without LCCSCT (50% vs 10%, p=0.025). In addition, simultaneous inactivation of *PRKAR1A* and *PDE11A* by small inactivating RNAs (siRNA) in HEK293 cells led to increased luciferase activity in a cAMP response elements (CRE)-based reporter assay. Our data suggest an effect of *PDE11A* variations on the risk for developing PPNAD and LCCSCT in the context of CNC. This is consistent with the role of PDE11A, as a susceptibility gene for adrenocortical and testicular tumors, at large. A synergistic effect of inactivating *PDE11A* and *PRKAR1A* mutations may be at play; both defects would lead to increased cAMP signaling, as suggested by the functional siRNA-based assay.

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SDHAF2 and TMEM127 genes in pheochromocytoma and paraganglioma predisposition. *Y. Ni[1,2], J. Moline[1], M. Orloff[1,3], J. Chen[1], C. Eng[1,2,3,4,5].* 1) Genomic Medicine Institute, CLeveland Clinic, Cleveland, OH; 2) Department of Molecular Medicine, Cleveland Clinic Lerner College of Medicine, Cleveland, OH; 3) Taussig Cancer Institute, Cleveland Clinic, Cleveland, OH; 4) CASE Comprehensive Cancer Center, Case Western Reserve University, Cleveland, OH; 5) Department of Genetics, School of Medicine, Case Western Reserve University, Cleveland, OH.

Pheochromocytoma (PC) and paraganglioma (PGL) are tumors of the autonomic nervous system, which can be hereditary or sporadic. Germline mutations in genes that encode succinate dehydrogenase subunits B, C, and D (SDHB, SDHC, SDHD) are well known to associate with PC and PGL. More recently, the Gly78Arg mutation in SDHAF2 encoding succinate dehydrogenase complex assembly factor 2, was identified in 2 familial PGL kindreds. In addition, germline mutations in TMEM127, a transmembrane protein-encoding gene, were shown to confer susceptibility to adrenal PC. We sought to determine the relative contribution of SDHAF2 and TMEM127 genes in predisposition to isolated and familial PC and PGL that were unrelated to RET, VHL or SDHB-D. Of 102 eligible probands, we previously identified 41 (40%) with germline SDHB-D deleterious mutations. Therefore, SDHAF2 and TMEM127 mutation and deletion analyses were performed in the remaining 61 PC-PGL patients (32 PGL, 29 PC; 5 multifocal and/or familial, 56 isolated, unifocal cases; 10-83y, mean 45y). No deletion was detected in any of the cases tested for these 2 genes. There were 2/61 (3%) with germline mutations/variants in SDHAF2: a potential splice variant (c. 371-3C>T) in an isolated case with metastatic thoracic PC diagnosed at the age of 26, and a deleterious Gly78Arg mutation in a 25yo with multifocal neck PGL and positive paternal family history. TMEM127 analysis revealed 4/61 (6.5%) cases with missense variants, 3 carrying the same start codon alteration, Met1Ile. Of these 3 cases, 2 had additional variants, Gly6Asp and Ser33Leu, respectively. The fourth case had a Gly6Asp variant. All 4 cases are isolated adrenal PC patients with relative early age at onset (10-58y, mean 34.75y). We compared the TMEM127 transcript level in these 4 cases to 6 healthy controls by quantitative RT-PCR. Mean mRNA level in all TMEM127 variant carriers was half that of the controls. This result is consistent with the loss-of-function of TMEM127 mutations. In summary, SDHAF2 and TMEM127 germline mutations were identified in addition to our original analysis of SDHB-D and increased the frequency of germline mutations in all incident PGL and PC patients from 40% to 46%. More specifically, TMEM127 variants were predominant in isolated adrenal PC cases. SDHAF2 mutations were associated with earlier onset familial PGL cases based on our and other groups' studies, while we reported the first isolated metastatic PC case with SDHAF2 variant here.

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No recurrent genomic aberrations in Ollier disease related enchondromas as assessed by high-resolution SNP array and expression array: a Genome-wide approach. *T.C. Pansuriya[1], J. Oosting[1], A.M. Cleton-Jansen[1], A.H.M. Taminiau[1], L. Sangiorgi[2], R. Sciot[3], P.C.W. Hogendoorn[1], K. Szuhai[1], J.V.M.G. Bovée[1].* 1) Pathology, Leiden University Medical Center, Leiden, Zuid Holland, Netherlands; 2) Rizzoli Orthopaedic Institute, Bologna, Italy; 3) University of Leuven, Leuven, Belgium.

Ollier disease is a rare, non-hereditary disease which is characterized by the presence of multiple enchondromas (EC), benign cartilaginous neoplasms arising within the medulla of the bone with an asymmetric distribution, and a risk of malignant transformation towards central chondrosarcoma (CS) up to 35%. The etiology of Ollier disease is unknown. Point mutations in *PTH1R* (G121E, A122T, R150C and R255H) have been reported in about 10% of Ollier patients. However, in our series all four reported point mutations in *PTH1R* were absent. We hypothesized that Ollier disease is a mosaic condition. An early post zygotic mutation in a tumor suppressor gene may explain the asymmetric involvement of the skeletal structures. Later on a second hit in some of these affected cells will subsequently lead to EC formation. We therefore undertook genome-wide copy number and loss of heterozygosity (LOH) analysis in 14 EC and 18 CS from 24 Ollier patients using Affymetrix SNP 6.0 array platform. We have included 16 healthy and 10 patient controls. The data were analyzed by R and Nexus software packages. In EC, a large number of small copy number changes were found. Because the number and size of these changes was not different from the controls, these are indicative of known CNVs. Paired analysis indicated absence of LOH in EC whereas most of the CS shared LOH at 6, 9p and 13q. No common genomic alterations were found for all ECs therefore we investigated the candidate genomic regions in each case separately. By using a SNP array approach we identified 5 regions, of which 3 remained valid after confirmatory tests. The 2 excluded regions were known CNV regions. Illumina expression array was performed on 5 EC, 14 CS and 6 controls. Thousands of genes were differentially expressed in EC compared to controls and between EC and CS. With pathway analysis, the p53 pathway (adj. p-value = 0.03) and biosynthesis of steroid pathway (adj. p-value = 0.07) differs between controls and Ollier tumours. Integration of SNP and expression data has allowed us to identify candidate regions which were validated. In conclusion, no genetic alterations are shared by all EC. Our data show conclusively that LOH and common copy number alterations are rare in EC which reflects genetic heterogeneity of the tumor. One could predict that instead small deletions, point mutations or epigenetic mechanisms seem to play a role in the origin of Ollier disease.

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Three novel mutations in *PDE8B*, a cAMP phosphodiesterase highly expressed in the adrenal cortex, in a cohort of patients with adrenal tumors. *A. Rothenbuhler[1], A. Horvath[2], F. Faucz[2], M. Almeida[2], M. Lodish[2], R. Libé[1], M. Nesterova[2], J. Bertherat[1], C. Stratakis[2].* 1) Institut Cochin INSERM U567; 2) NICHD, NIH, Bethesda, MD, USA.

Genetic aberrations in the cAMP signaling pathway have been found to play a role in many types of adrenal tumors (ADTs). Our group identified mutations of a phosphodiesterase (PDE) gene, *PDE11A*, in patients with micronodular adrenocortical hyperplasia (MAH). A genome-wide SNP genotyping study in individuals with adrenocortical hyperplasia leading to Cushing syndrome (CS) that was not caused by known defects indicated that, in addition to the 2q31-33 locus (*PDE11A*), a locus on 5q14, harboring another PDE, *PDE8B*, was likely to contain a disease-related gene. A single base substitution in PDE8B that resulted in a proline-to-histidine change in an evolutionarily-conserved residue of the protein (c.914A>T/p.H305P) was then identified in a female pediatric patient with CS due to MAH. We now studied 40 patients with secreting and non-secreting adrenal tumors for sequence alterations of the *PDE8B* gene and investigated PDE8B's expression in the adrenal cortex and its lesions. Three, previously non-reported PDE8B sequence variants were identified in three patients with secreting adrenal tumors; the first, a splice site mutation which leads to an estimated 20% decreased acceptor site activity of exon 14 (c1365-5 g/a), the second, a single base substitution, that led to a valine-to-isoleucine change in exon 18 (c.2089 G>A/p.V697I) and the third, also a single base substitution, that led to a arginine-to-histidine change in exon 2 (c.362G>A/p.R121H). In vitro transfection experiments showed that each of the missense mutations resulted in lower levels of PDE activity and higher levels of cAMP. Immunohistohemistry (IHC) studies showed an increase of PDE8B expression in the adrenal cortex of patients with adrenal lesions due to other defects of the cAMP-signaling pathway. In conclusion, we found three novel *PDE8B* sequence variations with potential impact on the ability of PDE8B to bind and/or degrade cAMP, and evidence of a counter-regulatory role of this PDE in cAMP signaling in adrenalocortical lesions. These data support a significant role of *PDE8B* in adrenal pathophysiology and/or tumorigenesis.

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Shared Genomic Segment analysis identifies a susceptibility locus for breast cancer at 14q23.3. *Z. CAI[1], L.A. Cannon-Albright[2], N.J. Camp[2], A. Thomas[2].* 1) Department of Biomedical Informatics, University of Utah, Salt Lake city, UT; 2) Division of Genetic Epidemiology, Department of Internal Medicine, University of Utah, Salt Lake city, UT.

Breast cancer is the second most prevalent cancer in developed countries. Family history is a major factor in terms of breast cancer risk. High-risk mutations have been found in BRCA genes, e.g. BRCA1 and BRCA2, but these only account for a small proportion of hereditary breast cancer cases. Other rare and common variants have also been suggested. However, even after accounting for all known and putative risk variants there remains substantial “missing heritability.” It is possible that further rare genetic variants exist. The shared genomic segment (SGS) method was developed to identify genomic regions shared by distantly related individuals in extended pedigrees using high-density genomewide SNP arrays. Genomic regions that are shared in excess by diseased individuals in extended high-risk pedigrees are good candidates to harbor disease loci. The SGS method measures allele sharing at consecutive genetic markers across the genome to identify those segments that are shared in excess of that expected by chance. Significance is assessed empirically to account for linkage disequilibrium between SNPs and pedigree structure. It has been shown to have good power to identify disease risk loci caused by rare genetic variations. We applied SGS method to a high-risk Utah breast cancer pedigree containing 10 cases separated by 33 meioses. This pedigree was assessed to contain a significant excess of breast cancer using a cluster analysis in the Utah Population Database. Additionally, risk of breast cancer in this pedigree was assessed not to be due to mutations in BRCA1 and BRCA2. A genomic region measuring 0.33 cM in length at 14q23.3 (65,713kb - 67,004kb NCBI Genome Build 36.3) was detected with high statistical significance (p<1e-6). This region has previously been shown to be differentially expressed across morphological breast cancer subtypes. Also, gene translocations have been identified in this region associated with breast cancer. In conclusion, this region identified by SGS is a strong candidate to contain a rare breast cancer risk variant.

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Lifetime environmental exposure to tobacco smoke, genetic susceptibility, and risk of lung cancer in non-smoking Taiwanese. *C. Hsiao[1], C. Chang[1], G. Chang[2], Y. Tsai[3], Y. Chen[4], M. Huang[5], W. Su[6], W. Hsieh[1], P. Yang[7], C. Chen[8], C. Hsiung[1].* 1) Division of Biostatistics and Bioinformatics, Institute of Population Health Sciences, National Health Research Institutes, Zhunan, Taiwan; 2) Division of Chest Medicine, Department of Internal Medicine, Taichung Veterans General Hospital, Taichung, Taiwan; 3) Department of Pulmonary and Critical Care, Chang Gung Memorial Hospital, Taipei, Taiwan; 4) Chest Department, Taipei Veterans General Hospital, Taipei, Taiwan; 5) Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; 6) Department of Internal Medicine, National Cheng Kung University Hospital, Tainan, Taiwan; 7) Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan; 8) Genomics Research Center, Academia Sinica and Graduate Institute of Epidemiology, National Taiwan University, Taipei, Taiwan.

Environmental tobacco smoke (ETS) has been suggested as a risk factor of lung cancer in nonsmokers. The ETS exposure mainly comes from three sources: childhood and adulthood household passive smoking, and adult work-place passive smoking. Although ETS and genetic factors may play important roles on lung cancer risk, the interactive effect between ETS and genetic factors on lung cancer risk needs to be explored. A total of 1,785 non-smoking lung cancer cases and 1,942 non-smoking healthy controls were enrolled from six medical centers in Taiwan between 2002 and 2009. Genetic polymorphisms of hOGG1 Ser326Cys were determined by the MassARRAY system. ETS history was obtained through personal interview based on a structured questionnaire. Multivariate logistic regression analyses were performed to estimate odds ratios (OR) and 95% confidence intervals (CI) for the risk of lung cancer in exposed vs. non-exposed ETS groups. Childhood ETS had non-significant effects on the risk of lung cancer (OR=1.2, 95% CI= 0.5-2.9) after controlling for adulthood ETS exposure (including adulthood household and work-place). Adulthood household and work-place had independent risk effects on lung cancer (adulthood household: OR=1.3, 95% CI= 1.1-1.6; work-place: OR=1.9, 95% CI= 1.6-2.4). On the other hand, considering the interaction between hOGG1 gene and ETS for the risk of developing lung cancer, comparing with the Ser/Ser genotype, the Cys/Cys genotype had higher risk in ETS exposure group (OR=2.0, 95% CI= 1.1-3.7), but not in the non-ETS group (OR=1.2, 95% CI= 0.6-2.8). It was concluded that the adulthood ETS has prominent effects on the risk of lung cancer, especially work-place passive smoking. In addition, hOGG1 Cys/Cys genotype has modifying effect on lung cancer risk by ETS exposure status among non-smokers.

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The Genetic Epidemiology of Melanoma Prognosis. *G. Cadby[1], S.V. Ward[1], J.M. Cole[2], M. Millward[3], L.J Palmer[1].* 1) Centre for Genetic Epidemiology and Biostatistics, UWA, Nedlands, Western Australia; 2) Western Australian Melanoma Advisory Service, Perth, Western Australia; 3) School of Medicine and Pharmacology, UWA, Nedlands, Western Australia.

Melanoma is the most aggressive form of skin cancer. Australia has the highest incidence of malignant melanoma in the world, and this appears to be rising. Epidemiological research has led to some understanding of the major environmental and genetic risk factors for melanoma, however the role of these factors in melanoma prognosis have not been fully investigated.

The Western Australian Melanoma Health Study (WAMHS) is a population-based case collection and linked biospecimen resource enabling investigation into the genetic epidemiology of melanoma. The WAMHS aimed to recruit all incident cases of invasive cutaneous melanoma in Western Australian adults from January 2006 to September 2009. The final WAMHS sample consisted of 1157 individuals who completed both the questionnaire and gave a blood sample for DNA.

In this analysis, we investigated environmental, host and genetic factors associated with Breslow thickness, which is a prognostic feature of melanoma. Thicker Breslow thickness is associated with poorer prognosis. Our main hypothesis was that the SNPs which increase melanoma-risk are also those which increase Breslow thickness.

A subset of 800 Caucasian WAMHS subjects were genotyped at 42 melanoma-risk SNPs. We investigated several environmental, host and genetic factors associated with Breslow thickness and found Breslow thickness was associated with increasing age (P<0.001), and the presence of naevi on the back (P<0.05). Four SNPs were also found to be associated with Breslow thickness. These were rs12203592 in IRF4, rs1800401 in OCA2, rs1042522 in TP53 and rs1733826 in BRAF. However, none of these associations remained significant after adjustment for multiple testing. Age at diagnosis, the presence of naevi and the four SNPs accounted for 6.8% of variation in Breslow thickness, indicating that there is a large amount of variation unexplained.

This study is the first of its kind to investigate the role of melanoma-risk SNPs in melanoma prognosis. As melanoma mortality and incidence are increasing in Australian adults, identification of the effect of environmental, host and genetic risk factors on prognosis will become increasingly important. Replication of these results, particularly in larger samples, is therefore required.

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***CASP8* promoter polymorphism is associated with high-risk HPV types and abnormal cytology but not with cervical cancer.** *K. Chatterjee[1], A-L. Williamson[1,2], M. Hoffman[3], C. Dandara[4].* 1) Division of Medical Virology and Institute of Infectious Disease and Molecular Medicine (IIDMM), University of Cape Town, Cape Town, Republic of South Africa; 2) National Health Laboratory Service, Groote Schuur Hospital, Observatory, Cape Town, Republic of South Africa; 3) School of Public Health and Family Medicine, University of Cape Town, Cape Town, Republic of South Africa; 4) Division of Human Genetics, Faculty of Health Sciences, University of Cape Town, Cape Town, Republic of South Africa.

Background: Only a small fraction of human papillomavirus (HPV) infected women progress to cervical cancer pointing to additional risk factors including host genetics that might play a role in development of cervical cancer. Caspase-8 (encoded by CASP8 gene) plays an important role in generating cell death signals leading to elimination of potentially malignant cells. Genetic variation in CASP8 might influence the susceptibility to cancer. CASP8 -652 6N ins/del polymorphism has been previously reported to influence the progression to several cancers including cervical cancer. The aim of this study was to investigate the role of CASP8 -652 6N ins/del polymorphism in susceptibility to HPV infection leading to pre-cancerous lesions and cervical cancer in South Africans. **Materials and Methods:** The CASP8 -652 6N ins/del polymorphism was investigated in 445 women of black African and mixed-ancestry origin with invasive cervical cancer and 1221 controls matched by age, ethnicity and domicile status. Genotyping for CASP8 -652 6N ins/del was done by PCR-RFLP. Papanicolaou test (Pap smear) was conducted on endocervical scrapings for the control women to test for their cytology status. **Results:** The CASP8 -652 6N del/del genotype did not show any significant association (P = 0.948) with cervical cancer cases. Further analysis within the controls showed a border-line association (P = 0.048) of this polymorphism with abnormal cytology in both ethnicities and significantly high-risk HPV infection (P = 0.030) among the black Africans. **Conclusion:** This is the first study of the role of CASP8 -652 6N ins/del polymorphism in cervical cancer in an African population. This result points towards the importance of variation in CASP8 and its associated genes in the susceptibility to HPV infection and development of pre-cancers.

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Ets1 as a potential target of microRNA-1 in cutaneous squamous cell carcinoma. *D. Gable, J. Fleming, A. Toland.* The Ohio State University Columbus, OH.

Cutaneous squamous cell carcinoma (SCC) is the second most common form of skin cancer, affecting 700,000 people in the United States annually. MicroRNAs are a type of RNA that regulate the expression of other genes. Using miRNA expression arrays, we identified lower levels of microRNA-1 (miR-1) expression in skin from FVB/N mice that are susceptible to chemically-induced skin cancer, compared to skin from Spret/EiJ mice that are resistant. Additionally, murine SCC cell lines showed greatly reduced levels of miR-1 compared to normal skin. We hypothesize that decreased expression of miR-1 in susceptible mice and SCC cell lines suggests a role of miR-1 as a tumor suppressor in skin. To test this hypothesis, we transfected a precursor for miR-1 into a murine SCC cell line, A5, and measured proliferation and apoptosis via an MTT assay and caspase 3/7 assay respectively at 24, 48, and 72 hours post-transfection. The growth rate for cells transfected with miR-1 significantly decreased at 48 to 72 hours post-transfection relative to those negative-control transfected cells at which time apoptosis significantly increased. These data are consistent with our hypothesis that miR-1 may act as a tumor suppressor in the skin. Using in silico prediction programs, we identified a number of genes whose expression may be regulated by miR-1. The transcription factor oncogene, Ets1, emerged as an interesting potential direct target of miR-1. Quantitative PCR and Western analyses revealed decreased Ets1 mRNA and protein expression in A5 cells transfected with miR-1 compared to negative control-transfected cells. Ets1 mRNA expression is also decreased in a normal keratinocyte cell line compared to A5 cells. The inverse correlation in expression suggests Ets1 is regulated by miR-1. Upon performing an MTT assay after co-transfecting A5 cells with a precursor to miR-1 and Ets1, Ets1 appears to increase proliferation independent of miR-1 regulation. Ongoing studies will determine whether Ets1 is a direct target of miR-1. We will also clarify the role of Ets1 in SCC cell apoptosis and migration, and identify other targets and tumor-related phenotypes associated with miR-1.

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A chemical genetics screen identifies novel drugs that target steroid biogenesis and receptor signaling which can inhibit the growth of glioma stem cells. *N. Ajewung[1], D. Poirier[2], D. Kamnasaran[1,3].* 1) Pediatrics Research Unit - Centre de recherche du CHUL-CHUQ; 2) Laboratory of Medicinal Chemistry, Oncology and Molecular Endocrinology - Centre de recherche du CHUL-CHUQ; 3) Department of Pediatrics, Laval University, Quebec, Quebec.

Giomas are among the top five causes of cancer related deaths, representing about ~60% of the cases in adults and ~30% in children with primary brain tumors. Furthermore, about five cancer syndromes are associated with patients having a higher risk for developing gliomas during their lifetime. Glioma stem cells represent a fraction of cells within a tumor mass which are postulated to be responsible for tumor re-growth. Most importantly, recent studies have associated glioma stem cells with impeccable treatment resistance mechanisms, leading to an overall poor survival among patients. Since a wide range of genes involved in steroid biogenesis and signaling are expressed in glioma stem cells, our objective was to investigate whether novel classes of drugs that target these gene products can be effective in inhibiting the growth of glioma stem cells. To accomplish this, we screened using a candidate chemical structure approach, a library of 400 drugs which can potentially inhibit steroid biogenesis and cell signaling. By using a panel of human glioma stem cells established from surgical specimens and from glioma cell lines, we discovered a potent drug that inhibits androsterone (male sex pheromone) biogenesis and with the ability to significantly reduce the viability of human glioma stem cells in a dose dependent manner over a one week period. Cells treated with this drug responded by undergoing apoptosis and cell cycle regulatory changes. Furthermore, significant inhibition of transformation was noted. Most remarkable, the toxicity on human astrocytes and neural stem/progenitor cells was minimal, in comparison to the effect on human glioma stem cells. In summary, we have discovered a novel drug from a chemical genetic screen which can significantly inhibit the growth of glioma stem cells, with minimal toxicity on non-transformed human astrocytes and neural stem/progenitor cells.

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Hereditary nonpolyposis colorectal cancer (HNPCC) mutations in patients diagnosed with ovarian cancer in a community setting: is it time to add ovarian cancer in clinical criteria for diagnosis of HNPCC? *S. Cohen, I. Shapira, R. Gralla, K. Sultan, V. John, H. Raftopoulos, J. Lovecchio, A. Menzin, J. Whyte, A. Sakaris, D. Contreras, V. Vinciguerra.* Cancer Genetics, Monter Cancer Center, North Shore-LIJ, Hofstra Medical School, Lake Success, NY.

Background: Up to 10% of ovarian cancers are due to inherited mutations. Of those 85% are due to BRCA 1 & 2 and 13% due to HNPCC mutations. Mutations in HNPCC carry 12% life-time risk for ovarian cancer, 10 fold higher than in the general population. In families with ovarian cancer diagnosis, finding mutations in either BRCA genes or HNPCC genes may provide an opportunity for risk reduction strategies. Objectives: To determine the incidence of HNPCC-related gene mutations in patients with personal or familial ovarian cancer and their likelihood to fulfill Amsterdam I/ II or Bethesda criteria. Methods: Over a 5-year period (2004 -2009), we analyzed the cause of referral for genetic testing and the mutations detected in 1193 consecutively tested individuals (excluding those from families who had been previously tested at our institution to limit the bias for mutation positivity). Forty-one subjects (3.4%) were referred for personal ovarian cancer cancer. The chart review included patient age, self-reported ethnicity, family history, genetic test results and method and which mutation if any was detected. Patients were initially tested for BRCA 1 & 2 mutations if negative they were tested for HNPCC. The ethnic distribution of the 41 ovarian cancer patients was: 16 Ashkenazi, 22 Non-Ashkenazi Europeans, 1 Latino and 2 Asians. Median age at diagnosis was 50 (range 45-60). Results: Twelve patients had deleterious mutations in the BRCA genes (29%; CI 15%-43%) and 5 had deleterious mutations in mistmatch repair genes (12%; CI 4%-26%). The mismatch repair gene mutations detected were two in MLH1 (MLH1 deletion exons 14-19, MLH1 2070insTT), two in MSH2 (MSH2A636P, MSH2 deletion exons 2-7 and 9-10) and one in MSH6 (3108delGT). None of the 41 subjects diagnosed with ovarian cancer at our institution fulfilled clinical criteria for diagnosis of HNPCC. Conclusions: By design Bethesda criteria are broader than Amsterdam I/II criteria and far more likely to lead to testing for HNPCC. Our results show that reliance upon clinical criteria would miss an opportunity to diagnose a large percentage of HNPCC patients. If Amsterdam clinical criteria were expanded to include ovarian cancer in patients with negative mutation testing for BRCA 1 & 2 genes, an additional 12% (5 out of 41) of patients would to have deleterious mutations in HNPCC genes. Genetic testing allows informed decisions on surveillance and preventive strategies for families at risk.

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Spectrum of germline TP53 mutations in breast cancer predisposition. *H. Hanson[1], S. Seal[1], A. Renwick[1], M. Ricketts[1], D. Hughes[1], G. Evans[2], D. Eccles[3], C. Turnbull[1], N. Rahman[1], FBCS(UK) collaboration.* 1) Section of Cancer Genetics, Institute of Cancer Research, Sutton, United Kingdom; 2) University Department of Medical Genetics, St Mary's Hospital, Manchester, United Kingdom; 3) Department of Clinical Genetics, University of Southampton School of Medicine, Princess Anne Hospital, Southampton, United Kingdom.

Germline *TP53* mutations cause Li-Fraumeni syndrome (LFS), a cancer predisposition syndrome associated with sarcomas, breast, brain and adrenocortical tumours. Mutations in LFS families are typically missense mutations that occur at hotspot codons located in the DNA-binding domain. The risk of breast cancer in classic LFS families is high. However, the contribution and mutational profile of *TP53* mutations in breast cancer cases that do not fulfill LFS criteria are unclear. To address this we sequenced *TP53* in 1525 breast cancer cases and in 1334 controls. The breast cancer cases consisted of 1317 familial and 208 non-familial cases and included 385 individuals affected below 31 years. None of the cases had a family history of classic Li-Fraumeni syndrome. We identified ten pathogenic mutations in breast cancer cases and none in controls (p=0.002). The mutations included nine truncating mutations and one pathogenic missense mutation, R337C, which is not in the DNA-binding domain. No hotspot mutations were identified. The family history of breast cancer was modest in the mutation-positive families. The prevalence of mutations was low in the familial breast cancer cases (7/1317) the young onset breast cancer cases (8/385) and the bilateral cases overall (5/330). However, it was appreciable in the small number of individuals with bilateral breast cancer with both breasts affected below the age of 31 years (4/6). We identified non-hotspot missense mutations, including variants in the DNA binding domain, with equal prevalence in both cases and controls. These data demonstrate that the phenotypic effects and penetrance of *TP53* mutations are highly variable and indicate that truncating mutations confer modestly increased risks of breast cancer but typically do not cause Li-Fraumeni syndrome. Furthermore, the identification of missense variants in controls indicates that more caution in attributing pathogenicity to non-hotspot mutations is required. These data have substantial implications for the clinical management of *TP53* mutations.

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Utilization of and referral for genetic services (GS) by 455 unaffected individuals at risk for inherited predisposition to cancer: Pathways to improve identification of individuals at risk for hereditary cancer syndromes. *I. Shapira, R. Gralla, K. Sultan, L. Weiselberg, V. Vinciguerra, H. Raftopoulos, V. John, A. Menzin, J. D'Olimpio, C. Devoe, L. Donahue, D. Budman, S. Cohen.* Oncology, Department of Cancer Genetics, Monter Cancer Center, North Shore-LIJ, Hofstra Medical School, Lake Success, NY.

Background Family history (FH) directs referral of unaffected individuals to GS and is the main strategy to identify hereditary cancer syndromes. Objectives: 1) To determine the rate of deleterious mutations in high risk individuals, based on pedigree, and 2) to identify the origin of their referral. Methods Over a 5-year period (2004 -2009) we analyzed the source of referral for genetic testing and the mutations detected in 1193 consecutively tested individuals. Of 1193 patients tested, 455 (38%) were unaffected by cancer. Of 455 unaffected individuals 80% (364) had pedigrees consistent with hereditary cancer syndromes and 20% (91) had relatives with known deleterious mutations in cancer syndrome genes. Of unaffected carriers 70% (67) had single site testing for known familial mutations. Referring source was: self 113 (25%), GYN 172 (38%), surgeon 111 (25%), PCP 39 (9%) cases, GI 20 (3%). Results Of the 455 individuals at high risk 79 (17%) were carriers of deleterious mutations: 33 BRCA1, 37 BRCA 2, 1 had 2-copies MYH, 1 APC, 3 MLH-1, 3 MSH-2 and 1 MSH-6 mutations. Of the 79 patients with deleterious mutations 26 (33%) were identified by single site testing, 22 (28%) by the Ashkenazi M3-panel, 26 (33%) were identified by sequencing and 2 (6%) by gene rearrangement tests. TEST # of PTS (%) BRCA1 SS 29 (6) BRCA2 SS 29 (6) M3 ONLY 151 (33) M3+REFLEX 64 (14) M3+REFLEX+BART 9 (2) COMP BRCA1/2 143 (31) COMP BRCA1/2+BART 8 (2) SEQ (MLH1/MSH2/MSH6) 9 (2) MLH1/MSH2/MSH6 SS 7 (1.7) APC SEQ 6 (1.7) APC SS 2 (1) MYH SEQ 1 (0.6) TOTAL 455 Table 1: Single site (SS), Ashkenazi panel (M3), Reflex Comprehensive Testing if M3 negative (REFLEX), BRCA rearrangement test (BART), Sequencing (SEQ). Conclusions: Single site testing of a known mutation costs ~ 400$ and in 67 patients (15%) gave rapid information about cancer risk. Of these 39% (26) unaffected individuals were identified as carrying deleterious mutations and 61% (41) individuals found out their cancer risk was average. Primary care physicians identified only 9% of patients with high risk for hereditary cancer syndromes. Discussion: Although FH is the cornerstone of high-risk patient referral to GS, primary care physicians referred only 9% of all patients at risk. FH, public awareness of cancer risk and education will identify at risk populations for referral for GS.

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Using Breast Cancer Pathology to Improve Identification of BRCA1 Mutation Carriers. *H. Talbot[1], J. Dunlop[2], A. Ashfield[3], P. Quinlan[3], L. Jordan[4], C. Purdie[4], J. Berg[1,2].* 1) Human Genetics, Centre for Oncology and Molecular Medicine, University of Dundee, Dundee, United Kingdom; 2) East of Scotland Clinical Genetics Service, Ninewells Hospital, Dundee; 3) Surgery and Oncology, Centre for Oncology and Molecular Medicine, Division of Medical Sciences University of Dundee Ninewells Hospital DUNDEE; 4) Department of Pathology, Ninewells Hospital, Dundee.

Introduction. Mutations in the BRCA1 and BRCA2 genes cause a high lifetime risk of breast cancer. Identifying mutation carriers is important for effective treatment, allowing identification of individuals at very high breast cancer risk who qualify for increased screening or prophylactic surgery. Currently, selection for testing is based on family history, with testing available in the UK if the patient has a 20% likelihood of having a BRCA1 or BRCA2 mutation. However, some evidence suggests that using information about breast tumour pathology can improve the identification of patients likely to carry a BRCA1 mutation. This study aimed to confirm the relationship between breast tumour pathology and BRCA1 mutations, and to examine the effect of including pathological data within two BRCA risk prediction models. **Methods**. We reviewed breast tumour pathology in 53 patients: 19 with BRCA1 mutations, 19 with BRCA2 mutations and 15 with a strong family history, but where no BRCA mutation was identified (BRCA0). Evans scores and BOADICEA mutation likelihood scores were calculated for all patients, prior to, and following, the addition of tumour pathology. To incorporate tumour pathology into BOADICEA scores, we used a simple Bayes approach. **Results**. We confirmed that women with BRCA1 mutations were more likely to have oestrogen receptor negative ($p = 0.0096$), progesterone receptor negative ($p = 0.0234$) and triple negative ($p = 0.0023$) breast cancer than those with BRCA2 mutations. The addition of tumour pathology did not significantly affect the identification of patients for BRCA1 testing when using Evans scoring. Using BOADICEA without pathology data, 4 patients with BRCA1 mutations did not meet the 20% threshold for testing. When adding in tumour pathology data to BOADICEA, 3 of these patients reached the testing threshold for BRCA1 testing, but one further patient fell below the testing threshold. We conclude that tumour pathology is an important factor that should be considered when deciding whether to offer testing for a BRCA1 mutation.

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BRCA mutation prevalence and performance of clinical risk assessment tools among Puerto Rican breast cancer patients. *J. Dutil[1], J. Gonzalez-Bosquet[2], M. Echenique[3], J. Matta[4], R. Sutphen[5].* 1) Department of Biochemistry Ponce School of Medicine Ponce, PR; 2) Gynecologic Oncology, Department of Women's Oncology Moffitt Cancer & Research Center Tampa, FL; 3) Cancer Center, Auxilo Mutuo Hospital San Juan, PR; 4) Department of Pharmacology and Physiology Ponce School of Medicine Ponce, PR; 5) University of South Florida College of Medicine Tampa, FL.

Approximately 5-10% of all breast cancers occur due to the inheritance of deleterious mutations in highly penetrant predisposition genes, primarily *BRCA1* and *BRCA2*. Little clinical or research testing of the BRCA genes has been reported in Puerto Rico. Thus, there is a need to further characterize the prevalence and spectrum of BRCA mutations. In order to achieve meaningful clinical testing of *BRCA1* and *BRCA2* in Puerto Rico, it is also necessary to assess the applicability of clinically available risk assessment tools in this population. Using direct sequencing, we screened an unselected sample of 200 newly diagnosed breast cancer cases for the presence of mutations in the *BRCA1* and *BRCA2* genes. Personal and family history of cancer (including cancer types and ages of diagnosis) were collected and used to estimate pre-test likelihood of mutation using the BRCAPRO and Myriad II models. The results are expected to be useful toward 1) understanding the prevalence and spectrum of *BRCA1* and *BRCA2* mutations in Puerto Rico and 2) developing accurate risk assessment tools for the clinical management of hereditary breast cancer syndrome in this population.

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A novel XPD mutation in a compound heterozygote with mild sun sensitivity. *T. Falik-Zaccai[1,2], R. Erel-Segal[2], L. Horev[3], O. Bitterman Deutsch[4], K. Zohar[1], L. Kalfon[1], H. Slor[5], G. Spivak[6], P.C. Hanawalt[6].* 1) Div Med Gen, Western Galilee Hosp, Nahariya, Israel; 2) Rappaport Faculty of Medicine, Technion, Israeli Institute of Technology, Haifa, Israel; 3) Department of Dermatology, Hadassah Medical Center, Jerusalem Israel; 4) Department of Dermatology, Western Galilee Hospital, Naharia, Israel; 5) Department Molecular Human Genetics, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; 6) Biology Department, Stanford University, Stanford, CA, USA.

The XPD protein plays a pivotal role in basal transcription and in nucleotide excision repair (NER) as one of the ten known components of TFIIH. XPD is involved in maintaining the integrity of the TFIIH complex, and its ATP-dependent 5'-3' helicase activity is essential for NER. Mutations in XPD can result in the DNA repair-deficient diseases xeroderma pigmentosum (XP), Cockayne syndrome (CS), trichothiodystrophy (TTD), cerebro-oculo-facial-skeletal syndrome (COFS), and in combined phenotypes such as XP/CS and XP/TTD. We describe here a 19-year-old individual with very mild sun sensitivity, no neurological abnormalities and no tumors, who carries R683Q and R616Q mutations in the two XPD alleles respectively. Codon 683 variations have been documented for a majority of XPD patients; R683W is the most commonly found mutation. R683Q has been described in four patients with variable symptoms, from mild to severe. Codon 616 mutations are rare. R616P or R616W substitutions cause loss of function; heterozygous patients with these mutations exhibit symptoms of XP, COFS or TTD, determined by mutations in the other allele. The novel mutation R616Q might also result in a null protein, thus our patient's presentation is likely a consequence of the R683Q mutation in a hemizygous state. We have determined that cells from this individual are just as UVC-sensitive as those from an XPD R683Q homozygous patient. Recovery of RNA synthesis after UV treatment and removal of UV-induced cyclobutane pyrimidine dimers from the overall genomes are impaired, implying deficiencies in both transcription-coupled and in global genomic NER.

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A Common French Canadian FH Mutation: Implications for Mutation Screening in HLRCC and Fumarate Hydratase Deficiency. *G. Graham[1], N. Kanigsberg[2].* 1) Department of Genetics, Children's Hosp Eastern Ont, Ottawa, ON, Canada; 2) Division of Dermatology, Ottawa Hospital, Ottawa, ON, Canada.

Fumarase is a TCA cycle enzyme and recently recognized tumour suppressor encoded by the fumarate hydratase (*FH*) gene. The co-inheritance of two *FH* mutations produces autosomal recessive fumarate hydratase deficiency, a rare inborn error of metabolism associated with infantile encephalopathy and early lethality. In contrast, heterozygosity for *FH* mutations predisposes to cutaneous leiomyomas, uterine leiomyomas and renal cell carcinoma, the autosomal dominant condition known as Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC). Identical fumarate hydratase mutations can contribute to both phenotypes. There are 180 *FH* mutation entries in the Leiden Open Variation Database (LOVD). While the majority are missense, frameshift or nonsense mutations, splicesite mutations, deletions and duplications have also been documented. We report our experience with 9 individuals ascertained primarily because of cutaneous leiomyomata, all of whom were found to have *FH* mutations. Seven were of French Canadian origin and 6 of the 7 were heterozygous for c.1293delA. The mutation converts a glutamine to a lysine at residue 432, causes a frameshift and results in the insertion of a stop codon 17 amino acids downstream. This mutation has been reported once previously, also in a French Canadian individual, and we therefore conclude that it represents a common mutation, likely reflective of a founder effect in this population. We discuss the clinical features and family histories of our patients as well as the implications of this finding for mutation screening in individuals of French Canadian ancestry presenting with a personal or family history indicative of either condition. Our preliminary experience would suggest that the penetrance for renal cell cancer associated with this mutation is low, as appears to be typical for other *FH* mutations causing HLRCC. To date there are no prospective studies demonstrating the sensitivity, efficacy, risks or cost-effectiveness of screening for renal malignancy in HLRCC. Until we have an improved understanding of the additional factors predisposing to carcinogenesis in this condition and an ability to predict which mutation carriers will develop renal cancers, we have chosen to screen all carriers of this and other mutations by CT/MRI. Given that our experience with this condition includes aggressive malignancy in a 15-year-old, we are offering predictive testing to at-risk family members as young as 10 years of age.

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Selenium and the Risk of Cancer in BRCA1 Carriers. *J. Lubinski[1], K. Jaworska[1], K. Durda[1], A. Jakubowska[1], T. Huzarski[1], T. Byrski[1], M. Stawicka[2], J. Gronwald[1], B. Gorski[1], W. Wasowicz[3], E. Kilar[4], M. Szwiec[5], D. Surdyka[6], E. Marczyk[7], P. Sun[8], S.A. Narod[8].* 1) Intl Hereditary Cancer Ctr, Pomeranian Med Univ, Szczecin, Poland; 2) Prophylactic and Epidemiology Center, Poznan, Poland; 3) Institute of Occupational Medicine, Lodz, Poland; 4) Regional Oncology Hospital, Swidnica, Poland; 5) Regional Oncology Hospital, Opole, Poznan; 6) Center of Oncology of Lublin Region, Lublin, Poland; 7) Regional Oncology Center, Krakow, Poland; 8) Womens College Research Institute and Dalla Lana School of Public Healt, University of Toronto, Canada.

It has not been established if dietary factors or nutritional supplements impact on the incidence of cancer in high-risk women. We randomised 1135 women with a BRCA1 mutation to 250 micrograms daily of elemental selenium as sodium selenite, or to placebo, in a double-blind trial. After a median follow-up period of 35 months (range 6 to 62 months), there were 60 incident cases of cancer diagnosed in the selenium-supplemented group, versus 45 cases in the placebo group (hazard ratio 1.4; 95% CI: 0.9 to 2.0). Selenium supplementation was not associated with a reduction in the risk of primary breast cancer (hazard ratio 1.3; 95% CI: 0.7 to 2.5), of contralateral breast cancer (hazard ratio 1.5; 95% CI: 0.7 to 3.2). or of ovarian cancer (hazard ratio 1.3; 95% CI: 0.6 to 2.7). The results of this study do not support the recommendation that selenium supplementation should be offered to BRCA1 carriers for chemoprevention. Part II Adnexectomy, genotypes and selenium level as markers of the risk of cancer In these part we conducted a nested case-control study of 68 women with breast cancer and 17 women with ovarian cancer and 170 controls matched 1 to 2. Cases and controls were matched for age at enrolment, past history of breast cancer, oophorectomy and whether they received selenium supplement or placebo during cancer chemoprevention trial. Combinations of clinical status, genotypes and selenium levels strongly associated with extremely low risk of cancer have been identified. The strongest associations have been found for GPX4 variants: a. all nTT and Se level 60-80μg/l - OR 0.32, p: 0.0009; b. all TT and Se level >80μg/l - OR 0.10, p: 0.047; c. for carriers without adnexectomy and with TT variant, Se level >80μg/l - OR 0.038, p: 0.014.

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A novel missense mutation in the PTEN gene in a patient with multiple melanoma and features of Cowden Syndrome. *L.M. PRADELLA[1], E. MARIANI[1], G. GASPARRE[1], L. AMATO[1], S. MICCOLI[1], A. LANZONI[2], C. ISHIOKA[3], K. SAIJO[3], G. ROSSI[4], G. ROMEO[1], D. TURCHETTI[1].* 1) Laboratory of Medical Genetics, Bologna, BO, Italy; 2) UO Dermatologia, Ospedale Bellaria, BO, ITALY; 3) Department of Clinical Oncology Institute of Development, Aging and Cancer Tohoku University 4-1 Seiryo-machi, Aoba-ku Sendai 980-8575, JAPAN; 4) UO Anatomia Patologica, Policlinico di Modena, ITALY.

A 38-years-old male patient was referred to the Cancer Genetics Clinic because of a personal history of multiple melanomas and moles. Indeed, since he was 26 he had more than 60 pigmented skin lesions removed, most of which were melanomas and melanocytic nevi at pathology examination. His history was also remarkable for subtotal thyroidectomy for follicular adenoma and hyperplasia at age 19, followed, 18 years later, by the removal of the remaining thyroid tissue, containing a follicular carcinoma. During the first thyroid surgery a large supraclavicular lipoma was also removed. Clinical examination revealed macrocephaly (>97th percentile), and palmoplantar keratoses. Molecular analysis of the CDKN2A gene failed to find mutations, but the analysis of the PTEN gene revealed the heterozygous substitution G1027A (V343M). The healthy parents and the sister of the patient do not carry the mutation. To our knowledge, this variant has not been reported before, but different substitutions of the same aminoacid (V343E; V343I) are known to be pathogenic. In silico prediction was performed using Polyphen and SIFT softwares: Polyphen predicted this variant to be probably benign while SIFT suggested it could affect protein function. To allow functional studies, PTEN complete cDNA was cloned on an expression vector under the control of a CMV promoter and the V343M substitution was obtained by site-directed mutagenesis. This construct will be used to evaluate the ability of the mutant protein to rescue a sick phenotype on a yeast model. Although melanoma has been occasionally reported in Cowden Syndrome patients, the phenotype of this patient, consistent with atypical mole malignant melanoma syndrome, is unexpected in a PTEN mutation carrier. If the functional assay showed an effect on PTEN function, further studies would be warranted to clarify the genotype-phenotype correlation.

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Double Heterozygosity for BRCA2 and PTEN Germline Mutations in Two Unrelated Probands. *C.G. Somerman, B.M. Boman, B.L. Grey, M.A. Parker, Z.J. Ali-Khan Catts.* Helen F. Graham Cancer Center, Christiana Care Health System, Newark, DE.

BRCA1 and BRCA2 are the most common genes associated with Hereditary Breast and Ovarian Cancer (HBOC). Germline mutations in BRCA1/2 are associated with an increased risk for the breast (female and male), ovarian, prostate and pancreatic cancer. Cowden syndrome (CS) is less common but also associated with hereditary breast cancer and is caused by mutations in the PTEN gene. CS is a hamartomatous tumor syndrome characterized by benign and malignant tumors of the breast, thyroid and uterus and non-cancerous lesions such as mucocutaneous trichilemmomas, acral and palmoplantar keratosis and papillomatous papules. Double heterozygosity is when an individual has two different gene mutations at two separate genetic loci. An extensive literature search using PubMed, Ovid and Google has revealed double heterozygosity for BRCA1 and BRCA2 mutations as well as with Lynch syndrome and HBOC but typically genetic testing for cancer syndromes stops when one mutation is identified. Here we report two unrelated probands that are double heterozygotes for BRCA2 and PTEN germline mutations which has not been previously reported. Proband A was diagnosed with a triple negative invasive ductal carcinoma at age 47, and contralateral fibrocystic changes and ductal hyperplasia. Her family history is significant for ovarian, colon and lung cancer, fibrocystic breast, endometrial fibroids and mental retardation. Proband B was diagnosed with an Estrogen and Progesterone receptor negative invasive ductal carcinoma at age 38, fibrocystic breast, multinodular goiter, 5 adenomatous colon polyps and macrocephaly. Her family history is significant for prostate, breast, brain, colon and lung cancer. Both probands met the diagnostic criteria for HBOC and underwent BRCA1/2 sequencing at a CLIA approved lab and BRCA2 deleterious mutations were identified. In addition, proband A and B met research eligibility criteria for PTEN testing and were found to have deleterious PTEN alterations. By taking a detailed history and elucidating HBOC and CS characteristics these two probands were offered both tests. The alterations in BRCA2 accounted for the history of breast, ovarian and prostate cancer however it did not account for the additional histories. CS does account for some of the additional benign findings. This illustrates the importance of obtaining a detailed personal and family history and exploring all possible genetic syndromes even in the presence of a known deleterious mutation.

500/T

The IMPACT study: Identification of Men with a genetic predisposition to Prostate Cancer: Targeted screening in BRCA1/2 mutations carriers and controls. Preliminary Results. *E.K. Bancroft[1,2], E. Castro[1], E.C. Page[1], R.A. Eeles[1,2], IMPACT Steering Committee and Collaborators.* 1) Institute of Cancer Research, Sutton, United Kingdom; 2) Cancer Gen Unit, Royal Marsden Hosp, Sutton, United Kingdom.

Background Men with a mutation in BRCA1 or BRCA2 genes have an increased risk of prostate cancer (PCa) by age 70 (3-5% and 20-23%, respectively). But the real incidence and prevalence of PCa in this population remains unknown and the accuracy and effects of targeted screening are still unclear. Objectives To establish an international PCa targeted screening study in BRCA1/2 mutation carriers in order to determine the sensitivity and specificity of PSA screening in this population. Methods Eligible men are 40 to 69 years old tested for known BRCA1/2 family mutation. Those who do not carry the mutation are eligible as controls. The estimated target population are 500 BRCA1, 350 and 850 controls. PSA is determined annually and the cut-off for biopsy is 3ng/dl. Results To date, 914 men have been recruited in 35 centres (282 controls, 330 BRCA1 and 292 BRCA2). Median age is 53 (40-69). Median PSA value is 0.9, with no differences among the three groups (Controls: 0.81 (0.07-7.4), BRCA1 0.9 (0.1-27); BRCA2 0.9 (0.2-14.7)) 75 men (8.2%) presented with a PSA >3ng/dl (21control, 20 BRCA1, 34 BRCA2). 56 biopsies have been performed diagnosing 23 tumors (5 controls, 5 BRCA1, 13 BRCA2). 85% of tumors diagnosed in BRCA2 were Gleason ≥7 and /or T2 and required radical treatment while in controls 50% were indolent. The estimated Positive Predictive Value (PPV) for PSA screening in BRCA2 is 43.3% vs 32% in controls. Conclusions IMPACT is the largest prospective study to evaluat PCa screening in BRCA carriers. Our data confirms that BRCA2 carries usually present with aggressive PCa and annual PSA determination with a cut-off to biopsy of 3 ng/ml is an accurate method for PCa screening in this population.

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Association of Paternal Genotype with Risk of New Mutation in RB1 gene in an Offspring. *A. Ganguly[1], E. Chao[1], S. Francisci[2,3], G. Bunin[4], C. Shields[5], M. Devoto[2].* 1) Department of Genetics, University of Pennsylvania, Philadelphia, PA; 2) Division of Human Genetics, CHOP, Depts. of Pediatrics and of Biostatistics and Epidemiology, University of Pennsylvania; 3) National Center for Epidemiology, Surveillance and Health Promotion, Istituto Superiore di Sanità, Rome, Italy; 4) Division of Oncology, Children's Hospital of Philadelphia, Philadelphia; 5) Wills Eye Institute, Thomas Jefferson University, Philadelphia.

Retinoblastoma (RB) is a malignant neoplasm of the retina, which occurs in infants and young children. This tumor is universally associated with mutation of the RB1 gene. In sporadic, bilateral RB, 94% of a new germline RB1 mutation occurs prior to conception on the paternal gamete. We hypothesize that paternal genotype contributes to risk of fathering a child with a de novo RB1 germline mutation.

To study association of common sequence variants, SNPs, with RB risk, we analyzed genomic DNA from fathers of children with de novo, bilateral RB. These individuals were recruited in Wills Eye Hospital, Philadelphia. We used an array-based approach (Affymetrix, SNP 6.0) to genotype more than 1 million loci in the cases and approximately 1100 ethnically and gender-matched controls.

Following genotyping, careful data cleaning was carried out checking for gender discrepancies, identity by descent (IBDs) to identify related individuals or duplicate samples, and sample heterozygosity to identify possible sample contamination. To ensure genotyping quality we excluded from the analysis individuals that had more than 10% of missing calls. SNPs that were not in Hardy-Weinberg equilibrium in the controls ($p £ 10^{-5}$), that failed genotyping for more than 10% of individuals, or with minor allele frequency (MAF) lower than 1%, were excluded from analysis. Association analyses were carried out with the 1 degree-of-freedom trend test on allele counts, using principal component analysis (PCA) to correct for potential sample stratification including individuals that disagreed with self-reported ethnicity. The final analysis included 95 cases. Genome-wide thresholds were used to identify 3 SNPs as significantly associated with the risk of a new mutation in RB1 gene in an offspring.

A single coding sequence SNP in a drug-metabolizing enzyme achieved genome wide significance with p-value of $8x10^{-13}$. The expression of this gene can vary twenty fold between individuals and is likely to have significant effects on the rate at which xenobiotics are metabolized. Further analysis of this gene and other results will be presented.

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Population Admixture Analysis of Hispanic Women at risk for *BRCA1* or *BRCA2* Mutations in High Risk Clinic Populations. *G. Larson[1], G. Rivas[1], J. Herzog[2], R. Ogaz[2], M.B. Terry[3], E. Ziv[4], S. Narod[5], J. Weitzel[2].* 1) Dept. of Molecular Medicine, Beckman Research Institute, City Hope, Duarte, CA; 2) Dept. of Clinical Cancer Genetics Beckman Research Institute City Hope, Duarte, CA; 3) Department of Epidemiology, Columbia University; 4) Department of Medicine, Institute for Human Genetics, University of California; 5) Women's College Research Institute, University of Toronto.

We have sought to characterize the ancestral origins of a variety of *BRCA1* and *BRCA2* mutations from high risk Hispanic women. Our prior work (Weitzel, et al CEBP 14:1666, 2005) has demonstrated conserved haplotypes through the *BRCA1* interval for several recurrent mutations from high-risk Hispanic patients, some exclusive to families with Mexican American or Spanish ancestry. To better define the ancestral origins of our patients in conjunction with the spectrum of *BRCA* mutations observed in our high-risk clinics, we have employed a set of Ancestry Informative Markers (AIMs) in conjunction with reference individuals from the Human Gene Diversity Panel (HGDP) to stratify breast cancer patients with Caribbean/African versus AmerIndian admixture. These data can be cross-validated with self-reported ancestry (country of birth, and city-when available). A set of ~ 100-125 AIMs can be used to differentiate these diverse populations with a high degree of confidence. This information will be used to identify germline *BRCA* mutations common to specific ancestral backgrounds, and to distinguish distinct subset populations that are included in the Hispanic category.

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Linkage study of prostate cancer in high-risk African American families from Louisiana. *E.M. Ledet[1], J.E. Bailey-Wilson[2], D.M. Mandal[1].* 1) Dept Genetics, Louisiana State Univ HSC, New Orleans, LA; 2) NHGRI, NIH, Baltimore, MD.

Prostate cancer is a complex multi-allelic disease and the most common malignancy in men throughout the world. In the United States, a lifetime risk of mortality from prostate cancer is 3% for white men and 4% for African-American men. Thus far, disease susceptibility loci have been identified for this cancer but definite locus-specific information is not established due to the tremendous amount of genetic heterogeneity. Previously, we performed a genome-wide linkage scan on three families using microsatellite markers and identified a region on chromosome 22q13. Given the small sample size of three families, this lod-score value does not reach the genome wide significance level. However, we have since continued recruitment and accrued 20 large high-risk African-American families with at least 3 affected individuals; additionally, 28 large high-risk Caucasian families have been recruited. Demographic information and relevant clinical information has been documented from the hospital pathological report on the affected. Recently an Infinium II HumanLinkage-12 panel (Illumina, Inc.) with 6,090 SNP markers was performed on 180 DNA samples, including 15 African American families and 4 Caucasian families. This panel is optimized for linkage detection for both monogenic and polygenic disorders; SNPs are distributed on every chromosome with an average gap of 441 Kb and 0.58 cM. Three samples were discarded from analysis due to poor array performance, for a total of 177 samples imported into BeadStudio version 3.3.7; a proprietary calling algorithm was used and SNPs which failed quality control or calling were removed from the data set. Quality control and pruning of released SNPs was performed using PLINK. Linkage analysis is ongoing on the African-American cohort with Merlin and Genehunter-Plus. In this study we intend to identify any markers associated with prostate cancer in high-risk African American families and document any correlation between clinical features, such as prostate specific antigen (PSA), 'age of onset' and/or Gleason score.

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Implications of germline HVR-II mitochondrial polymorphisms on Tunisian women affected with breast cancer. *B. Yacoubi Loueslati[1], W. Troudi[2], L. Cherni[2], K. Ben Rhomdhane[3], L. Mota-Vieira[4,5].* 1) Department of Biology, Faculty of Sciences of Tunis, ElManar University, Tunisia; 2) Laboratory of Genetics, Immunology and Human Pathology. Faculty of Sciences of Tunis, ElManar University, Tunisia; 3) Salah Azeiz Oncology Institute, Bab Saadoun, Tunisia; 4) Molecular Genetics and Pathology Unit, Hospital of Divino Espírito Santo of Ponta Delgada, EPE, Azores, Portugal; 5) Instituto Gulbenkian de Ciência, Oeiras, Portugal.

A high incidence of somatic mitochondrial DNA (mtDNA) polymorphisms has been recently reported in a wide variety of human cancers, with some of them proposed as potential markers for the early detection of breast cancer (BC). Nevertheless, little attention has been paid to the potential use of germline mitochondrial sequence variations as genetic risk factors for cancer. To evaluate the germline polymorphism of mitochondrial HVR-II region as genetic risk factor for BC, we performed a case-control study of 70 unrelated Tunisian BC women and 80 healthy age and sex matched blood donors, taking into account their menopausal status. Direct sequencing allowed us to detect a total of 351 polymorphisms for controls, and 248 variants for patients with 47 and 39 segregating sites, respectively. In both groups, more than 50% of the polymorphisms are due to four variants: 315 ins C, 309 ins C, 263 A>G, and 73 A>G. The HVR-II sequences are also classified into haplotypes on the basis of all polymorphisms. A total of 59 different haplotypes were found, twenty of them shared between patients and controls. Both groups had specific haplotypes - 18 in cases, and 21 in controls. Statistical analysis revealed a weak protective effect on BC risk for two mitochondrial polymorphisms - 152 T>C (OR = 0.33, 95% CI 0.12 0.91) and 263 A>G (OR = 0.17, 95% CI 0.06 0.47). In contrast, an increased risk of the occurrence of BC was detected, with the 315+C haplotype (OR = 11.66, 95% CI 1.44 252.23). Although more extensive studies, including different types of cancer and patients with different genetic makeup, are required, in order to improve the understanding of germline mtDNA polymorphisms in carcinogenesis, here we provide evidences that mitochondrial variants may interact to modify the risk for BC.

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Joint effects of germ-line TP53 mutation, MDM2 SNP309, and gender on cancer risk in family studies of Li-Fraumeni Syndrome. *C.C. Wu[1], R. Krahe[2], G. Lozano[2], B. Zhang[2], C.D. Wilson[2], E.J. Jo[3], S. Shete[1], C.I. Amos[1], L.C. Strong[2].* 1) Dept Epidemiology, Univ Texas MD Anderson CA Ctr, Houston, TX; 2) Dept Genetics, Univ Texas MD Anderson CA Ctr, Houston, TX; 3) Duncan Cancer Center, Baylor College of Medicine, Houston, TX.

Li-Fraumeni syndrome (LFS) is a rare familial cancer syndrome characterized by early cancer onset, diverse tumor types, and multiple primary tumors. Germ-line TP53 mutations have been identified in most LFS families. A high-frequency single-nucleotide polymorphism, SNP309 (rs2279744), in MDM2 was recently confirmed to be a modifier of cancer risk in several case studies: substantially earlier cancer onset was observed in SNP309 G-allele carriers than in wild-type individuals by 7-16 years. However, cancer risk analyses that jointly account for measured hereditary TP53 mutations and MDM2 SNP309 have not been evaluated in familial cases. Here, we designed a study to assess the associations of cancer incidence with germline TP53 mutation, MDM2 SNP309, gender, and their interactions in family studies and to estimate the cancer risk attributable to these factors simultaneously. A study population that comprised 19 extended LFS pedigrees ascertained with multiple germ-line TP53 mutations provides a unique opportunity to perform this cancer risk analysis in a quantitative way. We extended the novel statistical method in family studies that we recently developed that is structured for age-specific risk models based on Cox proportional hazards regression for censored age-of-onset traits. The dataset consisted of 463 individuals with 129 TP53 mutation carriers. Our analyses showed that the TP53 germ-line mutation and its interaction with gender were strongly associated with familial cancer incidence and that the association between MDM2 SNP309 and increased cancer risk was modest. In contrast with several case studies, the interaction between MDM2 SNP309 and TP53 mutation was not statistically significant. Our results showed that heightened MDM2 levels due to the SNP309 G-allele were associated with accelerated tumor formation in both carriers and non-carriers of germ-line p53 mutations.

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Fine-mapping epithelial ovarian cancer susceptibility loci at 8q24, 17q21 and 19q13. *H. Song[1], J. Tyrer[1], S. Ramus[2], G. Chenevix-Trench[3], M. Rossing[4], R. Ness[5], S. Kjaer[6], M. Garcia-Closas[7], J. Gronwald[8], P. Fasching[9], A. Whittemore[10], C. Pearce[11], S. Gayther[2], P. Pharoah[1], Ovarian Cancer Association Consortium (OCAC).* 1) Dept Oncology, Univ Cambridge, Cambridge, United Kingdom; 2) Department of Gynaecological Oncology, UCL EGA Institute for Women's Health, University College London, UK; 3) The Queensland Institute of Medical Research, Post Office Royal Brisbane Hospital, Australia; 4) Program in Epidemiology, Fred Hutchinson Cancer Research Center, Seattle, WA, USA; 5) The University of Texas, School of Public Health, Houston, TX, USA; 6) Department of Viruses, Hormones and Cancer, Institute of Cancer Epidemiology, Danish Cancer Society, Copenhagen, Denmark and Gynecologic Clinic, Rigshospitalet, University of Copenhagen, Denmark; 7) Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, Maryland, USA; 8) International Hereditary Cancer Center, Department Of Genetics and Pathology, Pomeranian Medical University, Szczecin, Poland; 9) University Hospital Erlangen, Department of Gynecology and Obstetrics, Germany; 10) Department of Health Research and Policy, Stanford University School of Medicine, Stanford, USA; 11) University of Southern California, Keck School of Medicine, Los Angeles, California, USA.

Background: A recent genome-wide association study (GWAS) has identified six epithelial ovarian cancer (EOC) susceptibility loci. The aim of this study was to use data from the 1000 genome project to fine map three of the EOC susceptibility loci at 8q24, 17q21 and 19q13 and to identify the potential causal variants for functional study. **Methods:** We narrowed the susceptibility locus to a region bounded by SNPs with r^2 of 0.1 with the top hit at each locus. We imputed genotypes for 5930 cases and 7164 controls for all the common SNPs within the region using data from our GWAS and 1000 genome project. Genotype frequencies in cases and controls were compared using a likelihood ratio test in a logistic regression model. The SNPs were ranked by test statistic. SNP with log likelihood within 6.9 of the top hit (odds 1000:1) were considered as potential functional variants. A set of tagSNPs were selected to tag the candidate causal variants. These were genotyped in 4681 invasive ovarian cancer cases and 5061 controls from 8 studies (from Australia, Europe and USA). **Results:** A total of 32, 19 and 26 tagSNPs were selected for genotyping for 8q24, 17q21 and 19q13 locus respectively. Among these 2 failed for 8q24 locus and 6 each failed for 17q21 and 19q13 locus respectively. The most strongly associated SNP at 8q24 was rs7814937 ($P = 1.66 \times 10^{-8}$), but 17 other SNPs has a log likelihood within 4.6 of rs7814937 (odds 100:1) including the top hits from the initial GWAS (rs1008821 and rs1516982). Similarly 4 SNPs at 17q21 and 15 SNPs at 19q13 remained plausible functional candidates. **Conclusions:** We have identified potential causal variants for ovarian cancer susceptibility at 8q24, 17q21 and 19q13. Studies of EOC in other ethnic groups may refine these further. Functional studies will be needed to determine causal mechanisms.

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Genetic Variants in Inflammation Pathway Genes Modulate Prostate Cancer Risk. *E.M. Kwon[1,2], C.A. Salinas[3,4], Z. Feng[3], S. Kolb[3], J.L. Stanford[3,4], E.A. Ostrander[1].* 1) Cancer Genetics Branch, National Human Genome Research Institute, NIH, Bethesda, MD; 2) Program in Human Genetics, Johns Hopkins University School of Medicine, Baltimore, MD; 3) Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 4) Dept. of Epidemiology, School of Public Health, University of Washington, Seattle, WA.

Chronic inflammation is an important mechanism for the development and progression of several types of cancer including that of the prostate. Genetic variants within the inflammation pathway have been previously associated with prostate cancer (PC) risk, but results have not been consistent. To further elucidate this mechanism in relation to PC, we selected 17 genes in the inflammation pathway and evaluated variants within each gene in relation to PC risk. A total of 144 tagging and amino acid altering single nucleotide polymorphisms (SNPs) were genotyped in Caucasian and African American men participating in one of two population-based, case-control studies (n = 1,458 cases; 1,350 controls). Eleven SNPs in seven genes (*CXCL12, IL4, IL6, IL6ST, PTGS2, STAT3*, and *TNF*) demonstrated a statistically significant association with overall PC risk in Caucasians. Four SNPs in genes *IL4, IL6ST, PTGS2,* and *STAT3* were independently associated after adjusting for covariates. The most significant result was with rs11574783 in the *IL6ST* gene where we observed a nearly 11-fold decrease in risk (OR=0.09, 95% CI 0.01-0.70). Cumulatively, these four SNPs conferred a three-fold elevation in PC risk among men carrying the maximum number of high-risk genotypes (OR=2.97, 95% CI 1.41-6.25). Risk estimates for seven SNPs (one each in *CXCL12, STAT3, VEGF*, and *IL6*, and three in *PIK3R1*), varied significantly according to disease aggressiveness ($p_{homogeneity}<0.05$). SNPs in *AKT1, PIK3R1* and *STAT3* were independently associated with more aggressive PC, demonstrating a 5-fold (95% CI 2.29-11.40) greater risk among carriers of all high-risk genotypes. In summary, our results suggest that SNPs in four inflammation-pathway related genes (*IL4, IL6ST, PTGS2,* and *STAT3*) are independently associated with PC susceptibility with three additional SNPs (in *AKT1, PIK3R1*, and *STAT3*) being independently associated with more aggressive PC. Furthermore, novel associations between PC risk and genetic variants were presented for *IL6ST, STAT4, AKT1*, and *PIK3R1* genes known to play a key role in inflammation. Thus, our results provide evidence to support the hypothesis that chronic inflammation contributes to the development and modulation of PC risk.

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Shared Functional Modules Detection in Ovarian Cancer. *S. Hong[1], H. Dong[1], L. Jin[1], M. Xiong[1,2].* 1) Fudan University, Shanghai, Shanghai, China; 2) Human Genetics Center, University of Texas School of Public Health, Houston, TX 77030.

Gene co-expression network and functional modules can reveal the potential gene-gene interaction relationships and thus provide a systematic view of gene regulation through a biological process such as tumor genesis, tumor metastasis. To study the gene-regulation pattern of ovarian cancer and understand the mechanism of it, we used two independent ovarian cancer datasets to construct the gene co-expression network and find the common patterns of them. One dataset was obtained from The Cancer Genome Atlas(TCGA) and the other was from Array Express. To estimate the partial correlation matrix of the network, a joint sparse regression model was used. For the reason that genes are usually organized into smaller groups to execute their specific functions as known pathways, we further detected modules from the constructed co-expression network using the dynamic tree cut procedure. We collected several hundreds of biologically meaningful pathways from KEGG and Biocarta and then used Fishers' exact test to look for modules which were significantly enriched with known pathways and also enriched with differential expressed genes. On the other side, we calculated the damage value and vertex degree to find the important genes. The miRNA target analysis was also implemented to search for the important genes using the miRNA data of the TCGA datasets. Our results showed that the two datasets had several common functional modules which were enriched with differential expressed genes as well as biological known pathways. These modules were mainly enriched with Cell Apoptosis, inflammation factor-related pathway, Immune Disorders. These shared pathways and modules may play an important role in ovarian cancer.

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A bladder and gastric cancer risk variant of *PSCA* is associated with increased mRNA expression and allelic expression imbalance in bladder tissue. *I. Kohaar, A. Mumy, P. Porter-Gill, L. Prokunina-Olsson.* Laboratory of Translational Genomics, NCI, NIH, Gaithersberg, MD.

A single nucleotide polymorphism (SNP) rs2294008 within the prostate stem cell antigen gene (PSCA) has been identified as a major susceptibility factor for urinary bladder cancer and poorly differentiated diffuse-type gastric cancer. The risk allele T of rs2294008 creates an alternative start codon that extends the signal peptide by nine amino acids. Strong overexpression of PSCA in bladder and prostate tumors compared to normal samples has been previously reported, however, it's unclear how this overexpression is related to the genetic risk variant. Here, we performed quantitative mRNA expression analysis in 39 normal and 42 tumor bladder samples, including 24 matched normal-tumor pairs. We show that mRNA expression of PSCA was significantly increased in tumors compared to matched normal bladder tissue by 5.7-fold (p=0.0060). We observed a 13.8-fold increase in expression in carriers of risk TT genotype (p=0.0080, n=8), compared to a 1.8-fold increase in carriers of non-risk CC genotype (p=0.66, n=7). Since rs2294008 is located within the transcribed sequence (5'UTR or exon 1 of PSCA), we performed an allelic expression imbalance (AEI) analysis in DNA and cDNA of 20 normal and 13 tumor samples heterozygous for rs2294008. The allelic ratio was similar in DNA of normal and tumor samples (60% and 59% of risk allele T, p=0.47) while it was different in cDNA of the same samples (55 % and 72% of risk allele T in normal and tumor samples, p= 0.0020). No AEI was observed in cDNA of normal samples compared to DNA (60 and 55% of risk allele T, p=0.19), while in cDNA from tumors there was a 2-fold increase in expression of risk allele T (p=0.039) while a 5-fold decrease in expression of non-risk allele C (p=0.0062), resulting in 72% of allele T in cDNA compared to 59% in DNA. In conclusion, we show that the risk allele T of rs2294008 is associated with increased mRNA expression of PSCA. The exact mechanism underlying this imbalance and its functional effect for cancer remains to be elucidated.

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Somatic mutations in GRM1 promote tumorigenesis. *M.D. Willard, M.L. Lajiness, I.H. Wulur, T.D. Barber.* Translational Science, Eli Lilly and Company, Indianapolis, IN.

Emerging data indicates that G protein-coupled receptors (GPCRs) have a critical but often under-appreciated role in cancer. Specifically, accumulating evidence suggests that modulation of GPCR function may promote cancer progression and metastasis. Metabotropic glutamate receptor type 1 (GRM1) and its role in physiological processes in the nervous system has been well-documented. Recently, the role of GRM1 in cancer progression has emerged. Somatic mutations altering the coding region of GRM1 have been detected in colorectal cancer and glioblastomas, but the functional significance of these mutations is unknown. Here we demonstrate that cancer-associated mutations of GRM1 lead to abnormal receptor localization, interactions, and activity, and consequently, abnormal cellular behavior. These findings suggest that somatic mutations in GRM1 promote tumorigenesis through deregulated receptor activity, and highlight the importance of evaluating GRM1 antagonists to block both the normal and mutant forms of the receptor.

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The GWAS-identified prostate cancer associated SNP, rs10993994, regulates microseminoprotein-beta (MSMB). *X. Xu[1], H. Lilja[2,3], R.J. Klein[1].* 1) Program in Cancer Biology and Genetics, Memorial Sloan-Kettering Cancer Center, New York, NY 10065, USA; 2) Department of Clinical Laboratories, Surgery (Urology) and Medicine (GU-Oncology), Memorial Sloan-Kettering Cancer Center, New York, NY 10065, USA; 3) Department of Laboratory Medicine, Lund University, Skåne University Hospital, 205 02 Malmö, Sweden.

Background: Recent genome wide association screens have identified a single nucleotide polymorphism (SNP), rs10993994, as a locus that confers genetic predisposition to prostate cancer. This SNP is located in the proximal promoter of micro-seminoprotein beta (*MSMB*). *MSMB* codes for one of major secretory products of the prostate, β-MSP/PSP94. Though the function of β-MSP is unknown, the protein and a peptide derivative have properties suggestive of tumor suppressors. We have previously shown that rs10993994 genotype is strongly correlated with physiological β-MSP levels in the serum and semen of young healthy men.

Objectives: We hypothesize that rs10993994 is the functional variant responsible for the prostate cancer association signal, and acts through reducing *MSMB* levels, a putative tumor suppressor. Consequently, our objective is to elucidate the regulatory mechanisms underlying rs10993994's influence on *MSMB*/β-MSP levels.

Results: Through promoter reporter activity analysis, we have determined that single nucleotide difference at rs10993994 causes a reduction in *in vitro* transcriptional activity. rs10993994 is located in a predicted CREB binding site; to determine if differential transcription factor binding is responsible for reduced transcription, we performed chromatin immunoprecipitation in the presence and absence of forskolin and found that the risk allele for prostate cancer appears to indeed abrogate a CREB binding site.

Conclusions: Our results indicate that rs10993994, a prostate cancer associated SNP identified from recent GWAS studies, is the functional variant responsible for the association signal and acts through reducing levels of β-MSP, a putative tumor suppressor.

One criticism of genome wide association studies has been the lack of functional follow-up to association signals. Our preliminary findings suggest a compelling possible causal pathway, in rs10993994's association with β-MSP levels and subsequent prostate cancer development. Our current studies include investigating the role of β-MSP in prostate cancer etiology.

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Functional glutathione peroxidase 3 polymorphisms associated with increased risk of taiwanese patients with gastric cancer. *I. Yang[1], J.Y. Wang[1,2,3,4], D.C. Wu[5,6], S.W. Huang[1,2], J.Y. Wu[5,6], S.H. Juo[1,7].* 1) Department of Medical Genetics, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; 2) Graduate Institute of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; 3) Department of Surgery, Faculty of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; 4) Cancer Center, and Division of Gastroenterology and General Surgery, Department of Surgery, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan; 5) Division of Gastroenterology, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan; 6) Department of Internal Medicine, Faculty of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; 7) Cancer Center and Department of Medical Research, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan.

Background: Glutathione peroxidase 3 (GPX3) can enhance an antioxidant's capacity and reduce genomic damage caused by oxidants and thus influence tumorigenesis. Hence, we investigated the role of GPX3 as a risk of gastric cancer. Methods: We first conducted a case-control study to test for the association between five tagging single nucleotide polymorphisms (SNPs) of GPX3 and the risk of gastric cancer in Chinese. Multivariate logistic regression analysis was performed to estimate the genetic effect with adjustments for age and sex. Functional studies were performed by using the luciferase reporter assay to assess functional consequences of the significant SNPs. Results: Among five SNPs (rs3763013, rs8177412, rs3805435, rs3828599, and rs2070593) genotyped in 227 cases and 844 controls, three SNPs were significant: intronic SNP rs3805435 (OR = 0.70, P = 0.037), intronic SNP 3828599 (OR = 0.68, P = 0.025), and 3' UTR SNP rs2070593 (OR = 0.48, P = 0.001). The two intronic SNPs rs3805435 and SNP rs3828599 were in linkage disequilibrium (D'= 0.91). Conclusions: The reporter assays showed significant difference in the luciferase expression between protective and risk alleles of two intronic SNPs (P = 0.004), whereas the 3'UTR SNP did not influence the luciferase expression. The present study suggests that the intronic SNPs at GPX3 can influence gene expression leading to an alteration of gastric cancer risk.

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Functional analyses of the effect on RNA splicing of unclassified sequence variants found in cancer susceptibility genes. *P. Gaildrat[1], A. Martins[1], S. Krieger[2], J.C. Théry[1], F. Révillon[3], A. Killian[1], E. Bohers[1], S. Baert-Desurmont[1], J. Tinat[1], A. Rousselin[2], P. Berthet[4], A. Chevrier[5], M.P. Buisine[6], T. Frébourg[1,5], A. Hardouin[2], M. Tosi[1].* 1) Inserm U614, Faculty of Medicine, Rouen, France; 2) Laboratoire de Biologie Clinique et Oncologique, Centre François Baclesse, Caen, France; 3) Laboratoire d'Oncologie moléculaire humaine, Centre Oscar Lambret, Lille, France; 4) Consultation d'Oncogénétique, Centre François Baclesse, Caen, France; 5) Unité de Génétique Clinique, CHU de Rouen, France; 6) Laboratoire d'oncogénétique et génétique moléculaire, CHRU de Lille, France.

The interpretation of the large numbers of variants of unknown biological and clinical significance found in genetic screenings represents a major challenge in medical genetics, and especially in oncogenetics. A large fraction of such unclassified variants (UVs) could influence mRNA splicing. Current efforts aimed at predicting the pathogenicity of germline sequence variants must include analyses of their effect on RNA processing and stability, not only for intronic variants, but also for those found in exons. Considering that patient RNA is not always available, we have used functional splicing assays based on hybrid minigenes transfected into cells in culture, to screen UVs of the mismatch repair genes involved in the Lynch syndrome. In a total of 153 UVs of MLH1 and MSH2 we found that 30 UVs (19.6%) induced a major splicing defect that was subsequently confirmed at the patient RNA level. Notably, among 62 variants encoding missense substitutions of MLH1 nine (14.4%) induced a major splicing defect, indicating that this type of functional screening should take place prior to testing for changes of protein function. We have previously studied 20 UVs of the breast and ovarian cancer susceptibility genes BRCA1 or BRCA2 (Bonnet et al., Journal of Medical Genetics 2008). In order to better define the roles of bioinformatics predictions, of minigene-based splicing assays and of functional assays on patient RNA, we have now studied an additional group of 53 UVs of BRCA1 or BRCA2. The hybrid minigene assay identified splicing alterations in 12 of the 53 UVs (5 with strong effects and 7 with weak effects). Comparisons with bioinformatics predictions confirmed that UVs at deep intronic positions or located at internal exonic positions cannot be predicted reliably, unless they create novel splice sites. We will describe several exonic variants of BRCA2 that affect novel splicing regulatory elements, in addition to the c.5434C to G variant in exon 23 of BRCA1 that we have recently characterized (Gaildrat et al., Journal of Medical Genetics, in press). Our results show that mapping exonic splicing regulatory elements in these major cancer susceptibility genes, by combined in vivo and ex vivo functional analyses of the effects of natural variants on splicing provides important clues for the prediction of the pathogenicity of exonic variants.

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NNK metabolism by short chain dehydrogenases/reductases and aldo-keto reductases. *A. Hull[1], J. Engle[2], A. Knipe[2], C. Gallagher[2,3], G. Chen[2], P. Lazarus[3], J. Muscat[2,3].* 1) Biology Dept, Lincoln University, Lincoln University, PA; 2) Dept of Public Health Sciences, Pennsylvania State University College of Medicine, Hershey, PA; 3) Dept of Pharmacology, Pennsylvania State University College of Medicine, Hershey, PA.

Lung cancer is the most common cause of cancer related deaths in the U.S. Smoking is estimated to account for 87% of all lung cancer cases. However, only some smokers develop cancer, indicating that they may be more susceptible to the effects of tobacco smoke carcinogens. One of the major carcinogens in tobacco smoke is the nicotine-derived nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). NNK is reduced to another potent lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), which can be glucuronidated by the uridine glucuronosyltransferase (UGT) enzyme family for excretion. Metabolism of NNK to NNAL is carried out by aldoketo reductase 1C1 and 1C2 (AKR1C1 and AKR1C2), and by the short-chain dehydrogenases/reductases carbonyl reductase 1 (CBR1) and hydroxysteroid dehydrogenase 11B1 (HSD11B1). The current study focused on examining the role of individual differences in DNA sequence, mRNA expression, and enzyme activity in CBR1, AKR1C1, 1C2, and HSD11B1 on NNK metabolism. Only very low-frequency coding SNPs exist in CBR1 and HSD11B1. Levels of CBR1 and HSD11B1 mRNA were found to vary significantly in 110 adjacent normal tissue liver samples from different individuals. Reductase activity assays performed with either cytosolic (to measure CBR1 and AKR activity) or microsomal (to measure HSD11B1 activity) fractions of human liver specimens demonstrated no correlation between expression levels of CBR1 mRNA levels and enzymatic activity in cytosol or between HSD11B1 and enzymatic activity in microsomes. The current data shows that variation in activity levels cannot be explained by either DNA sequence variation or mRNA expression. The lack of correlation between mRNA expression and enzymatic activity suggests a complex picture in which the contribution to total activity of the different enzymes varies significantly among individuals. By analyzing tagSNPs of AKR1C1, a haplotype was determined to be associated with lower lung cancer risk in men. TagSNP analysis of CBR1, HSD11B1 and AKR1C2 are underway to identify potential associations of haplotypes important in lung cancer risk.

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Evaluation of EGFR,PTENand P53 experssion and association with clinicopathological features in Iranian Colorectal Cancer Patients. *M. Abachi[1], M. Ghaffarpour[2,3,5], M. Salati[1], Z. Nour Mohammadi[4], F. Fereidooni[4], F. Mahboudi[1,6], A. Adeli[6], M. Houshmand[2,5].* 1) Nanocina LTD,Tehran Iran; 2) Medical Genetics Department, National Institute of Genetics Engineering and Biotechnology, Tehran, Iran; 3) Iranian Research Organization for Science and Technology (IROST), Tehran, Iran; 4) The cancer institute of Iran, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran; 5) Special medical center; 6) Pasteur Institute,Tehran Iran.

Background and Aim: Colorectal cancer (CRC) is one of the most important malignancies worldwide.The epidermal growth factor receptor (EGFR) signaling pathway is usually activated in colorectal cancer. EGFR is expressed in 30% to 85% of colorectal cancer patients. Dysregulation of EGFR signaling has been shown to stimulate cell proliferation, angiogenesis, and metastatic spread and to inhibition apoptosis. Tumour suppressor phosphatase and tensin homologue (PTEN) is an important negative regulator for the PIP3/Akt signalling pathway that promotes cell proliferation and inhibits apoptosis. Inactivation of PTEN has been demonstrated in a variety of cancers.It is frequently mutated more often in colon cancer (9%). The p53 protein regulates cell-cycle inhibition and apoptosis in response to DNA damage; thus, mutation or deletion of TP53 resulting in loss-of function often leads to uncontrolled cell growth The objective of this study was to investigate whether the loss of nuclear EGFR ,PTEN and P53 expression correlates with usual clinicopathological parameters. Materials and Methods: Patients and tissue specimens: Tissue samples were obtained from 27 consecutive patients with CRC who were diagnosed at Imam khomaini hospital,Tehran University,Tehran,Iran from February 2007 to January 2010 (10 men and 17 women; age range,38-88 years; with median age of 61.3 years). All tissues were frozen in liquid nitrogen immediately after surgery and stored at -80°C until the extraction of RNA. Histopathological examinations were performed and all tumours were confirmed as adenocarcinoma. Tumor histologic type and grade of differentiation were defined according to the World Health Organization criteria. Experssion analysis of EGFR,PTEN and P53 in tumor samples:Fresh tumors, containing at least 70% of neoplastic cells and their adjacents were extracted for genomic RNA using the QIAamp Mini kit (Qiagen in accordance with the manufacturer's instructions, and then was used as a template for first-strand cDNA synthesis by random priming. followed, EGFR,PTEN and P53 expression status was determined by Real time PCR . Results: In this study we demonstreated 67% overexpression of EGFR, 23% and 13% Down regulation of PTEN and P53 respectivly in colorectal cancer patients .It seems that the high level expression of EGFR play more important in Iranian colorectal cancer patients.

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Detection of BRCA1 and BRCA2 mutations by High-Resolution Melting curve analysis in breast cancer patients from Thailand and Pakistan. *J. Ahmad[1, 2], S. Sangrajrang[3], F. Le Calvez-Kelm[1], N. Kakar[2], C. Voegele[1], S. Daud[4], O. Sinilnikova[5], S.V. Tavtigian[6], F. Lesueur[1].* 1) Genetic Cancer Susceptibility, International Agency for Research on Cancer, Lyon, France; 2) Dept of Biotechnology and Informatics, BUITEMS, Quetta, Pakistan; 3) National Cancer Institute, Bangkok, Thailand; 4) National Center of Excellence in Molecular Biology, University of Penjab, Lahore, Pakistan; 5) Hospices Civils de Lyon, Centre Léon Bérard, Lyon, France; 6) Dept of Oncological Sciences, Huntsman Cancer Institute School of Medicine, University of Utah, Salt Lake City, UT, USA.

The spectrum of BRCA1 and BRCA2 mutations has been characterized in different populations worldwide with significant variation of the relative contribution of these genes to breast cancer between the populations. However, the contribution of mutations in these two genes to breast cancer patients in Pakistani and Thai populations remains relatively unexplored apart from a few genetics studies. Hence, we aimed to perform comprehensive BRCA mutation screening to characterize the frequency and spectrum of mutations within these two genes in a group of high-risk familial or early onset cases, which forms the largest group of breast cancer patients in these two Asian populations. 143 Thai cases and 85 Pakistani cases were enrolled in the study. The entire coding sequence of both genes was scanned for mutation using High Resolution Melting curve analysis followed by DNA sequencing. Two known BRCA1 mutations (p.E1250X and p.G1770fs) and two BRCA1 mutations that are not reported in the BIC database (p.S423fs and p.S1057fs) were observed. The nonsense mutation p.E1250X was identified in two unrelated Thai cases. The frameshift mutation p.S423fs was identified in a Thai case whereas the two other frameshift mutations were identified in two Pakistani cases. In the case of BRCA2, three 3 frameshift mutations (p.E1577fr, p.R1704fr and p.S2559fr) and one nonsense mutation (p.Q1107X) were identified. The nonsense mutation was identified in a Pakistani case and was already reported in BIC database, whereas the three frameshift mutations are novel. p.E1577fr was identified in a Pakistani case, p.R1704fr in two Thai cases, and p.S2559fr in one Thai case. In addition to the pathogenic mutations described above, we identified also some variants of uncertain clinical significance: one known inframe deletion in BRCA1 (p.K1110del), two evolutionarily unlikely missense substitutions (p.G1788C in BRCT domain of BRCA1 and p.G2901D in DNA binding domain of BRCA2) that are predicted to affect protein function, as well as a number of likely to be neutral missense variants, silent variants and intronic variants in both genes. In summary, our findings show that BRCA1 and BRCA2 account for a substantial proportion of hereditary breast cancer and early-onset breast cancer cases in Thailand and in Pakistan. Cost-benefit calculations would be required to determine the circumstances under which BRCA gene testing would be beneficial in these middle-income countries.

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Breast Ductal Lavage Revisited: The Case for Using Molecular Markers. *W.A. Alkhayal[1], S.C. Willey[1,4], C.X. Cocilovo[1,4], E.D. Feldman[1,4], M.K. Sidawy[2,4], A-M. Noone[3,4], M. Sharifi[4], J.D. Rone[4], L.R. Cavalli[4], B.R. Haddad[4].* 1) Surgery, Georgetown University Hospital, Washington, DC; 2) Pathology, Georgetown University Hospital, Washington, DC; 3) Biostatistics, Bioinformatics, and Biomathematics, Georgetown University, Washington, DC; 4) Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC.

Introduction: The diagnostic power of ductal lavage (DL) cytology for early detection of breast cancer is controversial. Our aim is to develop additional screening methodologies to complement cytology and improve early detection of breast cancer. To achieve our goal, we have established a unique biorepository of DL specimens linked to medical history, and tumor and blood samples from the same patient. These specimens are being used to optimize methods for simultaneous evaluation of multiple molecular markers in the same sample. The first phase of the study (establishment of the biorepository and cytology evaluation of DL) will be reported. Methods: One hundred twenty four patients with unilateral breast cancer were prospectively enrolled in an IRB-approved protocol at Georgetown University Hospital between April 2008 and April 2010. DL fluid was obtained from each patient in the operating room under anesthesia immediately before surgery. In each case two DL fluid samples were obtained: one from the breast with cancer and the other from the contralateral normal breast (control). Multiple aliquots from each DL sample were saved for future studies. One aliquot was used for cytology evaluation which was reported under the following categories: insufficient cells, benign cells, mild atypia, severe atypia, and malignant cells. Full clinical and demographic information was entered into a database. Results: Of the 124 women who were enrolled, 114 qualified for the study. Sufficient cells for cytology were present only in 47 (41%) of the affected breast specimens and in 43 (38%) of the non-affected control breasts. In the cases where cytology was possible, we found that in the affected breasts, 51% showed benign cytology, 30% mild atypia, 13% severe atypia, and only 6% malignant cells, compared to 65%, 28%, 7%, and 0%, respectively, in the control, non-affected breasts. Further statistical analysis is being conducted to assess the correlation of these findings with patients' clinical data and demographics. Conclusion: Our preliminary analysis confirms that cytologic evaluation of the DL fluid alone is not sufficient for early breast cancer detection. These findings support our ongoing studies to develop molecular biomarkers that will improve the diagnostic power of DL fluid. Furthermore, these molecular methods can be used to evaluate ductal fluid obtained through other approaches such as nipple aspirate fluid (NAF), and to study spontaneous nipple discharge.

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A Novel resequencing Diagnostic Microarray: RDMGGA1.0 chip, customized to diagnose mutations in patients with Breast, Ovarian, Colon, Skin and Multiple Cancers. *D. Bercovich[1], y. Plotsky[2], T. Guy[2], S. Allon-Shalev[3], AM. Lichanska[4], LA. Borsuk[4], C. Tibbetts[4].* 1) Human Genetic Lab, Tel Hai Academic College, Israel; 2) Galil Genetic Analysais (GGA), Kazarin 12900, Israel; 3) The Institute for Genetics, Ha'Emek Medical Center, Afula 18101, Israel; 4) TessArae, Potomac Falls, VA 20165 USA.

Despite the remarkable progress in identifying the genes causing the most common inherited cancers, current diagnostic algorithms do not incorporate all genes and known mutation analysis in the clinical evaluation of affected patients and relatives. In addition, the large genes size and the lack of highly predominant mutational hotspots for most populations frequently make mutation detection in these cancer genes outstandingly challenging, which is costly and time consuming. The interpretation is even more complex as a result of poor studies of gene-gene interactions and genotype-phenotype relationships. To fulfill this technological gap, we developed a new customized resequencing gene chip (RDMGGA1.0) that is focused on 11 genes: BRCA1, BRCA2, APC, MUTYH, MLH1, MSH2, MSH6, TP53, PTEN, P16 and KRAS simultaneously, in a single assay, with high call rate and accuracy. probes were designed to identify each base for all exons, 20 bases of intronic sequence bordering exons in the main tiles, and 3320 most frequent mutations were subtiled. The array uses the Affymetrix resequencing platform. Novel software was developed by TessArae for the data analysis. Amplicons were hybridized to the chip, and nucleotide detection was validated by standard capillary sequencing methods. Hybridization of amplicons with the chip produced high nucleotide sequence readout for all 11 genes in a single assay, with an automated call rate of over 98%. The accuracy of nucleotide calls was 99.99% when compared with capillary sequencing. The new resequencing chip enables efficient analysis of 11 genes with a high call rate and accuracy in one assay, and identifies disease-causing mutations.

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Breast cancer genetics: Lobular histology may be a negative predictor of BRCA mutations. *C. Delozier[1,2,3], A. Rahman[3], E. Ubias[1].* 1) Genetic Medicine, Central CA Faculty Med Group, Fresno, CA; 2) Saint Agnes Medical Center, Fresno, CA; 3) University of California San Francisco-Fresno Medical Education Program, Fresno, CA.

Lobular breast cancer represents 10-20% of all breast malignancies. Constitutional mutations in cadherin, a cell-adhesion molecule, result in a high risk of lobular breast and diffuse gastric cancers. However, BRCA-1 and BRCA-2 are the genes most frequently implicated, and tested for, in "monogenic" predisposition to breast cancer. Soon after clinical testing for BRCA mutations became available, a flurry of publications appeared comparing the histological characteristics of breast cancers in patients with BRCA-1 vs. BRCA-2 vs. BRCA-X (negative for BRCA mutations)patients. The next wave of publications was epidemiological, whereas the current focus is molecular profiling of breast cancers, primarily for somatic mutations. In the interim, histology has been displaced as a major consideration in genetic risk assessment. We believe that lobular histology may be as important a *negative* predictor of BRCA status as pre-menopausal age at diagnosis is a positive predictor. Although infrequently considered, there is some data from the literature to support this idea. Our personal experience is as follows: We have counseled and tested 174 breast cancer patients at significant risk of carying a BRCA mutation, for whom histology was available/adequate and BRCA results were unequivocal. Lobular breast cancer was underrepresented (10/174, 5.7%). Of these 174 breast cancer patients, 22 (12.7%) had a BRCA 1-2 mutation; four of these women had bilateral disease. All 26 tumors were of ductal histology. 152 patients (87.3%) were negative for BRCA 1-2 mutations. All ten lobular breast cancer patients were in this group. Histological analysis is an integral part of every cancer patient's evaluation. Why not take it into consideration in genetic risk assessment?

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Uveal Melanoma Exome Sequencing. *T. Ganguly[1], S. Sherrill Mix[1], E. Toorens[1], E. Chao[2], C. Shields[3], A. Ganguly[2].* 1) DNA Sequencing Facility, School of Medicine, University of Pennsylvania; 2) Department of Genetics, School of Medicine, University of Pennsylvania; 3) Wills Eye Hospital, Thomas Jefferson University, Philadelphia.

Uveal melanoma (UM) is a rare,adult onset primary malignant ocular disease with a significantly high degree of mortality. Monosomy 3 in the tumor is associated with poor prognosis.

We initially performed cancer exome sequence capture using Nimblegen method on two DNA samples,each pooled from four UM lesions with chromosome 3 disomy and four with monosomy respectively. We then followed by sequence capture of matched blood and tumor DNA on the Agilent Whole Exome capture platform. The captured DNA samples were sequenced on 454 and Illumina platforms. The results were analyzed using Gigabayes and Mosaik softwares. The software NextGene (Softgenetics, PA) was then used to eliminate duplicate reads representing bias in the capture step to eliminate false positives.

Cancer exome sequencing identified 211,701 and 158,653 changes within 12 basepairs of any coding exon on the Illumina platform for disomy 3 and monosomy 3 samples respectively. The numbers of changes were 151,037 and 151,706 respectively on the 454 platform. After filtering for optimal coverage (> 16 reads for Illumina; >8 reads for 454 platform), presence of reads in both directions and eliminating sequence changes in homopolymer regions, we found 18 mutations shared between the two sequencing platforms for the disomy 3 UM samples and 12 for the monosomy 3 UM samples. These results were all validated by Sanger sequencing. A frameshift mutation in BAP1 gene was observed in 25% of reads for both UM samples. The BAP1 gene is a tumor suppressor gene that functions in the BRCA1 growth control pathway. Its role in UM tumorigenesis is not known.

The genes with detected mutations were not same for all samples. These changes may represent somatic passenger mutations. The driver mutations, if present, are probably outside the cancer exome. We expect to identify the latter from the results of whole exome sequencing. The results translate basic cytogenetic observations into identification of clinically relevant biomarkers of metastasis and potential therapeutic targets in UM.

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Analysis of mutations of the Epidermal Growth Factor Receptor Tyrosine Kinase domain in Non-Small Cell Lung Cancer Brazilian patients. *J. Garcia Carneiro[1], L. Batos-Rodrigues[1], F.A. Bianchi-Leidenz[1], R. Melilo-Carolino[1], M.A. Bicalho[1,2], A. Vilhena[2], N. Amaral[2], L. De Marco[1].* 1) Molecular Medicine Dpt, UFMG, Belo Horizonte, Minas Gerais, Brazil; 2) Thoracic Section, Júlia Kubitscheck Hospital, Belo Horizonte, Minas Gerais, Brazil.

Lung cancer is a major cause of cancer-related deaths worldwide due to its high incidence, malignant behavior and lack of major advancements in treatment strategy. Accumulated evidences show that epidermal growth factor receptor (EGFR) is strongly implicated in the development and progression of numerous human tumors, including lung cancer. Somatic mutations in the tyrosine kinase (TK) domain of EGFR gene are associated to patient's response to TK inhibitors, such as gefitinib and erlotinib. Among all EGFR mutations already reported, small in-frame deletions in exon 19 and a point mutation in exon 21 at nucleotide 2573, leading to a substitution of leucine by arginine at codon 858 (L858R) account for ~90% of EGFR mutations associated with lung adenocarcinomas. Although EGFR mutations are well-studied worldwide, there are no sufficient data about their prevalence in the Brazilian population. Therefore, our study aimed to determine the prevalence of EGFR mutations in 42 Brazilian patients. Since ethnicity appears to play a significant role in determining the percentage of patients with mutations, we correlated our molecular findings with ancestry analyses. Our results showed the presence of exon 19 deletions in only 7.1% of cases. L858R mutation was not found in any of our cases. Ancestry analysis showed a strong African component in cases when compared to control group. For the case group, the proportions of Europeans, Africans and Amerindians were 0.8419 ± 0.0257 (mean ± SE), 0.1153 ± 0.0211 and 0.0427 ± 0.0105, respectively, while for the control group, the results were 0.9162 ± 0.0181, 0.0450 ± 0.0099 and 0.0386 ± 0.0101, respectively. Differences in proportions of African genomic ancestry between the two groups were significant (p value = 0.0004, p<0.05). Our data suggest that Brazilian patients with lung cancer have a low percentage of EGFR mutations that may be explained by the strong African component found in our samples.

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Frequency evaluation of PIK3CA mutations in exons 9 and 20 in Iranian colorectal cancer patients. *M. Ghaffarpour[1,2,6], E. Mohammadi Pargoo[3], M. Abachi[4], S. Sobhani[3], Z. Nour Mohammadi[5], F. Fereidooni[5], M. Houshmand[1,6].* 1) Medical Genetics Department, National Institute of Genetic Engineering and Biotechnology, Tehran, Tehran, Iran; 2) Iranian Research Organization for Science and Technology (IROST), Tehran, Iran; 3) Islamic Azad University Science and Research Campus, Tehran, Iran; 4) Nanocinna Ltd, Tehran ,Iran; 5) The cancer institute of Iran, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran; 6) special medical center,Tehran,Iran.

Background and Aim: Colorectal cancer (CRC) is one of the most important malignancies worldwide and it is the third most common cancer amongst Males and the fourth amongst females in Iranian cancer patients. PI3Ks are heterodimeric kinases involved in the control of cel=AClular growth, transformation, adhesion, and apoptosis. Somatic mutations of PIK3CA were described in CRCs with a frequency ranging from 13.6% to 32%.In CRCs, 3 mutational hot spots were described (E542K, E545K, and H1047R), covering about 80% of all PIK3CA mutations.The aim of this study was to identify PIK3CA gene mutations in exons 9 and 20 among Iranian CRC patients, and to consider whether they are linked with the clinicopathological parameters. Materials and Methods: Patients and tissue specimens: Tissue samples were obtained from 27 consecutive patients with CRC (10 men and 17 women; age range, 32-88 years; with median age of 61.3 years). Histopathological examinations were performed, and all tumors were confirmed as adenocarcinoma. Mutational analysis of PIK3CA in tumor samples: Fresh tumors, containing at least 70% of neoplastic cells and their adjacents were extracted for genomic DNA using the QIAamp Mini kit (Qiagen in accordance with the manufacturer's instructions. We searched for PIK3CA mutations in exons 9 and 20.PIK3CA exon 9 includes codons 542 and 545, PIK3CA exon 20 where the large majority of mutations occur in this genes.The PI3KCA mutational analyses were made by means of PCR sequencing. Results and conclusion: 21 (77.77%) patients had adenocarcinomas and 6 (22.23%) had mucinous adenocarcinomas. patients had stage I, II and III or IV disease with14.76%, 44.44%and 40.8% respectively and histological grade I, II and III with 66.6%,25.92% and 7.4%. respectively. Among the 27 samples analyzed (21were primary tumors and 6were metastases). The frequency of mutations in PIK3CA were present in 11.1% (3 of27) of the samples (11.1% mutation in exon 9(c.1633G>A E545K and no mutation in exon 20).The presence of PIK3CA mutation was not a significance associated with gender, histological grade, age, or cancer stage in patients with adenocarcinoma of the CRC(P>0.05).Although many study have been shown in CRCs, 3 mutational hot spots (E542K, E545K, and H1047R) ,our study indicated mutations were just in exon 9(E542K) and more samples should be investigate in exon 9,20 and other exons of PIK3CA gene.

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Genetic heterogeneity in *Tg-Interacting Factor (TGIF)* expression is a modifier of acute myeloid leukemia. *R. Hamid[1], J. Means[2], S. Brandt[2].* 1) Dept Pediatrics, Div Med Gen, Vanderbilt Univ Sch Med, Nashville, TN; 2) Dept Medicine, Vanderbilt Univ Sch Med, Nashville, TN.

TGIF is a homeobox repressor, which has been associated with holoprosencephaly however its role in any other human disease is not known. Interestingly *TGIF* is expressed in hematopoietic stem cells (HSC) and interacts with RA and TGF-β pathways both involved in HSC function. We have previously shown that there is considerable variation in *TGIF* expression in acute myeloid leukemia (AML) derived cell lines. In trying to further understand the basis of this observed variability in TGIF expression, we used real-time PCR to analyze 70 primary AML blast samples for TGIF mRNA abundance. We found that *TGIF* expression was a continuous variable with an eight-fold difference in *TGIF* mRNA levels between the lowest and the highest expressers. Sequence and southern blot analysis of TGIF genomic region in these 70 patients ruled out mutations or gene rearrangements as a cause of this variability in expression. We did notice several previously reported coding and promoter SNPS but none showed significant association with *TGIF* expression. We then hypothesized that the heterogeneity in *TGIF* expression is genetic/inherited in nature and as such present in normal individuals. To explore this hypothesis we determined *TGIF* mRNA abundance in peripheral blood mononuclear cells from 20 CEPH families (200 total individuals). Our data showed that in CEPH samples, *TGIF* expression was a continuous variable with a ~20 fold difference in expression between the lowest and the highest expresser. We then investigated whether the variability in *TGIF* expression in primary AML samples had a clinical consequence for these patients. Log-rank analysis showed that *TGIF* expression in AML patients, correlated significantly with overall survival ($P < 0.0001$). Analyzed as a continuous variable, lower *TGIF* expression was associated with a significantly shorter overall survival (5.5 months vs. 54.0 months) than those with higher expression ($P < 0.0001$). Finally, when patients were grouped by *TGIF* expression and level of expression related to survival, a dose-dependent relationship between TGIF expression and outcome was suggested. These data were confirmed in an independent cohort of 285 Dutch AML patients. In conclusion our data suggest that a) *TGIF* expression is likely a quantitative expression trait and b) as such it may act as a true modifier of AML. These finding have biologic implications for both AML and possibly holoprosencephaly.

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FREQUENCY OF THE TSER 2R/3R POLYMORPHISM IN THE TYMS GENE AND ITS ASSOCIATION TO BREAST CANCER IN A MEXICAN POPULATION. *B. Lazalde[1,2], G. Zambrano-Galvan[2], N. Jimenez-Soto[2], M. Reyes[2].* 1) Biomedical Res Unit, Mexican Inst Social Security, Durango, Mexico; 2) Faculty of Medicine, Universidad Juarez del Estado de Durango, Durango, Dgo. Mexico.

Background: It has been suggested that the TSER 2R/3R polymorphism (a 28-bp tandem repeat) in the TYMS 5'-untranslated region, is linked to modification in risk for some cancers. It also appears to affect the therapeutic and toxic effects of 5-fluorouracyle because thymidylate synthase, the product of TYMS, is its target. Aim: To study the frequency and association of TSER 2R/3R polymorphism of TYMS with breast cancer in a Mexican population. Metods: A hospital-based case-control study was conducted in 98 incident ductal breast cancers in women attended in the Cancer State Center of Durango, Mexico, group matched by age with 91 controls. TSER 2R/3R typification was done from genomic DNA by PCR. Hardy-Weinberg equilibrium and tests for association were done with a software of the Institute of Human Genetics of the Technological University of Munich. Results: Case and control groups were in Hardy-Weinberg equilibrium. Allelic frequencies were: 2R, 0.44 vs 0.39; 3R, 0.56 vs 0.61, and genotypic frequencies 2R/2R, 0.17 vs 0.11; 2R/3R, 0.53 vs 0.56; 3R/3R, 0.30 vs 0.33, for cases and controls respectively. Armitage's trend test: common OR=0.78, p=0.30. Significant allelic or genotypic differences between groups were not found. Conclusions: There was a high frequency of the TYMS TSER 2R/3R polymorphism in the studied groups, but association to breast cancer was not found. On the other hand, due to the high frequency found, the TSER 2R/3R genotyping could be worth with pharmacogenetic aim regarding treatment with 5-fluorouracyle and analogs.

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MiRNA-150 AND miRNA-155 expression patterns are distinct in acute myeloid leukemia versus blast phase of chronic myelogenous leukemia associated with inv(3)(q21q26). *P. Lennon[1], J. Han[1], S. Mulligan[1], P. Hu[1], C. Yin[2], P. Lin[2].* 1) Molecular Genetic Technology Program, School of Health Professions, UT-MD Anderson Cancer Center, Houston, TX; 2) Department of Hematopathology, UT-MD Anderson Cancer Center, Houston, TX.

Inv(3)(q21q26) is a rare recurrent cytogenetic aberration observed typically in acute myeloid leukemia (AML) or blast phase of chronic myelogeneous leukemia (CML- BP). The mechanism of how inv(3) causes leukemia is unknown. A unique feature of AML with inv(3) is that affected patients often have an increased platelet count but profound anemia due to dysregulation of megakaryopoiesis. MiRNA-150 has been shown previously to enhance megakaryopoiesis at the expense of erythropoiesis. MiRNA-155 has been shown to modulate megakaryopoiesis at the hematopoietic progenitor/precursor level by targeting some transcription factors, and that decline of miRNA-155 is required for proliferation and differentiation of megakaryocytes. We hypothesized that in AML or CML-BP associated with inv(3), miRNA-150 and miRNA-155 may contribute to or determine the clinical characteristics of these diseases. To test this, we analyzed miR-150 and miR-155 expression levels in 13 leukemic samples with inv(3) including AML (n=5), CML-BP (n=6) and CML-chronic phase (n=2) by using real-time reverse-transcriptase quantitative polymerase chain reaction. A case of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) was included as a control. Total RNA extraction was obtained from formalin-fixed paraffin embedded tissue sections. MiRNA-150 and 155 of the total extract were targeted for cDNA conversion (Applied Biosystems' Reverse Transcription Kit). Real-time polymerase chain reaction was used to examine their expression levels. Relative quantitations were analyzed by delta-delta CT method. We found significantly increased miR-150 expression in all 13 cases, particularly in AML and CML-BP. By contrast, expression of miR-155 was increased in CML-BP but decreased in AML and CML-CP. We conclude that miRNA-150 and miRNA-155 expression patterns are distinct among AML, CML-BP and CML-CP. The significantly increased level of miR-150 expression in AML and CML-BP in comparison to CML-CP suggests that miR-150 is implicated in the acute onset or blast transformation, and hence facilitates a more aggressive disease. Also, the differential expression of miR-155 in CML-BP versus AML and CML-CP suggests that it is more implicated in blast transformation than de novo disease. Further studies to compare miR-150 expression in AML and CML-BP cases with or without inv(3) are warranted to explore its potential as a marker for prognosis and targeted therapy.

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Oncogenesis of retinoblastoma associated to the SNPs of low frequency from RB1 gene in Mexican pediatric patients affected with hereditary and non hereditary disease. *M. Macias-Vega[1], J.C. Juarez[1], J.C. Ordaz[1], V. Bosch[1], M. Chávez[1], N. Carranza[1], C. Leal[1], P. Gariglio[2], R. Ocadiz[2], O.A. Pérez[1].* 1) Laboratorio de Oncologia Experimental, Instituto Nacional de Pediatria, Mexico D.F., Mexico; 2) Departamento de Patología Molecular, CINVESTAV IPN, Mexico D.F., Mexico.

BACKGROUND: The single nucleotide polymorphisms (SNPs) from RB1 gene have been classified in two groups: of high frequency (³ 5%) (normal alleles) and of low frequency (£ 5%) (risk alleles), some of them have been found like haplotypes, formed by risk alleles in heterozigosity and homozigosity associated with a high predisposition to develop the disease, with a differential response to the Chemotherapy and have been considered like prognostic markers in patients affected with breast cancer, hereditary retinoblastoma and ovarian cancer. OBJECTIVE To determine the frequency of the risk alleles from RB1 gene in pediatric patients. MATERIAL AND METHODS DNA samples from peripheral blood of 73 patients were analyzed, all samples were genotyped for 15 SNPs from RB1 gene by mean of allelic discrimination with Taq-Man probes. RESULTS The results show that in the control group the normal alleles occurred in homozigosity at 100% in five allelic markers C-779216, C-3042266, C-779170, C-33789354 and C-31604223 whereas in patients group it occurred only in one marker C-33789354, and only two markers more show a frequency nearest to 100% (C-779170 85%, C-31604223 95%), in relation to the risk allele, it was found in heterozigosity in 14 markers in the patients group while it occurred in 7 markers in the control group and it was found in homozigosity in 12 markers in the patients group and only in 6 markers in the control group. CONCLUSIONS Clearly the risk allele is find in high frequency both homozygosity and hetrozygosity in the patients group so the oncogenesis of retinoblastoma is associated with the risk alleles (SNPs) of the RB1 gene, however the extensive analysis of haplotypes will allow in a second phase to determine if any association exist among risk markers and clinical aspects of the disease like a different response of each patient to the pharmacology treatment, its utility like prognostic factor of the malignancy and for the opportune detection of haplotype carriers with high risk to inheriting the disease with at least one affected member as well as it application in diagnostic processes in human preimplantation treatments for assisted reproduction purposes. CONACYT-71020, INP-07/040.

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Genomic Signature of Metastasis in Prostate Cancer Health Disparity. *A. Pearlman[1], C. Campbell[1], E. Brooks[1], A. Genshaft[1], S. Shajahan[1], M. Ittmann[2], G. Bova[3], J. Melamed[4], R. Schneider[5], Y. Shao[6], H. Ostrer[1].* 1) Pediatrics Dept, NYU Langone Medical Center, New York, NY; 2) Pathology Dept, Baylor College of Medicine, Houston, TX; 3) Pathology Dept, Johns Hopkins University School of Medicine, Baltimore, MD; 4) Pathology Dept, NYU Langone Medical Center, New York, NY; 5) Microbiology Dept, NYU Langone Medical Center, New York, NY; 6) Biostatistics Division, NYU Langone Medical Center, New York, NY.

Prostate cancer is the most commonly diagnosed cancer in men. When detected early, prostate cancer can be cured, although most therapies, such as radical prostatectomy and radiation therapy, often result in significant side effects, including erectile dysfunction and urinary incontinence. Current biomarkers of metastatic potential have limited predictive value, resulting in life-preserving, albeit debilitating, radical treatments. In Caucasian American (CA) men, the disease is indolent in ~75% of diagnosed cases when left untreated. The disease is more prevalent and aggressive among African American (AA) men, resulting in a well-known racial health disparity. To investigate the genomic basis of metastatic disease and this disparity, we studied the somatic copy number alterations of primary tumors from 29 AA and 20 CA men and 52 hormone refractory metastasis samples from 12 CA patients with multiple metastases collected at autopsy. All 49 primary tumors had clinical attributes associated with invasive local disease, including stage II-IV and Gleason score 5-9 that were similar between the AA and CA groups. Unsupervised hierarchical clustering of a summarized metastasis profile along with the 49 primary tumors resulted in the compelling observation that 8 out of 29 AA primary tumors (28%) segregated with the metastasis cluster, whereas only 2 out of 20 CA primary tumors (10%) segregated with the metastasis cluster. This 2.8-fold ratio (bootstrap 95% confidence interval = 1.7-3.78, 800 iterations) of AA primary tumors over CA primary tumors was consistent with the previously reported rate of racial health disparity of mortality. A novel genome scanning method developed in-house identified genes undergoing natural selection in the metastases. The top candidate genes, KCNQ3, KCNB2, ASAH1, CDH13 and WWOX, that are potential drivers of the metastatic process and correlates of the racial health disparity may do so by facilitating the escape from anoikis through the control of potassium ion and ceramide concentrations. A risk model based on the top candidate metastasis signature genes predicted a metastasis cohort (n=14) with sensitivity-AUC = 0.95. These observations can be harnessed to gain a deeper understanding of the basic cellular biology of metastasis and can be used clinically to predict whether a primary tumor is likely to metastasize.

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Prevalence of common mutation frequency in codons 12, 13 of K-ras gene among Iranian colon cancer patients. *S. Sobhani[1], M. Ghaffarpour[2], Z. Nour Mohammadi[3], M. Houshmand[2].* 1) Islamic Azad University Science & Research Campus, Tehran , Tehran, Iran; 2) Medical Genetics Department, National Institute of Genetics Engineering and Biotechnology, Tehran, Iran; 3) The cancer institute of Iran, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran.

Back ground and Aims: Colorectal cancer (CRC) is the most gastrointestinal cancer in United States and Europe, and the third most common cause of cancer-related death. The development of colorectal cancer is a multiple process that can take place, because of the various mutations in proto-oncogenes, tumor suppressor genes, and epigenetic changes in DNA too. K-ras gene encodes a signal transducer protein which is a GDP/GTP binding protein. Mutation analysis in K-ras is known as a predictive biomarker in colorectal cancer. According to recent researches, somatic mutations in codons 12, 13 of K-ras gene are discovered in 40% - 50% human CRCs. The aim of this study was to estimate the contribution of K-ras gene mutations in codons 12, 13 in the incidence, and its association with clinicopathologic, sex, stage and familial history in Iranian colon cancer patients. Materials and Methods: Genomic DNA was extracted from tissue samples using QIAamp mini kit. Exon 1 which includes codons 12, 13 as two hot spots in this gene, was amplified with specific primers. After running the PCR products on 1.5% agarose gel, they were analyzed with automated sequencing method. Finally, we have blasted the sequences in ncbi, and statistical analysis of the data performed with SPSS software. Results: In this study, we have analyzed 18 (12F, 6M) tissue specimens of colon cancer patients using PCR/Sequencing method for codons 12, 13 of K-ras gene. 3 of 18 patients (16.6%) have shown a point mutation in codon 12 (2 G>A, 1 G>T) and none of them (0%) have shown mutation in codon 13. The presence of K-ras mutation does not have a significant association with gender, histological grade, age or cancer stage in patients with adenocarcinoma of the CRC (P>o.o5). Discussion: mutation in exon 12, 13 are not common in Iranian patients. We suggest whole genome sequence for our patients.

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Biomarkers of response to IGF1R inhibition in osteosarcoma. *K. Sol-Church[1], E.A. Kolb[2], D. Kamara[2], D.L. Stabkey[1].* 1) Dept Biomedical Res, Alfred I duPont Hosp Children, Wilmington, DE; 2) Cancer Therapeutics Laboratory, Alfred.I. duPont Hospital for Children, Wilmington, DE.

Objective: To evaluate signaling pathways influencing response in osteosarcoma (OS) tumors to the inhibition of the IGF-1 receptor (IGF1R). Methods: Recent preclinical studies have demonstrated that a subset of osteosarcoma xenografts is consistently sensitive to IGF1R inhibitors (OS1, OS2, OS9 and OS17) and a subset consistently resistant to IGF1R inhibitors (OS31 and OS33). This study investigates molecular pathways that differentiate the tumor lines. Results: Following inhibition of IGF1R with the inhibitor R1507, a fully humanized monoclonal antibody, xenograft tumor lines sensitive to IGF1R inhibition demonstrate a decrease in IGF1R, EGFR, AKT and MAPK phosphorylation within 48 hours of treatment. In contrast in the 2 resistant tumors OS31 and OS33, there is an increase in AKT and MAPK phosphorylation as well as EGFR mRNA and protein expression. Loss of TP53, a negative regulator of EGFR was also observed in both resistant tumors. In addition, a known Sp1 polymorphic binding site at -216 on the EGFR promoter is able to predict tumor response: IGF1R inhibitor sensitive tumors either carry the -216 T/T, and G/T genotype corresponding to higher constitutive EGFR protein expression. The 2 tumor lines resistant to IGF1R inhibition are homozygous G at this site. The G/G polymorphism predicts lower constitutive expression of EGFR but a rapid induction of EGFR expression and signaling in response to IGF1R inhibition. Transfection assays with the T and G genotypes using a luciferase reporter confirm these results. Pathway focused gene expression analysis and miRNA profiling are ongoing that will give clues as to the mechanism of response to chemotherapy in these tumor types. Conclusion: OS cells and tumors resistant to R1507 may survive in the presence of IGF-IR signal inhibition through an increase in EGFR expression mediating signaling involving MAPK. Efficiency of this change in EGFR expression can be predicted by a SNP in the EGFR promoter. Inducible mechanisms of resistance should be evaluated in sarcomas in response to potent targeted therapies.

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Clinical utility of inherited markers in determining need for repeat biopsy: results from placebo arm of the REDUCE® study. *J. Sun[1], A.K. Kader[1,2], P.J. Newcombe[3], B.H. Reck[4], S.T. Kim[1], T. Jin[1], Z. Zhang[1], S. Tao[1], G.T. Platek[4], C.F. Spraggs[3], J.C. Whittaker[3], V.E. Mooser[5], J.D. McConnell[2], S.L. Zheng[1], L.D. Condreay[4], R.S. Rittmaster[4], J. Xu[1,2].* 1) Center for Cancer Genomics, Center for Human Genomics, Wake Forest University School of Medicine, Winston-Salem, NC, USA; 2) Dept of Urology, Wake Forest University School of Medicine, Winston-Salem, NC, USA; 3) GlaxoSmithKline Research and Development, Harlow, UK; 4) GlaxoSmithKline Research and Development, Research Triangle Park, NC, USA; 5) GlaxoSmithKline Research and Development, King of Prussia, PA, USA.

Purpose: Management of men following negative prostate biopsy for prostate cancer is challenging. The predictive performance of currently available clinical parameters such as prostate specific antigen (PSA) for prostate cancer is limited. Recently, 33 PCa risk-associated single nucleotide polymorphisms (SNPs) have been identified from genome-wide association studies. We sought to assess whether supplementing existing predictors with the prediction of prostate cancer on subsequent biopsy. Methods: Study subjects included 1,654 men in the placebo arm of the four-year randomized REduction by DUtasteride of prostate Cancer Events (REDUCE®) trial, where all subjects had PSAs between 2.5-10.0 ng/mL, a negative prostate biopsy at baseline and underwent scheduled prostate biopsies at years 2 and 4. Results: Of 1,654 men who had at least one prostate biopsy over four years, 410 (25%) and 124 (7%) were diagnosed with prostate cancer and high-grade PCa (Gleason grade ≥ 7), respectively. Differences in the genetic score between men with positive and negative biopsies were highly significant even after adjusting for other clinical variables ($P = 3.58 \times 10^{-8}$). The AUC for prostate cancer prediction of the genetic score was 0.59, higher than any other individual clinical parameters including PSA (AUC = 0.54). When the genetic score was added to the best clinical model including five parameters (age, family history, free/total PSA ratio, prostate volume, and number of cores at base biopsy), the AUC increased from 0.60 to 0.64. The differences in detection rates between men with lower or higher genetic risk at each quartile of estimated risk based on the best clinical model ranged from 9.31% to 13.66% for prostate cancer and 2.89 to 6.16% for high-grade prostate cancer, providing strong evidence for the added value of genetic markers in risk prediction. Conclusions: For men with an initial negative biopsy, genetic markers may be used to supplement existing predictors to better predict for prostate cancer and high-grade prostate cancer on subsequent biopsy.

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Altered miRNA expression in lymphoblastoid cell lines of Finnish familial prostate cancer cases. *T. Wahlfors[1], H. Mattila[1], D. Fischer[2], M. Schindler[2], H. Oja[2], J. Schleutker[1].* 1) Lab Cancer Gen, Inst Med Tech, University of Tampere, Tampere, Finland; 2) Tampere School of Public Health, University of Tampere, Finland.

Identifying new risk factors and explaining their functionality in prostate carcinogenesis and prostate cancer prognosis would have instant clinical utility, especially with aggressive disease status. Non-coding RNAs, like miRNAs can be informative for the identification of men who may be at elevated risk for prostate cancer and men at risk for developing a more aggressive disease outcome. Based on the current knowledge predisposition to PrCa is arising from multiple alterations in the genome and most often these variations are low penetrant and challenging to find. Since considerable amount of miRNAs are located within intronic regions and regulated by the host gene promoter, miRNA expression profiles gives us one possibility to study disease related variations in non-protein coding chromosomal areas and could lead to identification of regulatory variants. miRNA expression from 186 lymphoblastoid cell lines from both affected (N=111) and unaffected (N=75) individuals from Finnish multiplex prostate cancer families were included in this study. Isolation of total RNA was done using standard Trizol protocol and Agilent Human miRNA V2 Oligo Microarray kit was used for miRNA detection. After quality checking and data normalization different statistical methods were used for data analyses for example Ward's method for clustering and Random Forest method for ranking. Selected set of miRNAs were used for validation with TaqMan MicroRNA Assays. Based on the initial clustering analysis we were able to separate the healthy controls (N=36) from the cancer patients (N=63). Healthy individuals were members from 17 families and cases from 38 families making a total number of analyzed families 56. Case and control cohorts did not overlap concerning the family history. We identified six miRNAs (miR-770-5p, miR-202, miR138-2, miR431, miR-513c and miR487b) that were mostly affecting to clustering. Those miRNAs and 13 other miRNAs (based on expression differences or ranks from Random Forest) were chosen for further validation. Validation results of miR-151-5p, miR-151-3p, miR-29c, miR-557 and miR-195 were consistent with the array results. Other validation results were either opposite or the expression was too low to draw any conclusions. Confirmed miRNAs warrant further studies to identify the mechanism underlying and validation in larger sample set.

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Gene expression profile for early relapse patients of colorectal cancer. *Y. Wang[1], I.P. Yang[1], J.W. Wang[1,2,3,4], S.H. Juo[1,5].* 1) Department of Medical Genetics, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; 2) Graduate Institute of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; 3) Department of Surgery, Faculty of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; 4) Cancer Center, and Division of Gastroenterology and General Surgery, Department of Surgery, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan; 5) Cancer Center and Department of Medical Research, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan.

Colorectal cancer (CRC) is one of the most frequent cancers and a common cause of cancer-related deaths in the developed world. The recurrence rate among patients with early-stage CRC is more than 20%. The overall 5-year survival rate of CRC patients ranges from 40% to 60%. Here we developed a multiplex, quantitative gene expression assay to test for a gene expression profile comprising 21 candidate genes for early relapse (defined as a recurrence of 12 months after surgery) of CRC. These 21 genes were previously identified from a microarray study and the majority of them are involved in metastasis. The multiplex assay is performed on the GenomeLab™ GeXP Genetic Analysis System (Beckman Coulter Inc.) where the gene expression level was compared with a reference gene. Using as little as 40ng of total RNA extracted from frozen fresh tissues, the GeXP platform can detect as low as 0.5-fold change in expression level. Thus it can provide a more subtle change that is hard to be achieved by tranditional quantitative real-time polymerase chain reaction. In addition, the GeXP multiplex feature allows genes of interest and an internal control to be analyzed in a single well for improved accuracy. A total of 60 frozen tissue samples of CRC were analyzed and they are consisted of adenocarcinoma (98%) and mucinous carcinoma (2%). The results indicated that fibroblast growth factor 2 (FGF2), matrix metallopeptidase 9 (MMP9) and pitrilysin metallopeptidase 1 (PITRM1) had higher expression levels in the patients with early relapse than those with non-early recurrence (p£0.05). Fibroblast growth factor-2 (FGF2) is a prototypic angiogenesis inducer, matrix metallopeptidase 9 (MMP9) and pitrilysin metallopeptidase 1 (PITRM1) both are proteinase. The present study augments that the previous results from the microarray study where several genes were implied in early relapse of CRC. Using this multiplex gene expression system, we are allowed to quantitatively interrogate several candidate genes simultaneously. We are currently underway to further confirm of the importance of these three genes in an independent samples.

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MICA-STR genetic diversity and its association with ovarian cancer in a northern Chinese population. *Y. Fan[1], W. Yu[1], P. Ye[1], Z. Wang[2], Q. Meng[1], Y. Duan[1], X. Liang[1], W. An[1].* 1) Institute of Transfusion Medicine, Blood Center of Dalian, Dalian, Liaoning, China; 2) Department of Gynecologic Ontology, Dalian Obstetric and Gynecological Hospital, Dalian, China.

MHC class-I chain related gene A (MICA) maps 46 kb centromeric to HLA-B and is highly polymorphic. The MICA-NKG2D system plays an important role in the anti-tumor immune responses. Ovarian cancer, a polygenic disease, is the leading cause of death among malignant gynecological tumors, and genetic factors are critical for the induction of this malignancy. We postulated MICA gene as a susceptibility factor for ovarian cancer. In this study, 97 unrelated patients newly diagnosed with ovarian cancer and 102 randomly selected healthy controls were enrolled. All subjects were of Han ethnicity and were local residents in Liaoning province, northern China. Peripheral blood with EDTA anticoagulant was collected after each person's informed consent. Genomic DNA was isolated from blood samples by commercial DNA extraction kit. The short tandem repeat polymorphism of exon 5 of MICA gene (MICA-STR) was determined using fluorescent polymerase chain reaction-capillary electrophoresis. The allele frequencies of the five MICA-STR variants in the control group were 10.8% (A4), 22.1% (A5), 34.8% (A5.1), 17.6% (A6), and 14.7% (A9), respectively. The frequency of the MICA-A5 allele in ovarian cancer patients was significantly higher than that in the controls (33% vs. 22.1%, P=0.015, odds ratio=1.739, 95% confidence interval=1.113-2.718). The results suggest that the MICA-A5 allele appears to increase the susceptibility to ovarian cancer in this population. The potential interaction between MICA and other genetic factors remains to be further studied.

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Chromosomal abnormalities in ossifying fibromyxoid tumor of soft parts. *S. Gebre-Medhin[1], F. Vult von Steyern[2], O. Brosjö[3], H. Domanski[4], N. Mandahl[1], F. Mertens[1].* 1) Deparment of Clinical Genetics, University and Regional Laboratories, Skåne University Hospital, Lund University, Lund, Sweden; 2) Deparment of Orthopedics, Skåne University Hospital, Lund University, Lund, Sweden; 3) Department of Orthopedics, Karolinska University Hospital, Stockholm, Sweden; 4) Deparment of Pathology, University and Regional Laboratories, Skåne University Hospital, Lund University, Lund, Sweden.

Ossifying fibromyxoid tumor (OFMT) of soft parts is an uncommon, rarely metastasizing tumor of uncertain cellular origin which predominantly occurs in the extremities of the adult. Clinically, the tumor often presents as a small painless subcutaneous mass displaying a partial peripheral ring of calcification upon radiography. Microscopically, there are often nests and cords of round-shaped cells in a myxoid stroma surrounded by an incomplete layer of ossified tissue. Among the few cases of OFMT that have been cytogenetically examined to date no recurrent clonal chromosomal abnormalities have been identified. Thus, although OFMT, based on histological and ultrastructural grounds, have been suggested to possibly harbor a common cytogenetic rearrangement, this remains to be shown. In the present work we report three new cases of OFMT which were investigated using cytogenetic, fluorescent in situ hybridization (FISH), and short nucleotide polymorphism (SNP) array analyses. Together with previous published karyotypes our findings indicate that rearrangements involving the short arm of chromosome 6 are common in OFMT.

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Gene mutation and protein functional alteration in nevoid basal cell carcinoma syndrome and familiar keratocystic odontogenic tumor. *Y. Lu, XD. Wang, HG. Zhu, WM. Ye, SL. Zhang, D. He, MB. Zhang, TT. Zhang, GF. Shen, WT. Chen.* Oral and Maxillofacial Surgery, Ninth People's Hospital,Shanghai Jiaotong University School of Medicine, Shanghai, China.

Objective: To investigate PTCH gene mutation and alteration of PTCH protein function in nevoid basal cell carcinoma syndrome (NBCCS) families and familiar keratocystic odontogenic tumor families. The activity of Hedgehog pathway in tumor sample of proband from each family was also studied. Methods: NBCCS and familiar keratocystic odontogenic tumor families were collected according to major and minor criteria. Mutation of PTCH and PTCH2 gene were detected by PCR and directly sequence analysis. Alteration of PTCH protein function after gene mutation was forecasted by bioinformatics analysis. Gli protein, one of key molecule in Hedgehog pathway, was detected by immunohistochemistry in tumor sample of proband from each family. Results: Seven families were collected, including five NBCCS families and two familiar keratocystic odontogenic tumor families. In two NBCCS families, a new 3bp deletion mutation of PTCH c.1537_1539delGAT or c.1540_1542delGAT (p.513delD or p.514delD) and a nonsense mutation of PTCH c.2776ACC>ACT (p.W926end) were detected. In two familiar keratocystic odontogenic tumor families, a missense mutation of PTCH c.3277 CGA>GGA (p.G1093R) and a new splice mutation of PTCH c.339+1G>C (NM 000264.3, 'A' in promoter ATG as the first base in the sequence) were detected. Mutation of p.513delD or p.514delD might affect transmembrane domain of PTCH protein. Mutation of p.W926end might affect the second functional domain of PTCH protein. Mutation of p.G1093R might affect active site of PTCH protein. Expression of Gli protein was detected in all cancer samples of probands from all families. Conclusions: Multiple keratocystic odontogenic tumors were main and first reason to hospital in collected NBCCS families. Keratocystic odontogenic tumors were the only symptom in familiar keratocystic odontogenic tumor families. Different mutation might affect PTCH protein function through different way. No PTCH mutation was detected in three NBCCS families, so maybe other gene take responsible for NBCCS in these families. Key molecule of Hedgehog pathway might be therapy target for NBCCS and Keratocystic odontogenic tumor.

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microRNA regulates the responses to ionizing radiation by controlling MAPK signaling in lung cancer cells. *H. ARORA[1], W.Y. PARK[1], H.Y. SUNG[1], A.K. PARK[1], S. JIN[2], S.M. DONG[3], J.H. AHN[4].* 1) BIOMEDICAL SCIENCE, SEOUL NATIONAL UNIVERSITY, SEOUL, Korea; 2) Departments of Radiation Biology, Gilin University College of Medicine, China; 3) Division of Translational & Clinical Research I, Research Institute, National Cancer Center; 4) Department of Biochemistry, Ewha Women University College of Medicine, Seoul 158-907, Korea.

To understand the microRNA-mediated posttranscriptional control of the responses to ionizing radiation (IR), we examined time-series profiles of genome-wide microRNA expression upon 2 Gy γ-irradiation in radioresistant H1299 and radiosensitive H460 human lung cancer cell lines. We selected differentially expressed microRNAs (DEmiRs) according to the statistical analysis (ANOVA, $p<0.05$) in H1299 cells and H460 cells. The enrichment analysis showed that MAPK signaling pathway is targeted by DEmiRs in both cell lines. However, the reported target genes in these two pathways were differentially regulated in a concerted manner. We cross validated our analysis and found that over 60% of the mRNAs that were predicted by our analysis are significantly changed. From these results, we suggest that IR-induced regulation of microRNA might affect the regulation of MAPK signaling pathways in a concerted manner, which may confer the resistance to ionizing radiation.

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SUCCINATE DEHYDROGENASE SUBUNIT B (SDHB) IMMUNOSTAINING IN GASTROINTESTINAL STROMAL TUMORS (GIST) ASSOCIATED WITH CARNEY-STRATAKIS SYNDROME, CARNEY TRIAD, AND WILD-TYPE GIST. *M.B. Lodish[1], K. Janeway[2], J. Gaal[3], E.R. Ball[1], M. Raygada[1], S.Y. Kim[4], C.A. Stratakis[1].* 1) Developmental Endocrinology , NICHD, NIH, Bethesda, MD; 2) Dana Farber Cancer Institute, Boston, MA; 3) Medical Center Rotterdam, The Netherlands; 4) National Cancer Institute, Bethesda, MD.

Gastrointestinal Stromal Tumors (GIST) are rare neoplasms of the GI tract arising from the interstitial cells of Cajal. While most adult GIST patients have activating mutations in *KIT* or *PDGFRA* and respond to therapy using tyrosine kinase inhibitors (TKI), pediatric GIST patients do not have mutations in these genes, are thus termed wild-type (WT), and do not respond as well to TKI therapy. Mutations in genes *SDHB,-C,-D* (SDHx) encoding subunits of succinate dehydrogenase (mitochondrial oxidation complex II) have been implicated in the pathogenesis of WT GIST. We recently reported that up to 29% of patients with WT GIST harbor germline SDHx mutations, in the absence of co-existing paraganglioma (PGL)(1). SDHB immunohistochemistry (IHC) has been shown to have a sensitivity of 84-100% to detect the presence of an SDHx mutation (2). We investigated whether SDHB IHC could effectively discriminate between SDHx-related and non-SDHx-related GIST in a cohort of patients with WT GIST, including GIST from patients with Carney-Stratakis syndrome (CSS) and Carney Triad (CT). CSS is characterized by the presence of PGL and GIST inherited in an autosomal dominant manner. SDHx germline mutations are present in the majority of patients with CSS. Carney triad (CT) is a rare syndrome that is associated with GIST, PGL, and pulmonary chondroma; the causative genetic defect remains unknown. SDHB IHC was perfomed on 20 WT GISTs from 20 unrelated patients. Nine of these patients had wild-type GIST alone, the remainder had CSS or CT. Results of IHC were compared to SDHx mutational status. All GISTs from patients with CSS/CT were SDHB negative by IHC (n=11). Negative SDHB immunostaining was seen in 5 tumors from patients with WT GISTs, only one of whom had a known SDHx mutation. A total of 4 GISTs from 4 patients were SDHB positive by IHC and none of these harbored germline SDHx mutations. Although SDHx mutations are not present in patients with CT, the negative SDHB expression in GIST samples isolated from these patients implicates the SDH pathway in the pathogenesis of this disease. We conclude that SDHx mutations are present frequently in pediatric and young adult patients with wild-type GIST; an absence of SDHB immunoreactivity is observed in 100% of GISTs associated with SDHx mutations and in all CT/CSS related GISTs. (1) Lodish MB et. al, 2009. Hormone Res. 72:95. Suppl. 3 (2) van Nederveen FH et. al, 2009. Lancet Oncol. 10:764-71.

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Prediction of microsatellite instability in colorectal cancer based on pathologic and genetic data. *N. Roslin[1], M. Mrkonjic[2,3], A. Hyde[4], C. Greenwood[1,2], D. Fontaine[4], S. Raptis[2,3], A. Pollett[3], P. Laird[5], V. Pethe[3], T. Chiang[1], D. Daftary[6], E. Dicks[4], S. Thibodeau[7], S. Gallinger[2,3,6], P. Parfrey[4], H. Younghusband[4], T. Hudson[2,8], J. McLaughlin[2,3,6], R. Green[4], B. Zanke[6,9], J. Beyene[1,2], A. Paterson[1,2], B. Bapat[2,3].* 1) Hospital for Sick Children, Toronto, ON, Canada; 2) University of Toronto, Toronto, ON, Canada; 3) Mount Sinai Hospital, Toronto, ON, Canada; 4) Memorial University of Newfoundland, St. John's, NL, Canada; 5) University of Southern California, Los Angeles, CA, USA; 6) Cancer Care Ontario, Toronto, ON, Canada; 7) Mayo Clinic, Rochester, MN, USA; 8) Ontario Institute for Cancer Research, Toronto, ON, Canada; 9) Ottawa Hospital Research Institute, Ottawa, ON, Canada.

Approximately 15% of colorectal cancer (CRC) tumours show high frequency of microsatellite instability (MSI-H). These cancers show different clinicopathological features from microsatellite stable (MSS) tumours, may respond differently to chemotherapy, and are common in patients with HNPCC. To identify MSI-H tumours, Jenkins et al. (2007, Gastroenterology, 133:48-56) proposed MsPath, a logistic regression model to predict MSI status based on clinical features. The MsPath score, based on model parameter estimates, ranged from 0 (1% probability of MSI-H) to 6.6 (91% probability). The authors recommended a threshold of 1.0 (4% probability) to send samples for MSI testing, in order to have high sensitivity. rs1800734, located in the promoter region of *MLH1*, has been shown to be associated with MSI status (unpublished data; $p=3.38x10^{-6}$, OR=1.74 (95% CI 1.38, 2.20) for the minor allele). We evaluated the predictive abilities of MsPath in a sample of 401 CRC tumours from patients in Newfoundland (NL), re-fit a model which included rs1800734 in the sample from NL, and assessed the predictive abilities of the models in 310 CRC tumours from Ontario (ON). MsPath scores for all samples were calculated using the model published by Jenkins et al. (2007). Logistic regression was used to fit the 6 variables in MsPath and rs1800734, using an additive coding for the SNP. Models were assessed by constructing a receiver operating curve, and calculating the area under the curve (AUC). All analyses were performed using R 2.6.1 (www.r-project.org). In NL, MsPath scores were higher in MSI-H tumours than in MSS/L tumours (mean 3.5 and 1.5, respectively). The AUC was 0.86. Using the cutoff of 1.0, 162 (40%) of the samples would not be sent for MSI testing; 2 of these were MSI-H (sensitivity 94%). The score had a specificity of 44%. When the model was re-estimated in the NL data, including the SNP, rs1800734 was significant at the 5% level, and appeared to be associated with MSI status independently of the variables in MsPath. In the ON data, addition of the SNP did not significantly improve the predictive ability of the model. The model proposed by Jenkins et al. had an AUC of 0.86 in the ON data; the AUC was also 0.86 when rs1800734 was added to the model. Although rs1800734 was significant when added to the model, it did not improve the predictive ability of MsPath. Thus, it is of little benefit to add this SNP to MsPath.

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"Micronesian" Autosomal Recessive Aplastic Anemia. *M. Dasouki[1], S. Abhyankar[2], R. Calado[3].* 1) Dept. of Pediatrics & Medicine, Univ Kansas Med Ctr, Kansas City, KS; 2) Dept of Medicine, Univ Kansas Med Ctr, Kansas City, KS; 3) Hematology Branch. National Heart, Lung, and Blood Institute. National Institutes of Health. Bethesda, MD.

Aplastic anemia is a clinically and molecularly heterogeneous disorder which occurs in various bone marrow failure (BMF) syndromes. Known causes of BMF include Fanconi anemia and the recently described telomeres related disorders. Telomeres are made up of a variable number of tandem repeats (TTTAGGG in humans), show inter-individual length variability and are involved in maintaining genetic stability. Patients with idiopathic aplastic anemia have shorter telomeres than normal controls. Genes known to cause aplastic anemia include: the telomerase RNA component gene (TERC), telomerase reverse transcriptase gene (TERT), interferon-gamma gene (IFNG), NBS1, PRF1 and SBDS. Here, we describe a large Micronesian family with 11 siblings, three of whom developed aplastic anemia at an average age of 15 years. Two of these affected siblings (a sister & brother) died at 9 and 21 years of age respectively. The surviving affected sister is 21 years old now, and was diagnosed with aplastic anemia 5 years ago. She showed no physical signs of Fanconi anemia. Peripheral blood lymphocytes spontaneous, as well as MMC & DEB induced chromosome breakage analysis was normal. Telomere length determined by qRT-PCR was normal in the proband, her parents and her two "HLA matched" phenotypically healthy brothers. These results suggest a new form of recessively inherited aplastic anemia in this Micronesian family. SNP array homozygosity mapping followed by candidate gene DNA sequencing or whole exome sequencing may allow the identification of the genetic cause of this novel aplastic anemia. The telomere complex related aplastic anemias will be reviewed.

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Germline CDH1 mutation can be co-segregated with inherited invasive lobular breast carcinoma in the absence of diffuse gastric cancer. *Y. Bignon[1], X. Ze Ming[1,2], L. Lai Sheng[1,2], C. Lacquet[1], F. Penault-Llorca[1], N. Uhrhammer[1].* 1) Dept Oncogenetics, LOM UMR 484 INSERM, Clermont-Ferrand, France; 2) Sun Yat-Sen University Cancer Center, 651 Dongfeng Road, East, Guangzhou, P. R. China,510060.

Present data are highly suggestive but do not unequivocally prove the co-segregation of germ-line CDH1 mutations and inherited invasive lobular breast cancer (ILBC). In order to clearly demonstrate the role of constitutional CDH1 mutations in ILBC, we conducted a screening study on a large family with five ILBC cases, including two bilateral ILBC cases. Noteworthy no diffuse gastric cancer (DGC) was observed in this family. Screening of the CDH1, BRCA1 and BRCA2 germ-line mutations was performed on blood samples from a Caucasian family with five pathologically confirmed ILBC cases but without DGC case. When available, loss of heterozygosity (LOH) and immunohistochemistry (IHC) analyses were performed on the corresponding tumor samples. Follow-up data is available for all living familial members. Other families with CDH1 germline mutations were reviewed in the local database of our center. The key words 'CDH1, germ-line mutation, lobular breast cancer, diffuse gastric cancer' were employed for searching Pubmed and google scholar. Results: No BRCA1 or BRCA2 mutation was found. A deleterious CDH1 germ-line mutation,c.283C>T (pQ95X),was found in the three living women with ILBC, and a healthy 71-year-old male. The mutation was also present in two obligate carriers, non of them died of DGC. According to the "two hits" model, loss of the second CDH1 allele in one of the breast tumors was attested by LOH and IHC studies. Four patients with both ILBC and DGC in hereditary DGC families have been reported in the literatures, with ILBC occurring either synchronously or prior to DGC , the lapse from the diagnosis of ILBC to the diagnosis of DGC was 8-9 years in non-synchronous cases, whereas two of the three living ILBC cases in the present family had ILBC diagnosed more than 12 years ago. A second family with a germ-line CDH1 mutation (c.1582del) without any DGC cases was found in our database, the proband was affected by a pathologically confirmed ILBC at age 48, after 15 years of follow-up there was no evidence of DGC in this woman or her relatives. No follow-up data for other ILBC cases with CDH1 mutation was available in the literatures. In conclusion, germline CDH1 mutation can be co-segregated with ILBC in the absence of DGC. The risk of DGC in these families and the underlying molecular mechanism leading to this phenotypic divergence, such as the epistatic effects of other genes, merit further study.

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Incidence of germ-line SUFU mutations in sporadic medulloblastoma. *A. Remenieras[1], J. Bombled[1], G. Pierron[2], M. Barrois[1], J. Grill[4], P. Varlet[3], O. Delattre[2], B. Bressac-De Paillerets[1], L. Brugières[4].* 1) Department of Biopathology, Institut Gustave Roussy, 94805 Villejuif, France; 2) Unité INSERM 830. Laboratoire de Génétique et Biologie des Cancers Section de Recherche Institut Curie, 75005 Paris, France; 3) Department of Neuropathology, Sainte-Anne Hospital, UMR Inserm U894; Psychiatry and Neurosciences Center, University Descartes Paris 5, France; 4) Department of Pediatric Oncology, Institut Gustave Roussy, 94805 Villejuif, France.

Medulloblastoma (MB) is the most common pediatric brain tumor. Five histologic subtypes are described: classic MB, desmoplastic/nodular MB, MB with extensive nodularity (MBEN), anaplastic and large cell MB. Involvement of the sonic hedgehog signaling pathway in the genesis of these tumors has been well established with the presence of somatic mutations in Patched 1, suppressor of fused (SUFU) or smoothened mainly in the desmoplastic/nodular subtype. Several cases of patients with desmoplastic/nodular MB or MBEN carrying germ-line mutations of the SUFU gene have been described including two families previously described by our group. To assess the incidence of SUFU germ-line mutations according to the age at diagnosis and histologic subtype in patients with sporadic MB, the 12 exons of SUFU were analyzed by direct sequencing of genomic DNA in all 119 consecutive patients treated for a MB in the pediatric department of the Institut Gustave Roussy and for whom a blood sample was available. In this series, 50 patients were under 4 at diagnosis. Patients with a familial history of MB were excluded from the analysis. Additionally a Q-PCR was performed to identify genomic rearrangements. Putative splice site mutations were subsequently studied by transcript analysis. Six mutations were identified by sequencing: 3 were frameshift mutations, 1 was a nonsense mutation and 2 were intronic (one targeted exon 11 splice site (c.1297-1G>C) and the other was a c.318-10delT). In the last case, transcript analysis indicates that c.318-10delT mutation induces skipping of exon 3. Mutations were inherited from a healthy parent in 3/5 cases in which the parents could be tested and it was a de novo mutation in the 2 other cases. The characteristics of patients with a germ line mutation of the SUFU gene were a young age at diagnosis(6/6 under 4 at diagnosis) and a desmoplastic/nodular or MBEN histology (6/6 patients). Additonally, 11 different unknown variants were identified in 13 patients. Some of them may be ethnogeographic polymorphisms as we identified them more than once in patients with African origin. No rearrangement was identified by Q-PCR. Germ-line mutations of the SUFU gene are not a rare event in patients diagnosed during the first years of age with a desmoplastic/nodular MB or MBEN even in the absence of familial history of cancer.

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Inactivation of SDHD in the pituitary tumor of a patient with acromegaly and familial paragangliomas due to a novel SDHD mutation. *P. Xekouki[1], MQ. Almeida[1], A. Lytras[2], M. Azevedo[1], E. Ball[1], B. Pasini[3], K. Pacak[4], A. Horvath[1], G. Tolis[2], CA. Stratakis[1].* 1) Section on Endocrinology Genetics, Program on Developmental Endocrinology Genetics (PDEGEN), Eunice Kennedy Shriver National Institute of Child Health & Human Development (NICHD), National Institute of Health (NIH), Bethesda, Maryland 20892,USA; 2) Division of Endocrinology & Metabolism, "Hippocrateion" General Hospital, Athens, Greece; 3) Department of Genetics, Biology and Biochemistry, University of Torino, Italy; 4) Reproductive and Adult Endocrinology Program, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892, USA.

Mutations in the subunits B, C and D of succinate dehydrogenase (SDH) mitochondrial complex II have been associated with the development of paragangliomas (PGLs), Carney-Stratakis syndrome (CSS), as well as with renal and papillary thyroid cancer (SDHB). In addition, a case of testicular seminoma has been reported in association with SDHD mutation. Here, we report a unique case of acromegaly and multiple familial extra-adrenal PGLs. The proband, a 37 year-old male, presented with a pituitary macro-adenoma. During pre-surgical imaging for trans-sphenoidal surgery (TSS), bilateral carotid body masses with characteristics typical of PGL were revealed. Biochemical evaluation showed elevated plasma and urinary levels of catecholamines and further imaging showed multiple PGLs; bilateral adrenalectomy revealed extra-adrenal PGLs in close proximity to the adrenal glands, rather than the expected pheochromocytomas. During TSS, a GH-secreting macroadenoma was partially removed. The patient was placed on somatostatin (SMS) analogue therapy with near-complete remission of acromegaly. Genetic analysis revealed a novel SDHD mutation (c.298_301delACTC), leading to a frame-shift and a premature stop codon at position 133 of the protein; the patient's peripheral leukocytes mRNA confirmed SDHD haploinufficiency. MEN1, AIP and CDKN1B mutations were not found; two common polymorphisms were detected in the CDKN1B (c. 326 T>G p.V109G) and AIP (c. 682 C>A, p. Q228K) genes. SDHD Loss-of-heterozygosity (LOH) in the GH-secreting adenoma was investigated by quantitative real-time RT-PCR. SDHD protein expression was assessed by Western blot and immunohistochemistry (IHC). Amplification of the SDHD WT allele was significantly reduced in the pituitary tumor in relation to the patient peripheral blood (SDHD WT allele copy number, 0.3 ± 0.04 vs. 1.0 ± 0.01; $p < 0.0001$). SDHD expression by Western blot and IHC was decreased in the pituitary tumor with SDHD mutation, compared to a GH-secreting adenoma negative for SDHD mutation. In conclusion, we describe the first kindred with a germline SDHD pathogenic mutation, inherited PGLs and acromegaly due to a GH-producing pituitary adenoma. SDHD LOH and down-regulation of protein in the GH-secreting adenoma supports SDHD's involvement in the pituitary tumor formation in this patient.

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Genome Wide Scan of Malignant Melanoma Identifies Additional Pigmentation Gene Influencing Risk. *C.I. Amos[1], J.E. Lee[2], W.V. Chen[1], E. Pugh[3], K.F. Doheny[3], C.C. Laurie[4], L.-E Wang[1], Q. Wei[1].* 1) Dept Epidemiology, MD Anderson Cancer Ctr, Houston, TX; 2) Dept Surgery, MD Anderson Cancer Ctr, Houston, TX; 3) Center for Inherited Disease Research, 333 Cassell Drive, Suite 2000, Baltimore, MD; 4) Department of Biostatistics, Box 359461, University of Washington, Seattle, WA.

Malignant melanoma causes 75% of skin-cancer related death and siblings of cases have an approximately 4-fold higher melanoma risk, suggesting the importance of genetic factors in its etiology. We therefore conducted a genome-wide association analysis of melanoma using samples and data from 1781 melanoma cases from M.D. Anderson Cancer Center and 1050 age and sex-matched healthy controls. Samples were anlyzed using an Omni 1M Quad V1-0_B SNP chip and standard quality control filters were applied with assistance from the GENEVA coordinating center including removing SNPs and samples with low call rates (<95% and < 90% respectively), and removing non-Caucasians and genetic outliers. After filtering, 818237 SNPs had minor allele frequency >0.01, passed the Hardy-Weinberg Disequilibrium test (p> 10-5) and were retained for analyses. Analyses were conducted using logistic regression in PLINK. Results identify known causal regions such as MC1R (rs4785751, p=3.4 x 10-10) and CDK2NA region (rs1889680, p=4.89 x 10-7). However, in addition one SNP in HERC2 reaches genome wide significance (rs12913832, p=7.9 x 10-8) and several additional interesting candidate loci are highly suggestive: DACH1 (rs17613530, 5.7 x 10-6) and CSMD1 (rs2617055, p= 5.9 x 10-6). HERC2 influences hair and eye pigmentation (iris color), and skin sensitivity to sun and freckling. We are further investigating the effects of these SNPs according to skin pigmentation type and history of sunburns. These results further expand knowledge about the role of skin pigmentation in determining risk for sporadic melanoma.

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Copy Number Variation Associated with Risk of Aggressive Prostate Cancer. *F. Demichelis[1,2], S.R. Setlur[3], S. Banerjee[4], C.X. Chen[1], D. Oldridge[1], B. Stenzel[5], J.Y.H. Chen[3], N. Kitabayashi[1], M. Poptsova[1], G. Schaefer[5], J. Bektic[5], G. Bartsch[5], C. Lee[3], H. Klocker[5], M.A. Rubin[1].* 1) Department of Pathology and Laboratory Medicine, Weill Cornell Med College, New York, NY; 2) Institute for Computational Biomedicine, Weill Cornell Med College, New York, NY; 3) Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA; 4) Department of Public Health, Weill Cornell Med College, New York, NY; 5) Department of Urology, Innsbruck Medical University, Innsbruck, Austria.

Prostate cancer (PCA) remains a major public health problem with over 219,000 cases diagnosed per year in the U.S. and 27,000 expected deaths. There is widespread consensus that we need biomarkers to identify aggressive PCA at the earliest point. Multiple genome-wide association studies and follow-up validation studies have discovered a few dozen SNPs associated with PCA risk, however their effect is low to moderate with unknown biological implications. Herein, we report for the first time a comprehensive survey to discover germline Copy Number Variants (CNVs) associated with risk of aggressive PCA. **Methods**: Germline DNA from 742 men with PCA (cases) and 811 men with negative prostate biopsies (controls) from the PSA Screening Tyrol Cohort (Austria) was profiled using a genome-wide oligonucleotide platform (Affymetrix 6.0). SNP-based analysis was applied to control for ethnicity and genotype similarity (e.g., first degree relatives). 5,239 CNV loci (30% previously unreported) were evaluated by applying a computational approach for the Identification and genotyping of germline Changes in Copy Number (IgC2N). We explored for age and PSA-adjusted CNVs associated with increased risk of developing PCA and aggressive PCA. **Results**: Twenty-two of 56 previously reported PCA risk SNPs were validated including loci at 6q, 7p, 7q, 8q (region 1 and 3), and 17q. Of the investigated CNVs, 43% had frequencies below 10%. Sixty-seven CNVs showed significant association with one of the two phenotypes (FDR<10%). For the majority of the CNVs that showed an association, the minor allele frequencies were < 20% and the best tagged SNP had an R^2 < 0.5. 47% of the top ranked CNVs overlapped or were in proximity of gene coding regions and 4 of these showed significant allelic RNA expression imbalances in a set of 50 prostatic tissue samples. Gene ontology analysis of the top ranked CNVs for aggressive PCA risk nominated the calcitonin gene-related polypeptide receptor, which is known to function as a proangiogenic growth factor, suggesting a possible underlying mechanism for cancer progression. **Conclusions**: We have identified CNVs in gene coding regions as having significant associations with the risk of PCA and more importantly aggressive PCA. Germline risk factors might help guide clinicians toward active surveillance or definitive treatment (eg, surgery, radiation therapy). Three candidate CNVs have been identified and are being validated on a large US PSA screening cohort.

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Genetic analysis of neuroblastoma in African American patients. *M. Devoto[1,4], V. Latorre[1], S. Diskin[2], M. Diamond[2], H. Zhang[3], H. Hakonarson[1,3,4], J. Maris[2,4].* 1) Division of Human Genetics, The Children's Hospital of Philadelphia, Philadelphia, PA; 2) Division of Oncology, The Children's Hospital of Philadelphia, Philadelphia, PA; 3) Center for Applied Genomics, The Children's Hospital of Philadelphia, Philadelphia, PA; 4) Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, PA.

In the last two years, genome-wide association studies in neuroblastoma patients have revealed several loci associated to increased risk of disease. These include a region of chromosome 6p22 containing the predicted genes FLJ22536 and FLJ44180, the genes BARD1 on chromosome 2q35 and LMO1 on chromosome 11p15, and a copy number variable region on chromosome 1q21. These studies have all been carried out in North American patients of European descent, which constitute the majority of neuroblastoma cases. Extension of these findings to other ethnic cohorts is important to confirm their validity beyond the Caucasian population. The different pattern of linkage disequilibrium present in other ethnic groups may also help to better define the limits of the association signals and identify specific causal variants. We have collected a group of 326 self-reported African-American neuroblastoma patients and genotyped them using the same high-density SNP chips that were used for the study of the patients of European descent, namely the Illumina HumanHap 550K and 610Quad. A group of 2500 African-American unaffected children genotyped on the same platforms was identified to use as controls in the association tests. Standard quality control procedures were applied to remove samples with low call rates and high heterozygosity, and SNPs with low call rates, excess deviation from Hardy-Weinberg equilibrium in controls, and minor allele frequency less than 0.01. Association analysis was performed by stratified Cochran-Mantel-Haenszel chi-square test after multi-dimensional scale clustering of cases and controls in homogeneous groups based on genome-wide data to account for population substructure. Among the known loci, the one that was most consistently replicated was BARD1, with six SNPs showing p<0.05 (smallest p=0.0004 for rs7587476). Two SNPs in the FLJ22536/FLJ44180 region had nominally significant p-value (smallest p=0.02 for rs1928174), and only one in LMO1 (p=0.02 for rs4237769). Overall these results support the hypothesis that the same genetic factors affect risk of neuroblastoma in both populations, but a larger number of cases is necessary to definitely confirm the findings from the Caucasian cohort in the smaller African-American sample.

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The genetics and epidemiology of colorectal cancer consortium (GECCO): An international collaboration. *T.A. Harrison[1], T.J. Hudson[2], A.T. Chan[3], P.A. Newcomb[1], B.J. Caan[4], C.M. Ulrich[5], L. Hsu[1], D.J. Duggan[6], M.L. Slattery[7], S.B. Gruber[8], G. Rennert[9], G.E. Goodman[10], R.L. Prentice[1], S.J. Chanock[11], R.B. Hayes[12], A. Hazra[13], J. Ma[13], S. Bézieau[14], M. Hoffmeister[15], S. Gallinger[16], B.W. Zanke[2], R.E. Schoen[17], E. White[1], J.C. Figueiredo[18], G. Casey[18], L. Le Marchand[19], J. Chang-Claude[20], J.D. Potter[1], H. Brenner[15], U. Peters[1].* 1) Publc Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA; 2) Ontario Institute for Cancer Research, Toronto, ON, Canada; 3) Gastrointestinal Unit, Massachusetts General Hospital, Boston, MA, USA; 4) Division of Research, Kaiser Permanente, Oakland, CA, USA; 5) Division of Preventive Oncology, German Cancer Research Center, Heidelberg, Germany; 6) Genetic Basis of Human Disease Division, Translational Genomics Research Institute, Phoenix, AZ, USA; 7) Department of Internal Medicine, University of Utah School of Medicine, Salt Lake City, UT, USA; 8) Division of Molecular Medicine and Genetics, University of Michigan, Ann Arbor, MI, USA; 9) Department of Community Medicine and Epidemiology, Carmel Medical Center, Haifa, Israel; 10) Swedish Cancer Institute, Seattle, WA, USA; 11) Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, USA; 12) New York University School of Medicine, New York, NY, USA; 13) Channing Laboratory, Brigham and Women's Hopsital, Boston, MA, USA; 14) Centre Hospitalier Universitaire (CHU) de Nantes, Pôle de Biologie, Service de Génétique Médicale, Nantes, France; 15) Division of Clinical Epidemiology and Aging Research, German Cancer Research Center, Heidelberg, Germany; 16) University Health Network, Toronto General Hospital, Toronto, ON, Canada; 17) Division of Gastroenterology, Hepatology, and Nutrition, Presbyterian University Hospital, Pittsburgh, PA, USA; 18) Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA; 19) Epidemiology Program, Research Cancer Center of Hawai'i, University of Hawai'i, Honolulu, HI, USA; 20) Division of Cancer Epidemiology, German Cancer Research Center, Heidelberg, Germany.

As with many complex diseases, it is recognized that low-penetrance variants likely play an important role in conferring risk of colorectal cancer. Large-scale genome-wide association studies (GWAS) and replication studies are needed to elucidate inherited risks, and understand how known environmental contributors to disease risk may modify the effects of associated genetic variants. The Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO) is a collaborative effort comprised of a coordinating center and scientific researchers from well-characterized cohort and case-control studies conducted in North America, Australia, and Europe. Currently, the consortium comprises the following studies with inclusion of over 30,000 participants: the Assessment of Risk for Colorectal Tumours in Canada (ARCTIC) study; the Carotene and Retinol Efficacy Trial (CARET); the Colon Cancer Family Registry (C-CFR); Darmkrebs: Chancen der Verhütung durch Screening (DACHS); the Diet, Activity, and Lifestyle Study (DALS); the Health Professionals Follow-up Study (HPFS); the Multiethnic Cohort (MEC); the Molecular Epidemiology of Colorectal Cancer (MECC) study; the Nurses' Health Study (NHS); the Physicians' Health Study (PHS); the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial; the VITamins And Lifestyle (VITAL) cohort; the Women's Health Initiative (WHI); a case-control study from the University of Nantes in France; and a case-control study from the University of Hawai'i. This international consortium aims to accelerate the discovery of colorectal cancer-related variants by replicating and characterizing GWAS findings, conducting a large-scale meta-analysis of existing and newly generated GWAS data, and performing targeted sequencing and fine mapping. A key strength of GECCO is the ongoing harmonization of detailed clinical, epidemiologic, and outcome data across consortium studies. This will allow evaluation and characterization of GWAS findings with respect to interactions with environmental factors. Future plans include investigation of rare variants, survival studies, and incorporation of gene-expression and tumor characteristics. This international collaboration of studies encompassing over 30,000 research participants with detailed environmental data provides an excellent opportunity to investigate how genetic variants and environmental risk factors contribute to the etiology of colorectal cancer.

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Dissecting the genetic susceptibility architecture of breast cancer using GWAS information and gene expression data. *C. Hicks, A. Pannuti, L. Miele.* Cancer Institute, University of Mississippi Medical Center, Jackson, MS.

Genome-wide association studies (GWAS) are a powerful technique for identifying common genetic variants associated with breast cancer. However, to date, data from GWAS have not been maximally leveraged and integrated with gene expression data to infer the causal association between gene expression and the disease. We present a novel integrative genomics approach that leverages information from GWAS with gene expression data to dissect the genetic susceptibility architecture of breast cancer. We hypothesized that genes containing SNPs associated with breast cancer share the genetic susceptibility architecture functionally, interact with each other and their downstream targets in biological pathways; and that combining GWAS information with gene expression data could increase the power to identify novel genes which could not be identified using GWAS alone. We tested these hypothesizes using 130 genes containing 400 SNPs identified from 40 GWAS covering 120, 000 cases and 130,000 controls; and two gene expression data sets on Caucasian (42 cases and 143 controls) and Asian (43 cases and 43 controls) populations. We performed both supervised and unsupervised analysis assuming the gene and pathway as units of association. We identified 70 candidate genes in the Caucasian population and 30 genes in the Asian population with good evidence ($P<0.05$) of distinguishing breast cancer patients from controls. Pattern recognition analysis revealed that candidate genes are functionally related and identified a novel set of genes which have similar expression profiles with candidate genes. Pathway prediction revealed that genes containing SNPs associated with breast cancer interact with each other and their downstream targets in intricate biological pathways. Among the identified pathways included the P53, apoptosis, kinase and AKT pathways. Additionally, genes containing SNPs with large ($P<10-5$) and small effects ($P<10-3$), had shared patterns of expression and were involved in the same biological pathways. We found differences in patterns of gene expression between Caucasians and Asian populations. Results show that integration of gene expression data with GWAS information provides putative functional bridges between GWAS and pathways, thereby serving as a potential powerful approach to identifying biological mechanisms underlying GWAS findings in breast cancer, and to infer the causal association between gene expression and the disease.

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Gene-asbestos interaction and lung cancer risk from a genome-wide association study. *C. Liu.* Dept Environmental Hlth, HSPH, Boston, MA.

Epidemiologic studies of occupational and population-based case-control studies have consistently observed increased lung cancer risk with occupational asbestos exposure, while the molecular mechanisms of nonmutagenic carcinogens including asbestos remain largely unknown. To evaluate the association between lung cancer linked to asbestos and genetic variability, we conducted a genome-wide association analysis in 1,000 Caucasian cases and 1,000 Caucasian controls using Illumina Human 610-Quad BeadChips. Cumulative lifetime asbestos exposure score (AES) was calculated from self-reported duration and intensity of occupational and nonoccupational exposures. A total of 12.1% of cases and 8.5% of controls had "high" AES (determined by a priori cut point).

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Association scan of all trait-associated SNPs identifies association between pancreatic cancer and SNP in childhood diabetes gene, HNF1A. *B. Pierce, H. Ahsan.* Health Studies, Univ Chicago, Chicago, IL.

Stringent significance thresholds are required in genome-wide association studies (GWAS) because many statistical tests are conducted. As a result, weakly associated SNPs with p-values above genome-wide significance thresholds are not detected, even in large studies. One way to partially alleviate the multiple testing penalty is to focus association studies on subsets of SNPs with high prior probabilities of association. Using data on 1,763 pancreatic cancer cases and 1,802 controls of European descent from the Cancer Genetic Markers of Susceptibility (CGEMS) GWAS study (PanScan-I), we examined associations with pancreatic cancer risk for 1,663 SNPs that have been shown in GWAS studies to associate with human phenotypes other than pancreatic cancer (according to http://www.genome.gov/GWAStudies/). This method has two key advantages: (1) the SNPs tested are very likely to be (or tag) functional variants and (2) the number of SNPs tested is drastically lower than a typical GWAS, resulting in less stringent p-value thresholds. Association tests were conducted using a log-additive genetic model and logistic regression adjusted for age, sex, and two axes of ancestry derived from principal components analysis. Using this method, we have identified many novel candidate SNPs for pancreatic cancer based on departure from QQ plot expectations. Our strongest association signal comes from HNF1A and is statistically significant after a Bonferroni correction. HNF1A is also known to harbor genetic variants that influence risk for maturity-onset diabetes of the young and circulating CRP concentrations. PanScan-II data will soon be available, and we will attempt to replicate this finding prior to this presentation.

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Identification of loci associating with histiocytic-dendritic cell neoplasms using a canine genome wide study. *A.L. Shearin[1], H.G. Parker[1], E. Cadieu[1,2], B. Hedan[2], M. Breen[3], J. Cullen[4], A. Grone[5], G. Rutteman[5], C. Andre[2], E.A. Ostrander[1], E.V. Schmidt[1,6,7].* 1) Cancer Genetics Branch, NHGRI, NIH, Bethesda, MD; 2) Institut de Génétique et Développement de Rennes, Université de Rennes, Rennes, France; 3) Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC; 4) Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC; 5) Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands; 6) Cancer Research Center at Massachusetts General Hospital, Boston, MA; 7) Department of Pediatrics, Harvard Medical School, Boston, MA.

While comparatively rare, histiocytic-dendritic cell neoplasms remain devastating. Their status as human orphan diseases urges adaptation of novel genetic strategies to identify their pathogenesis. Breeding bottlenecks and strong selective pressures have caused strong genetic predispositions for many cancers in specific canine breeds. The 25% incidence of histiocytic sarcomas in Bernese Mountain dogs (BMD) offers a unique opportunity to understand the genetic basis of dendritic cell neoplasms. We performed sequential genome wide association studies (GWAS), initially using 240 BMD samples from the U.S. followed by a confirmatory study using 234 European BMDs. Comparing 19,000 informative SNPs, a single-marker chi-squared analysis corrected for population structure and kinship yielded significant associations with the disease phenotype at 2 loci ($p<1.1 \times 10^{-7}$ and $p<2.5 \times 10^{-6}$). Focused genotyping and sequencing of the most highly associated region identify a 300 kb candidate region. This region contains a disease-associated haplotype for which 75.0% of cases are homozygous versus 20.8% of controls. However, this haplotype is also tightly linked ($r2 \geq 0.8$) with additional individual segments spread over the whole region, making causative mutational analysis difficult. Indeed, ten linked SNPs over the whole region predict 75% of the cases with a false positive rate of 5%. Using GM-CSF/IL4-dependent dendritic cell cultures, we are performing functional analyses of candidate genes in the region. Notably, the segments spread across the region that are linked to its core haplotype contain predicted transcriptional regulatory elements. We are therefore testing the effects of these DNA segments using standard transcriptional analyses to evaluate the possibility that multiple associating regulatory SNPs are involved in the pathogenesis of the histiocytic sarcomas. Since the candidate region is syntenic with several genome-wide human disease associations, our results may offer interesting comparative insights into genetic association studies. A second locus identified in the BMD is largely confined to the European population, possibly because this locus has approached fixation in the U.S. BMD population. Future studies of genetic interactions between these two loci offer the potential to understand regulatory pathways that cause poorly understood histiocytic-dendritic cell neoplasms.

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Identification of multiple genetic variants related to nasopharyngeal carcinoma predisposition through genome-wide scan. *W. Su[1], K. Tse[1], M. Yang[1], Y. Chang[1], Y. Shugart[2,3].* 1) Chang Gung Molecular Medicine Research Center, Chang Gung University, Kwei-Shan, Tao-Yuan, Taiwan; 2) Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 3) Genomic Research Branch, Division of Neuroscience and Basic Behavioral Science, National Institute of Mental Health, National Institutes of Health, Bethesda, MD.

Nasopharyngeal carcinoma (NPC) is a multi-factorial malignancy closely associated with genetic factors and Epstein-Barr virus infection. Recently, we have conducted a genome-wide association study (GWAS) in 277 NPC patients and 285 healthy controls within the Taiwanese population, analyzing 480,365 single-nucleotide polymorphisms (SNPs) using Illumina HumanHap 550 BeadChip. Twelve statistically significant SNPs were identified and mapped to chromosome 6p21.3 in the major histocompatibility complex region. These association signals were replicated in two independent sets of case-control samples. Two of the most significant SNPs (rs2517713 and rs2975042; $p = 3.9 \times 10^{-20}$ and 1.6×10^{-19}, respectively) were located in the HLA-A gene. Moreover, we detected novel significant associations between NPC and two genes: specifically, gamma aminobutyric acid b receptor 1 (GABBR1) (rs29232; $p = 8.97 \times 10^{-17}$) and HLA-F (rs3129055 and rs9258122; $p = 7.36 \times 10^{-11}$ and 3.33×10^{-10} , respectively). Notably, the association of rs29232 remained significant (residual $p < 5 \times 10^{-4}$) after adjustment for age, gender, and HLA-related SNPs. Furthermore, higher GABAB receptor 1 expression levels can be found in the tumor cells in comparison to the adjacent epithelial cells ($p < 0.001$) in NPC biopsies, implying a biological role of GABBR1 in NPC carcinogenesis. To our knowledge, it is the first GWAS report of NPC showing that multiple loci (HLA-A, HLA-F, and GABBR1) within chromosome 6p21.3 are associated with NPC. Following the GWAS study, we made an effort to search systematically for copy number variations (CNVs) that confer increased risk to NPC using the same dataset. We generated CNV calls using four different softwares and used 15 internal duplicated samples to look for best filtering criteria. CNVs were selected if same CNVs were identified from two independent softwares to increase credibility of prediction. Association testing and follow-up replication analyses confirmed the CNVs were associated with NPC.

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Excess Germline Copy Number Variations Detected in Familial Pancreatic Cancer. *J.A. Willis, S.H. Olson, R.C. Kurtz, R.J. Klein.* Memorial Sloan-Kettering Cancer Center, New York, NY.

Background: Pancreatic adenocarcinoma is a rapidly fatal disease. Identifying the human germline variations involved in pancreatic cancer may help to uncover novel therapeutic targets and to develop clinical screens. Although single-nucleotide polymorphisms (SNPs) have been associated with pancreatic cancer risk, the contribution of germline copy number variations (CNVs) remains unclear. To explore this contribution, we performed genome-wide CNV discovery and analysis using a case-control cohort. Methods: 263 pancreatic cancer cases and 203 healthy, unrelated controls were recruited for this study at the Memorial Sloan-Kettering Cancer Center. DNA was collected from each individual via blood, saliva, or buccal sample and genotyped on the Illumina Human CNV370-duo SNP array. Normalized probe data (log R ratio and “B allele frequency”) from the array were processed separately for each individual by a hidden Markov model (PennCNV) to generate CNV calls. We used logistic regression to test for differences in overall CNV burden, adjusting for sample quality, ancestry, DNA source, age, and experimental batch effects. Results: Our discovery experiment yielded a total of 32,146 CNV calls in the study cohort. Quality-control filtering was applied at the sample-level by excluding those individuals with B allele frequency drift >0.002, log R ratio standard-deviation >0.24, and greater than 200 CNV calls. A total of 40 samples (9,619 CNVs) were removed in this filtering step. Our final dataset was derived from 244 cases and 182 controls, and is comprised of 22,527 CNV calls (14,017 deletions; 8,510 duplications). Our preliminary analysis has identified a significant excess of CNV burden in cases ($n = 32$) having multiple family history of pancreatic cancer versus controls (case-control burden ratio = 1.34; per-CNV odds ratio = 1.013, $p = 0.032$). Conclusions: Excess germline CNV burden was identified in cases with multiple family history of pancreatic cancer. To understand the biological basis of this association, we are exploring whether specific CNV loci (or gene targets) are commonly detected in this group.

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A rare deletion on chr18q22.1 contains a palindromic AT-rich repeat and may increase risk of ileal carcinoids. *K. Walsh[1], A. DeWan[1], M. Choi[1], L. Hsu[1], M. Jurkiewicz[1], J. Yao[2], M. Kulke[3], K. Oberg[4], J. Hoh[1].* 1) Division of Chronic Disease Epidemiology, Yale University School of Public Health, New Haven, CT; 2) Department of Gastrointestinal Medical Oncology, MD Anderson Cancer Center, Houston, TX; 3) Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA; 4) Department of Endocrine Oncology, University Hospital, Uppsala, Sweden.

Carcinoid tumors are rare neuroendocrine tumors with high genomic instability, most frequently found in the small intestine. Little is known about the genetic factors involved in the disease, but loss of chromosome 18q22-qter is an early event in ileal carcinoid tumorigenesis. We hypothesized that rare constitutional copy-number variants (CNVs) may predispose some individuals to develop ileal carcinoids. We applied multiple CNV calling algorithms to Illumina 300K array data to identify CNVs involved in carcinoid pathogenesis. Although no CNVs were significantly associated with case-status, a rare 40kb deletion at Chr18q22.1 was identified in five cases but no controls. qPCR analysis on the full case-control set identified this deletion in 14/226 cases (6.6%) and 2/98 controls (2.1%). This intergenic deletion is located in a genomic region which is lost in 60% of all ileal carcinoid tumors. The deletion is not flanked by any regions of high sequence homology, making non-allelic homologous recombination an unlikely mechanism for the genesis of this CNV. At the center of the deleted region is a palindromic AT-rich repeat, which may play a role in formation of this CNV. Palindromic repeats can cause DNA to form cruciforms and are known to induce double-strand breaks, causing non-Robertsonian translocations. However, it is unknown if palindromic repeats can induce other types of chromosomal aberrations, such as CNVs. To explore this, data from the Database of Genomic Variants and the UCSC genome browser were used to determine if palindromic repeats are associated with CNVs in the human genome. Dinucelotide repeats greater than 30 bases were analyzed, of which 6,291/37,429 (16.8%) were palindromic (ATn or TAn). Compared to other dinucleotide repeats, palindromic repeats were more commonly found within CNVs ($p = 0.016$). When stratified by CNV type, palindromic repeats were significantly associated with deletions ($p = 0.014$), but not duplications ($p = 0.333$). After controlling for length and the overlap with genes, LINEs, SINEs, and LTRs, the association between palindromic repeats and CNVs remained significant in a logistic regression model ($p = 0.0176$). Further work is needed to determine if this candidate CNV influences carcinoid risk, and if palindromic repeats promote CNV formation. Because this association is driven by copy-number deletions, potential mechanisms must account for the formation of deletions without reciprocal duplications.

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Mining GWAS Data to Find Etiologic Variation Common to NSCL/P and Breast Cancer. *B. Erickson[1], K.K. Ryckman[2], J.C. Murray[1,2].* 1) Department of Molecular & Cellular Biology, University of Iowa, Iowa City, IA; 2) Department of Pediatrics, University of Iowa, Iowa City, IA.

Background: Epidemiologic data has shown an increased incidence of breast cancer in women born with nonsyndromic cleft with or without cleft palate (CL/P). Several studies of both breast cancer and nonsyndromic CL/P have found association within intron 2 the *FGFR2* locus. Several GWAS studies have been published on breast cancer and CL/P and we mined this data for other sites of genetically associated variation shared between these two disease phenotypes. **Objective:** Investigate common regions of GWAS signals between recent breast cancer and CL/P studies. **Methods:** Seven breast cancer GWAS studies and five CL/P GWAS studies were identified for use in this study. We generated a list of SNPs associated at significance ($< 10^{-5}$) ordered by genome position from published data and dbGAP. Regions were noted as having common GWAS signals between breast cancer and CL/P studies if SNPs were located within a common linkage disequilibrium (LD) block, or in cases where LD block structure was less defined, SNPs separated by less than 1.5 Mb. In regions with common GWAS signals, we are using TaqMan genotyping and sequencing to identify rare SNPs associated with CL/P that may have functional importance. **Results:** Only 35kb separate breast cancer GWAS (rs2981582) and CL/P GWAS (rs2912787) SNPs near *FGFR2*. These SNPs are within one LD block in the CEU Hapmap population. Chromosome 2 was the location of the next closest common GWAS signal between CL/P (rs11900952) and breast cancer (rs17030257) 135kb apart SNPs surrounding the thyroid adenoma associated gene (*THADA*). Chromosome 10q21.2 near Zinc Finger Protein 365 (*ZFN365*) was a common GWAS site with a CL/P SNP (rs1475084) separated from a breast cancer SNP (rs10995195) by 317kb. The final common GWAS signal site was at 8q24 between CL/P (rs7015145) and breast cancer (rs13281615) SNPs separated 1.4 MB. **Conclusions:** The proximity of GWAS signals suggests an element in or near intron 2 of *FGFR2* plays a role in both CL/P and breast cancer. The common GWAS signals in close proximity on 10q21 and 2p21 are currently being genotyped and sequenced with breast cancer signals prioritize focusing smaller regions to find a CL/P etiologic variant. While the separation of the SNP sites near 8q24 may suggest different etiologic variants near that locus contributing to each disease, a common etiologic variant risk for CL/P and breast cancer could still be identified with closer genotyping and sequencing.

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Discovering epistatic genetic interactions associated with neuroblastoma. *M. Capasso[1], K. Bosse[2], S.J. Diskin[2], Y.P. Mosse[2], H. Hakonarson[3], A. Iolascon[1], M. Devoto[4], J.M. Maris[2].* 1) CEINGE Biotecnologie Avanzate, Napoli, Italy; 2) Children's Hospital of Philadelphia, Center for Childhood Cancer Research, Philadelphia, PA, USA; 3) The Children's Hospital of Philadelphia, Center for Applied Genomics, Philadelphia, PA, USA; 4) The Children's Hospital of Philadelphia, Division of Genetics, Philadelphia, PA, USA.

Background. We have demonstrated that polymorphisms in the BRCA1 interacting gene BARD1 are associated with high-risk neuroblastoma (NBL) using a genome-wide association approach (Capasso, Nat Genet 2009). The mechanism by which BARD1 impacts susceptibility to NBL remains undefined. Here, using a two-locus analytic method, we sought to identify genes that might increase susceptibility to NBL development by their interaction with BARD1.

Methods. We performed an interaction analysis based on two datasets comprising 1433 cases and 3221 controls, and 1627 cases and 2575 controls, respectively, genotyped with the Illumina HumanHap 550K. Six databases (BIND, BIOGRID, MINT, HPRD, STRING, IntAct) were queried to identify the proteins known or predicted to interact with BARD1. A regression analysis method implemented in PLINK was utilized to test for pairwise interactions between BARD1 SNPs and SNPs of these genes.

Results. A total of 109 proteins were reported in the six databases as known or predicted to interact with BARD1. Twenty-two of these were included in three or more of the databases, and were the focus of subsequent interaction analyses. In the first dataset, the most significant interaction was found between the intronic SNP rs919581 of the PTN gene (pleitropin) and rs17487792 of BARD1 (OR=1.45, $P=9\times10^{-5}$). The PTN SNP showed no effect in the single SNP analysis (OR=0.97, P=0.50) while the BARD1 SNP showed highly significant association with NBL (OR=1.36, $P=2\times10^{-16}$). The statistical interaction between the two SNPs was confirmed in the second dataset (OR=1.45, $P=1\times10^{-4}$). The OR of the highest-risk genotype (homozygote at the minor alleles for rs17487792 and rs919581) relative to the most common genotype at the two loci combined was 9.69. SNP rs17487792 is in high LD (r2=0.96) with rs2070096, which is predicted to regulate BARD1 splicing. Experiments are ongoing to test if BARD1 isoforms related to this SNP affect physical interaction with PTN.

Conclusions: PTN and BARD1 variants may act in an epistatic fashion to promote NBL tumorigenesis. Future work will focus on identifying the mechanism of this interaction, and discovering if somatically acquired alterations in these genes may contribute to a high-risk NBL phenotype.

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Variation at 9p24.3 (*DMRT1*) is associated with susceptibility to testicular germ cell tumor. *S.L. Ciosek[1], P.A. Kanetsky[1], N. Mitra[1], S. Vardhanabhuti[1], D.J. Vaughn[1], M. Li[1], R. Letrero[1], K. D'Andrea[1], D.R. Doody[2], J. Weaver[3], A. Albano[4], C. Chen[2,5], J.R. Starr[2,5,6], D.J. Rader[1], A.K. Godwin[3], H. Hakonarson[4], M.P. Reilly[1], S.M Schwartz[2,5], K.L. Nathanson[1].* 1) University of Pennsylvania School of Medicine, Philadelphia, PA; 2) Fred Hutchinson Cancer Research Center, Seattle, WA; 3) Fox Chase Cancer Center, Philadelphia, PA; 4) The Children's Hospital of Philadelphia, Philadelphia, PA; 5) School of Public Health, University of Washington, Seattle; 6) School of Medicine, University of Washington, Seattle, WA.

Testicular germ cell tumor (TGCT) is the most common malignancy diagnosed in young men. Risk factors include family history and undescended testis. We previously have shown that the rare gr/gr deletion increases risk for TGCT (Nathanson 2005). In genome-wide association studies, we and our UK colleagues independently identified SNP markers in or near *KITLG* and *SPRY4* as risk alleles for TGCT (Kanetsky 2009; Rapley 2009). *BAK1* also was found to increase risk. Here we report the results of our expanded GWAS that included 72 additional (a 26% increase) TGCT cases. In total, we compared 349 TGCT cases to 919 controls and found 6 markers at 5 novel autosomal loci that surpassed our secondary significance threshold (5×10^{-6}). After imputation to determine those hits likely to be falsely positive, we brought 2 markers each from 3 loci (at or near *DMRT1*, *PFTK1*, *SLC25A31*) into replication. The replication set consisted of 439 cases and 960 controls from a population-based case-control study in Washington state and parents of 204 of the cases. We observed an association between TGCT and rs7040024 ($P_{trend}=2.1 \times 10^{-6}$) and rs755383 ($P_{trend}=2.1 \times 10^{-4}$), both of which map to *DMRT1*; markers mapping to *PFTK1* and *SLC25A31* did not replicate. The per-allele odds ratio for the major A-allele in rs7040024 was 1.69 (95% CI 1.36, 2.10), and homozygous carriage of the major A-allele increased risk nearly three-fold (OR 2.96; 95% CI 1.52, 5.73) as compared to homozygous carriage of the minor C-allele. In a case-parent analysis, inheritance of two A-alleles at rs7040024 was associated with over a three-fold risk (OR 3.59; 95% CI 1.58, 8.12). Associations did not differ by tumor subtype, and there were no differences found when the cases were stratified by family history of TGCT or personal history of undescended testis. *DMRT1*, or doublesex and MAB3 related transcription factor 1, codes for a DNA binding protein that is expressed in the nuclei of Sertoli cells, spermatogonia and spermatoctyes of adult males. *DMRT1* is conserved across many species, and is known to play a role in testis determination in mammals, as well as sex determination in vertebrates. Of particular interest, all TGCT susceptibility loci identified to date affect pathways that regulate the survival, proliferation and migration of primordial germ cells. Additional genetic studies as well as studies to elucidate the biological basis of the increased risk of TGCT associated with the identified loci are planned.

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Fine-Scale mapping of breast cancer loci: From tag SNP to causal variant. *M. Ghoussaini[1], A. Cox[2], E. Dicks[1,3], P. Pharoah[1,3], D. Easton[1], A. Dunning[1], BCAC (Breast Cancer Association Consortium).* 1) Department of Oncology, University of Cambridge, Strangeways Research Laboratory, Worts Causeway, Cambridge, CB1 8RN; 2) Institute for Cancer Studies, University of Sheffield, Sheffield, UK; 3) Department of Public Health and Primary Care, University of Cambridge, Strangeways Research Laboratory, Worts Causeway, Cambridge, CB1 8RN.

More than 260 new disease loci, including 18 breast cancer loci, have been discovered via Genome Wide Association Studies (GWAS) but, for almost all of these, identifying the causal variant remains a challenge. Identification of the causative variants is central to understanding the genes and mechanisms involved in cancer pathogenesis. We generated a catalogue of all 20,020 common variants in the LD blocks, with a frequency of 2% or greater in Europeans, defined by 18 known breast cancer susceptibility variants, using data from the 1000 Genomes Project (www.1000Genomes.org). These regions range in size from 30 to 1050Kb. Two loci contain no known genes, four contain a single gene and the majority contain several. Total numbers of common variants per locus vary widely (452 to 2,696). The number of SNPs that are candidates for being the primary causative variant (defined as correlated with the best hit with r2 more than 0.1) range from 42 to 675 per locus. An example is the 8q24 breast cancer locus, which spans 150Kb and contains no known genes. It is located ~ 800 kb downstream of the FAM84B gene and ~400 kb upstream the pro-oncogene c-MYC. Using data from the 1000 Genomes Project, we catalogued 603 variants in this locus, of which 227 have a detectable correlation with GWAS tag SNP rs13281615 and were thus considered to be potentially causative. Sixty-eight tag SNPs for these 227 variants have been genotyped in 13,410 cases and controls from the SEARCH study. A new variant (rs1562430) was found to be more significantly associated with breast cancer than the original GWAS tag SNP (p-trend=5.8x10-7, OR=0.85,CI= [0.80-0.91]) versus (p-trend=2.2x10-5, OR=1.14, CI= [1.07-1.21]), correlation between the two variants is 0.40. Further refinement of the set of potential causal variants may be possible through genotyping in case-control studies of different ethnicities.

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Genome-wide association analysis of familial lymphoma. *V. Joseph[1], T. Kirchhoff[1], A. Dutra-Clarke[1], V. Devlin[1], N. Hansen[1], J. Przybylo[1], J. Bhatia[1], M. Kedmi[1], J. Maragulia[1], S. Mukherjee[2], DB. Yehuda[3], L. Engel[4], J. Brown[5], O. Paltiel[3], R. Klein[2], A. Zelenetz[1], K. Offit[1,2].* 1) Dept. Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY; 2) Sloan-Kettering Institute, Memorial Sloan-Kettering Cancer Center, New York, NY; 3) Hadasaah University, Jerusalem, Israel; 4) Department of Epidemiology and Biostatistics, Memorial Sloan-Kettering Cancer Center, New York, NY; 5) Dana Farber Cancer Center, Boston, MA.

The incidence of lymphoma has doubled over the past two decades in the U.S; however, the etiology of the most common types of the disease remains unknown. There is increasing evidence that points to genetic predisposition in the development of lymphomas. Genome-wide association analysis (GWAS), have proven to be useful tools for the identification of common inherited susceptibility loci for human cancers. With increased public availability of population control data, GWAS designs now significantly benefit from reduction in genotyping and cost. In this study, we sought to discover novel germ line variants predisposing to lymphoma using GWAS. We carried out a two-stage association analysis to identify common genetic loci conferring risk for development of lymphomas. In the first stage we have performed a GWAS using Affymetrix 6.0 SNP arrays on 292 kindreds of lymphoma showing family history of the disease, affecting at least 2 first degree relatives. The phase 1 is comprised of 46 patients (probands) with Hodgkin's disease (HD) and 246 cases of non-Hodgkin's lymphoma (NHL), of which 26% are follicular subtypes and 35% diffuse large cell subtypes. As controls, we have used publically available data acquired from dbGAP initiative on 1013 individuals genotyped by Affymetrix 6.0 as part of schizophrenia and bipolar disorder GWAS. To reduce population heterogeneity, both cases and controls chosen were of European (EU) ancestry. After rigorous QC and population stratification adjustments, in stage 1, we identified 200 SNPs with p<10E-4; one of which reached the genome-wide level of significance (p<10E-7). The top 150 independent loci were passed to a replication stage on 2500 lymphoma cases and 2500 controls, all of EU ancestry. Approximately 20 SNPs replicated initial associations observed in Phase 1. Using multivariate analysis, we are currently testing these findings for associations with lymphoma subtypes. There is preliminary evidence of germ line variants in humans increasing risk for lymphocytic malignancies. Moreover, for a subset of SNPs, we observe significant association with multiple subtypes indicating a more common genetic origin of lymphoid malignancies. The observed associations point to involvement of novel molecular pathways which may provide new insights into the molecular pathogenesis of lymphoma.

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Genome Wide Association Study to Identify Single Nucleotide Polymorphisms (SNPs) Associated with the Development of Erectile Dysfunction in African-American Men Following Radiotherapy for Prostate Cancer. *S. Kerns[1], H. Ostrer[1], R. Stock[2], W. Li[3], J. Moore[2], A. Pearlman[1], C. Campbell[1], Y. Shao[4], N. Stone[2, 5], L. Kusnetz[2], B. Rosenstein[2, 6].* 1) Department of Pediatrics, New York University School of Medicine, New York, NY; 2) Department of Radiation Oncology, Mount Sinai School of Medicine, New York, NY; 3) Queens/Elmhurst Hospital Center, Department of Radiation Oncology, Jamaica, NY; 4) Division of Biostatistics, New York University School of Medicine, New York, NY; 5) Department of Urology, Mount Sinai School of Medicine, New York, NY; 6) Department of Radiation Oncology, New York University School of Medicine, New York, NY.

Purpose: Clinical factors do not fully explain the variability in development of normal tissue toxicities resulting from radiotherapy for prostate cancer. A predictive tool incorporating genetic factors could therefore assist clinicians and patients in weighing the benefits of radiotherapy with the risks of developing chronic side effects. Methods: A genome-wide association study was performed to identify SNPs associated with erectile dysfunction (ED) among a cohort of African-American prostate cancer patients treated with external beam radiation therapy. All men were followed-up for a minimum of one year after treatment with the five-item Sexual Health Inventory for Men (SHIM) questionnaire to assess erectile function. Only men with a pre-treatment SHIM score ≥ 16, indicative of good erectile function, were included in the study. Of the 79 patients included, 27 patients were defined as cases based on a post-treatment SHIM score £ 7, and 52 patients were defined as controls based on a post-treatment SHIM score ≥ 16. Results: We identified the rs2268363 SNP, located in the follicle stimulating hormone receptor gene, as significantly associated with ED using a chi-square association test and correcting for multiple comparisons (unadjusted p-value = 5.46x10-08; Bonferroni p-value = 0.028). We identified four additional SNPs that tended toward significant association with unadjusted p-value < 10-06. A multivariate logistic regression model that incorporated estimated ancestry and four of the top-ranked SNPs was a more accurate classifier of ED than a model that included only clinical variables. Conclusions: To the best of our knowledge, this is the first genome wide association study to identify SNPs associated with adverse effects resulting from radiotherapy for prostate cancer. It is also important to note that the SNP that proved significantly associated with ED is located within a gene whose encoded product plays a role in gonad development and function. Another key finding of this project is that the majority of the top-ranking SNPs were specific to people of African ancestry and would therefore not have been identified among a cohort of patients of European ancestry. This study supports the feasibility of using a genome-wide approach to investigate genetic predisposition to radiation injury and warrants validation with a larger and separate cohort of prostate cancer patients.

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Genome-wide association study to detect CNV associations with Cutaneous Melanoma. *Y. Xu[1], E. Pugh[2], B. Peng[1], A. Schnell[2], E.Y. Lu[1], K. Hetrick[2], W. Chen[1], J.E. Lee[3], L. Wang[1], Q. Wei[1], C.I. Amos[1].* 1) Dept Epidemiology, MD Anderson Cancer Ctr, Houston, TX; 2) Center for Inherited Disease Research, Baltimore, MD; 3) Dept Surgery, MD Anderson Cancer Ctr, Houston, TX.

Cutaneous Melanoma (CM) causes 75% of deaths from skin cancer and has a significant genetic component as evidenced by family studies and genome-wide association analyses, which have identified several causal or associated loci. In order to further study the genetics of CM, we conducted a genetic analysis of cases and controls recruited from the University of Texas M.D. Anderson Cancer Center (UTMDACC). 2053 cases of CM were collected at the UTMDACC starting in 1993. 1063 controls were collected from relatives and friends of patients visiting the UTMDACC during the same time period. These controls were not biologically related to patients included in this study. Genotyping was performed on Illumina HumanOmni1-Quad_v1-0_B array at the Center for Inherited Disease Research (CIDR), and quality controls were applied by the Gene Environment Association Studies (GENEVA) and UTMDACC. For the melanoma data, the median call rate was 99.97% and the error rate estimated from 69 pairs of study sample duplicates was 1e-5. We applied copy number variation (CNV) calling algorithm PennCNV on the data, and the concordance of CNVs for the same study sample duplicates was 73.61%. We performed genome-wide association tests based on CNV calls from PennCNV. We found that the most significant signal located in the region of SPIRE2 gene on chromosome 16q slightly centromeric to the MC1R region. Copy number changes have been validated for a sample of subjects in this region using an RT-PCR quantitative DNA analysis technique.

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Development of breast cancer risk model using haplotype analysis of the Ashkenazi Jewish population. *E. Rinella[1], S. Pramanik[2], S. Guha[2], C. LeDuc[2], H. Ostrer[1], Y. Shao[3], A. Pearlman[1], C. Campbell[1], M.B. Terry[4], R. Senie[4], I. Andrulis[5], M. Daly[6], E. John[7], W. Chung[2].* 1) Human Genetics Program, NYU School of Medicine, New York, NY; 2) Pediatrics, Columbia University Medical Center, New York, NY; 3) Division of Biostatistics, NYU School of Medicine, New York, NY; 4) Epidemiology, Columbia University, New York, NY; 5) Ontario Cancer Genetics Network, Cancer Care Ontario, Toronto, Ontario, Canada; 6) Clinical Genetics, Fox Chase Cancer Center, Philadelphia, PA; 7) Cancer Prevention Institute of California, Stanford University School of Medicine & Stanford Cancer Center, Stanford, CA.

Breast cancer risk has a high heritability, yet the genetic markers revealed thus far only account for 20-25% of familial breast cancer cases. The ability to establish genetic risk models is critical for optimizing screening and treatment of breast cancer, but many more variants must be identified. This study utilizes data mining techniques to build on GWAS results by examining IBD (Identity By Descent) segments and haplotypes among homogeneous cohorts of Ashkenazi Jewish (AJ) women. Methods: AJ women with invasive breast cancer and a family history of breast cancer were recruited along with age-matched AJ controls at New York City research institutions. Affymetrix 500K SNP arrays were used to genotype a discovery cohort of 238 breast cancer cases and 237 controls. Individual SNP analysis was conducted using standard case/control association analysis and validated in a replication cohort of 239 cases and 294 controls. The cohorts were combined for haplotype analysis (using an Expectation-Maximization algorithm) and IBD analysis (using GERMLINE/DASH). Association analysis was performed on haplotypes and clusters of shared segments. Biological significance of haplotypes, IBD segments, and individual SNPs was determined using GSEA (Gene Set Enrichment Analysis). Results: IBD segments of (≥30 SNPs) and haplotypes (≥2 SNPs) appearing in at least 10% of the 1008 study subjects were included for analysis. 147 haplotypes containing at least 10 SNPs were significantly associated with breast cancer risk (p=0.00998 - $5.31x10^{-6}$; OR=1.26 - 1.96). These results enhanced the individual SNP association analysis that revealed 73 SNPs (with minor allele frequencies of at least 5%) that were significantly associated with breast cancer (p_C= 0.001 - $6.16x10^{-7}$; OR=1.29 - 4.45). The haplotype analysis confirmed individual SNP results, both spanning 18 common genes (many not previously reported in association with breast cancer risk) and common pathways involved in apoptosis and transcription. Haplotype analysis reveals an additional 211 genes not covered with the individual SNP analysis. Conclusions: An advantage to studying a homogeneous population of cases and controls, such as the AJ women presented here, is the ability to perform haplotype and IBD analyses. Building disease risk models based on such analyses reduces complexity thereby revealing regions associated with breast cancer that were not detectable using the individual SNP analysis commonly found in GWAS.

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The MutS homolog hMSH4 interacts with eIF3f in mammalian cells. *YL. Chu, X. Wu, C. Her.* School Molecular Biosciences, Washington State University, Pullman, WA.

Accumulating evidence suggests that members of the mismatch repair family participate in diverse cellular functions in addition to their primary action in DNA damage recognition and repair. Being a member of this family of proteins, the molecular mechanisms involved with the human MutS homolog 4, hMSH4, are poorly defined. It is known that hMSH4 is promiscuous and the heterocomplex composed of hMSH4-hMSH5 is capable of binding to synthetic Holliday junctions. In addition, mice lacking the Msh4 gene display defective chromosome synapsis, leading to testicular and ovarian degeneration and, therefore, male and female sterility. Notwithstanding a low but readily detectable level of hMSH4 in mitotic tissues, the biological function of hMSH4 is largely unknown. In order to better understand the molecular mechanisms associated with hMSH4 in human cells, we have set out to identify potential hMSH4 interacting partners. To this end, screening of a human ovary two-hybrid cDNA library has led to the identification of eIF3f (eukaryotic initiation factor 3 subunit f)—known to play a major role in stabilizing the 40S ribosomal subunit. Intriguingly, recent studies have also demonstrated that eIF3f is involved in the regulation of apoptosis, and the reduction of eIF3f has been documented in several types of human cancers, although the biological mechanism behind this is still unclear. Our preliminary studies have suggested that hMSH4 interacts with eIF3f in human cells, presumably mediated through the N-terminal region of hMSH4. In addition, our confocal microscopic study has indicated that hMSH4 and eIF3f co-localize primarily in the cytoplasm under normal cellular conditions. Functional analysis of this newly discovered interaction in DNA damage response and cell cycle regulation is presently ongoing in the laboratory. It is conceivable that the interplay between hMSH4 and eIF3f may play a role in the pathogenesis and/or proliferation of cancer cells. Furthermore, the results of our current study are likely to be an important steppingstone for elucidating additional molecular functions of hMSH4.

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Protein kinase A regulates caspase-1 via Ets-1 in bone stem cell-derived lesions: a link between cyclic AMP and pro-inflammatory pathways in osteoblast progenitors. *M.Q. Almeida[1], K.T. Tsang[1], C. Cheadle[2], T. Watkins[2], JC. Grivel[3], M. Nesterova[1], R. Goldbach-Mansky[4], C.A. Stratakis[1].* 1) Section on Endocrinology and Genetics, Program on Developmental Endocrinology & Genetics (PDEGEN), Eunice Kennedy Shriver National Institute of Child Health & Human Development (NICHD), National Institutes of Health (NIH), Bethesda, MD 20892; 2) Genomics Core, Division of Allergy and Clinical Immunology, School of Medicine, Johns Hopkins University, Mason Lord Bldg., Center Tower, Rm. 664, 5200 Eastern Avenue, Baltimore, MD 21224; 3) Program on Physical Biology, NICHD, NIH, Bethesda, MD 20892; 4) Translational Autoinflammatory Disease Section, NIAMS, NIH, Bethesda, Maryland 20892, USA.

Patients with genetic defects of the cyclic (c) AMP-signaling pathway and those with neonatal-onset multisystem inflammatory disease (NOMID) develop “tumor-like” lesions of the long bones. The molecular basis of this similarity is unknown. NOMID is caused by inappropriate activation of a caspase-1 activating inflammasome. The present study demonstrates that NOMID bone lesions are derived from the same osteoblast progenitor cells that form fibroblastoid tumors in mice and humans with defects that lead to increased cAMP-dependent protein kinase (PKA) signaling. NOMID cells showed high PKA activity, and an increase in their cAMP signaling led to PKA-specific activation of caspase-1. Increased PKA led to inflammation-independent activation of caspase-1 via over-expression of the proto-oncogene and early osteoblast factor, Ets-1. In NOMID cells, as in cells with defective PKA regulation, increased prostaglandin E2 (PGE2) led to increased cAMP levels and activation of Wnt-signaling; increased Wnt-signaling underlies tumor formation in a variety of settings associated with inappropriate PKA activation. Caspase-1 and PGE2 inhibition led to a decrease in cell proliferation of both NOMID and cells with abnormal PKA. These data reveal a previously unsuspected link between abnormal cAMP signaling and defective regulation of the inflammasome and suggest that caspase-1 and PGE2 inhibition may be therapeutic targets in bone lesions associated with defects of these two pathways.

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Notch Gain of Function Contributes to the Osteosarcoma in a Mouse Model. *J. Tao[1], S. Chen[1], M. Jiang[1], T. Bertin[1], F. Gannon[2], B. Lee[1,3].* 1) Human & Molecular Genetics, Baylor College Medicine, Houston, TX 77030; 2) Pathology, Baylor College Medicine, Houston, TX; 3) Howard Hughes Medical Institute, Houston, TX.

Human osteosarcoma (OS) is the most common primary bone cancer, comprising approximately 20% of all bone tumors and about 5% of pediatric tumors overall. Most OS occurs sporadically and our understanding of its molecular basis is still limited. From a signaling perspective, dysregulation of several evolutionally conserved pathways including Wnt, TGF/ BMPs, SHH and FGFs has been found in human OS tumor samples and cell lines. Recently, our and other studies suggest that activation of Notch signaling contributes to the pathogenesis of human OS. Notch signaling plays an important role in the developmental processes and adult tissue homeostasis by regulating cell fate determination, proliferation, differentiation and apoptosis. Notch, a transmembrane receptor, releases its intracellular domain (NICD) into the nucleus to regulate transcription of target genes such as the Hey and Hes family of transcription factors. Altered Notch signaling has been associated with several cancers in which the data suggest that Notch can act both as an oncogene and tumor suppressor gene depending on its expression levels and timing. Our previously established human tumor xenografts in nude mice showed decreased tumor growth after chemical or genetic inhibition of Notch signaling, suggesting that its inhibition may be a therapeutic approach for the treatment of OS. To generate a mouse osteosarcoma model and examine the role of Notch signaling in bone tumorigenesis, we established a bitransgenic mouse line that constitutively expressed a single copy of Notch NICD in osteoblasts. The resulting bitransgenic Notch gain-of-function (GOF) mice developed osteosclerosis at age of two months. Histomorphometric and molecular analysis of cavarial and long bones indicated that Notch could stimulate proliferation of immature osteoblasts while inhibiting their differentiation into mature osteoblasts. This gain of function phenotype was reminiscent of osteoblastic tumors. Indeed, aging studies of these GOF mice showed that they spontaneously developed osteoma at 100% penetrance. Of 24 aged mice to date, we have detected multifocal malignant tumors in six of them by gross examination. Pathological analysis confirmed that one of them was telangiectatic osteosarcoma, featured by poorly differentiated osteoblast-like cells, tumor osteoid, necrosis and hemorrhage. Together, our preliminary data support our hypothesis that Notch activation may be a dominant mechanism for OS pathogenesis.

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cAMP and KIT-KITLG signaling interact in testicular tissue and possibly cooperate in predisposition to testicular germ cell tumors: a novel function of PDE11A in testis. *M. Azevedo[1], E. Bornstein[1], A. Horvath[1], c. Kratz[2], M. Nesterova[1], M. Greene[2], C. Stratakis[1].* 1) NICHD/NIH, Bethesda, MD; 2) NCI/NIH, Bethesda, MD.

Testicular germ cell tumors (TGCTs) account for 95% of the cases of testicular cancer, the most common solid malignancy affecting young males. Somatic activating mutations in the proto-oncogene KIT are found in patients with TGCTs, whereas common variations in the KITLG gene have been associated with these tumors. Phosphodiasterase (PDE) 11A (PDE11A) is expressed in several tissues, but is most highly expressed in testis (and prostate). PDE11A’s enzymatic activity, thus, appears to be an important regulator of cAMP signaling in testicular tissue. Recently, we reported PDE11A mutations (and functional polymorphisms) as predisposing factors for the development of TGCTs (1). In this study, we investigated whether the two pathways of cAMP and KIT/KITLG signaling interact in testicular tissue. A TGCT cell line (NTERA-2) was transiently transfected with plasmid DNA expressing either the wild type (WT) or the mutated forms of PDE11A described in patients with TGCTs (R52T, F258Y, G291R, Y727C, R804H, V820M, R867G and M878V); we then determined cAMP levels, PDE and PKA activity, and RNA and protein levels for KIT, KIT/KITLG, and related molecules. cAMP levels were significantly higher in the NTERA-2 cells transfected with all the PDE11A mutations and relative PDE activity was lower. KITLG expression was consistently increased in the presence of PDE11A mutations. Preliminary results suggest that PDE11A and KITLG TGCT-linked alleles co-segregate in families with TGCT. We conclude that KITLG expression consistently increases in the presence of PDE11A-inactivating defects, both at the RNA and protein levels, in TGCTs. This is the first demonstration, in any tissue, of a functional link between the cAMP and the KIT signaling pathways in predisposition to cancer.

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Continued Identification of Ethnic Specific Differences in Breast Cancer and Normal Breast Tissue. *L. Baumbach[1], M.E. Ahearn[1], C. Gomez[1], A. Mejias[1], M. Jorda[1], T. Halsey[2], J. Yan[2], K. Ellison[2], K. Mulligan[2], R. Kittles[3], A. Ashworth[4], M. Pegram[1].* 1) Univ Miami School of Medicine, Miami, FL; 2) Almac Diagnostics, Durham, NC; 3) University of Illinois at Chicago, Chicago, IL; 4) Breakthrough Cancer Research Center, London, UK.

Ethnic-specific disparities in stage of presentation/survival rates exist in breast cancer patients; the cause is unknown. We are continuing to investigate possible genetic contributions to these disparities, we are investigating genomic changes in breast cancer (BC) samples from multi-ethnic cohorts of African-American (AA), Hispanic (His), native African (Kenyan) and non-Hispanic white (Caucasian) women with “triple negative” (ER-, PR-, Her2/Neu-) BC. The overall goal is to identify differentially expressed genes between tumor and adjacent normal tissue that are common or unique among the three groups. We are also analyzing normal breast tissue samples (reduction mammoplasty residual tissue) from AA, His and Cauc women to investigate gene expression patterns across ethnicities in normal breast tissue. Pathology samples are cut from FFPE (Formalin Fixed Paraffin-embedded tissue) blocks marked by pathology as self-matched normal vs. tumor tissue. Almac Diagnostics performs RNA isolation, cDNA preparation, and hybridization of tumor/normal cDNAs to a breast cancer focused gene expression array (Breast Cancer DSA Research Tool). From the Breast Cancer DSA arrays data, a two-way ANOVA (disease state and ethnicity) identified 6479 transcripts with a p-value less than 0.01. Data QC indicated that samples clustered well with respect to ethnicity and adjacent normal vs. tumor tissue. We have identified ethnic-specific expression patterns in the matched normal and tumor samples. Ethnic specific differences between the adjacent normal tissues were also seen and ethnic specific expression differences were noted in small collection of normal breast samples. The same BC samples are being analyzed by array CGH to assess copy number variations in relation to the gene expression differences. In addition, DNA from the AA samples is being evaluated with a panel of ancestry-informative DNA markers. These analyses indicate that high quality gene expression data can be obtained from FFPE samples, and ethnic specific gene expression differences have detected in tumor and matched normal breast tissue across ethnic groups. Validation of these results has important implications for addressing BC health disparities, as well as tailored approaches to prediction, prevention and treatment.

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A miRNA inhibitor screen for *KRAS*-selectively lethal miRNAs. *R. Borkowski[1], L. Du[3], A.F. Gazdar[3,4], J.D. Minna[3,4], A. Pertsemlidis[2,3].* 1) Program in Genetics and Development, UT Southwestern Medical Center, Dallas, TX; 2) McDermott Center for Human Growth and Development, UT Southwestern Medical Center, Dallas, TX; 3) Simmons Comprehensive Cancer Center, UT Southwestern Medical Center, Dallas, TX; 4) Hamon Center for Therapeutic Oncology Research, UT Southwestern Medical Center, Dallas, TX.

Lung cancer, especially non-small cell lung cancer (NSCLC), is currently the leading cause of cancer fatalities in the United States, accounting for 30% of the fatalities in males, and 26% in females. Additionally, the five year survival rate for individuals affected by lung cancer has only improved by 3% in the past 35 years. As suggested by the survival data, very few effective treatment options are available for NSCLC, and many vary dramatically in efficacy depending on the genetic status of the tumor. In particular, the presence of mutant *KRAS* in NSCLC serves as a negative predictor for drug efficacy. *KRAS* mutations are found in ~22% of NSCLC tumors, and tumors harboring these mutations are categorically refractory to treatment with Epidermal Growth Factor Receptor (EGFR) Tyrosine Kinase Inhibitors (TKI) gefitinib (Iressa) and erlotinib (Tarceva); furthermore, there are no effective therapies targeted for *KRAS* mutant NSCLCs.

In order to identify genetic susceptibilities specific to *KRAS*-dependent NSCLC, we use systematic miRNA depletion to identify miRNAs, and their target genes and pathways, necessary for the survival of this genetic subset of tumors. A miRNA inhibitor screen can serve to identify not only independent miRNAs involved in selective survival, but also genes and pathways involved that would be obscured by genomic redundancy in a conventional RNAi screen. Using a comprehensive library of miRNA inhibitors coupled with an endpoint assay for cell viability, we have identified several miRNAs that selectively decrease the viability of *KRAS*-dependent NSCLC cell line NCI-H1155 with minimal effect in the wild-type *KRAS* cell line NCI-H1437. These candidates are being validated in an expanded panel of ten cell lines including four *KRAS* mutant, four *KRAS* wild-type, and two immortalized human bronchial epithelial cell lines. miRNAs that, when inhibited, selectively decrease the viability of the refractory tumors will elucidate the pathways and processes necessary for mutant *KRAS*-dependent survival, and will expose individual miRNAs and mRNAs as therapeutic targets.

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Identification of the rno-miR-30 family members as a potential central player during 4-nitroquinoline 1-oxide-induced tongue carcinogenesis in rat. *W. Chen[1,2], T. Zhang[1], P. Zhang[1,2], J. Zhang[1], D. Ye[1,2], Q. Xu[1,2], W. Qiu[1].* 1) Department of Oral & Maxillofacial Surgery, Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China; 2) Shanghai Key Laboratory of Stomatology, Shanghai, China.

Objective: Constituents of tobacco can cause DNA adduct formation, and are implicated in the development of oral squamous cell carcinoma (OSCC). However, there are few published studies on the mechanism(s) underlying tobacco associated oral carcinogenesis. It has been widely known that MicroRNAs have an important role in regulation of tumor generation and development. The present study was aimed to analyze the expression profiling of microRNAs and their target genes in rat cancer tissues, which was induced by 4-nitroquinoline 1-oxide (4NQO) and a useful model that mimics tobacco-related human OSCC. Methods: Twenty male Sprague-Dawley (SD) rats were administrated with 0.002% 4NQO in drinking water for 36 weeks, whereas ten rats fed with drinking water were utilized as a control. 15 rats from the 4NQO-treated group developed tongue squamous cell carcinoma, which were confirmed by pathological examination. miRNA and mRNA gene expression profiling studies were pursued using RNA pools derived from 7 tumor and 7 normal tissues, respectively. The significantly expressed miRNAs and mRNAs were verified by RT-PCR followed by gene ontology (GO) analysis in an attempt of building the miRNA-gene correlation network, and miRNA-go-network. Results: 57 miRNAs and 243 mRNA transcripts exhibited differential expression profiles between tumors and normal tongue tissues. Among them, the differential expressions of three miRNAs and three mRNA were validated. In tumor tissues, the expression level of the members of the rno-miR-30 family (rno-miR-30a, -30a*, -30b-5p, -30c, -30d, -30e and -30e*) is only 8% to 37% of their counterparts in the control group. High enrichment GOs including differentiation-related targeted genes and miRNA-gene networks revealed genes of Ubadc1, Ank2, Mybpc1, Ldhb, Cpt2, and Cabc1 as the primary six target genes, which were all known regulated by the rno-miR-30 family. Of the rno-miR-30 family, the three members (rno-miR-30a*,-30d, and -30e*) were the first three highest degree of miRNAs based miRNA-gene networks, and the members (rno-miR-30 b-5p, -30c, -30d, and -30e) were the four of the highest degree of miRNAs uncovered by miRNA-go-network. Conclusions: Rat tongue squamous cell carcinoma induced by 4NQO relates to 57 miRNAs and 243 genes. Decreased expression of the rno-miR-30 family may play a fundamental role in carcinogenesis development especially in biochemical process of oxidation reduction and ion transport.

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Genome-Wide Linkage Scan of Colorectal Cancer Families with MMR Deficiency. *M.S. Cicek[1], B.L. Fridley[1], D. Serie[1], W. Bamlet[1], J.M. Cunningham[1], N.M. Lindor[1], J.D. Potter[2], S.N. Thibodeau[1], E.L. Goode[1], the Colon Cancer Family Registry.* 1) Mayo Clinic College of Medicine, Mayo Clinic, Rochester, MN; 2) Fred Hutchinson Cancer Research Center, Seattle, WA.

Substantial evidence from segregation and linkage analyses excluding families with known mutations supports the existence of unidentified susceptibility loci for colorectal cancer (CRC). As part of a larger search to identify novel CRC regions, we assessed linkage in 548 families with no identified germline mismatch repair (MMR) mutations including 129 families with phenotypic evidence of defective MMR (dMMR). We studied families from the Colon CFR, a multi-site NCI-supported consortium, and from Newfoundland, defining dMMR families as those with at least one cancer case with tumor microsatellite instability (MSI-H) or loss of immunohistochemical expression for at least one of following MMR genes: *MLH1, PMS2, MSH2, or MSH6*. Family members (N=737) with an average family size of 5.7 and mean per family of 2.6 and 3.1 for 337 affected and 400 unaffected, respectively, were genotyped using the Affymetrix 10k 2.0 Array or the Illumina Linkage Panel 12 (10,091 pooled SNPs with r2<0.1) and analyzed using MERLIN. Assuming a dominant mode of inheritance, an HLOD of 6.49 in the chromosome 2 *MSH2/MSH6* region (114.3 cM) and an HLOD of 10.91 on the chromosome 3 *MLH1* region (93.3 cM) were observed. The data confirm strong linkage to these known genes. Subsequent MMR gene sequencing on these 129 dMMR families detected 42 families with pathogenic mutations and four families with missense mutations of unknown significance (T764N on *MSH6*, T117R and I219V on *MLH1* and G669V on *MSH2*). In addition, of 51 families exhibiting *MLH1/PMS2* loss, 11 families (22%) segregated pathogenic *MLH1/PMS2* mutations; similarly, of 26 families with *MSH2/MSH6* loss, 14 families (54%) segregated pathogenic *MSH2/MSH6* mutations. Not all families with pathogenic mutations yielded elevated LOD scores, suggesting incomplete linkage-informativeness for some families. Among the remaining 83 dMMR families with no detected mutations, evidence of linkage at *MLH1/PMS2* genes was seen for 65% of the families and at *MSH2/MSH6* genes for 59% of the families; however, other novel regions are suggested.

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The metastasis gene MMP-9 is a direct target of the chromatin modifier SMYD3. *A. Cock-Rada, S. Medjkane, N. Janski, J.B. Weitzman.* UMR 7216 - Epigenetics and Cell fate, University Paris Diderot, Paris, France.

Epigenetic mechanisms involved in tumor initiation and progression are becoming an increasing focus of study in the search for new cancer therapies, due to their potential reversibility. Matrix Metalloproteinase-9 (MMP-9) is a proteolytic enzyme that degrades the extracellular matrix and plays an important role in tumor progression and metastasis formation by regulating processes like tumor growth, angiogenesis, escape from immune surveillance, cell migration and invasion. MMP-9 is regulated by several transcription factors but its transcriptional regulation by epigenetic-modifying enzymes is relatively unexplored. SMYD3 is a histone H3K4 methyltransferase with DNA binding capacity, which has been shown to contribute to the transcriptional activation of several genes involved in cancer. It has been shown to be important for tumour proliferation in colorectal, hepatocellular and breast carcinomas and in cellular invasion in cervical carcinomas. We are studying the role of SMYD3 in the regulation of MMP-9 and tumor invasion. In order to identify new regulators of MMP-9 expression at the chromatin level, we characterized several activating and repressive histone marks on the MMP-9 promoter by Chromatin Immunoprecipitation (ChIP) in a reversible bovine cancer model. We observed that tri-methylation of histone H3K4 is an important event in the activation of MMP-9. We studied the expression of several H3K4 methyltransferases and demethylases by quantitative PCR and observed a positive correlation between the expression of MMP-9 and the gene coding for SMYD3. Using over-expression and knockdown techniques to modulate SMYD3 levels, we observed an effect on MMP-9 expression and protein activity in bovine transformed leukocytes and in human fibrosarcoma cells (HT1080). We observed by ChIP that SMYD3 binds to the MMP-9 promoter. We generated HT1080 stable cell lines with shRNA against SMYD3 and observed a decreased binding of SMDY3 to the MMP-9 promoter, along with a decrease in H3K4me3 marks. We are now studying the effect of SMYD3 knockdown on the invasive phenotype of these cells, using matrigel invasion and migration assays. These results identify SMYD3 as a new regulator of MMP-9 in cancer progression.

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mir-21 Regulate Sprouty1 Expression in Prostate Cancer Cells. *M. Daremipouran[1,2], B. Kwabi-Addo[2].* 1) Genetics and Human Genetics, Howard University, Washington, DC; 2) Howard University Cancer Center Washington, DC.

Background: Sprouty1 is a negative regulator of fibroblast growth factor signaling with a potential tumor suppressor function in prostate cancer (PCa) cell lines. Biochemical analysis shows that Sprouty1 is downregulated in prostate cancer tissues in comparison to normal prostate tissues. We have previously shown that in some cases DNA methylation is a potential mechanism for the downregulation of Sprouty1 expression, suggesting other mechanisms of gene inactivation could also affect Sprouty1 expression. Recent studies have shown that microRNA-21 (mir-21) is overexpressed in various types of cancers including prostate cancer. It is suggested that mir-21 may have a potential role in regulating the malignancy and metastatic abilities of prostate cancer cells by regulating the tumor suppressors such as; Sprouty1. To elucidate additional molecular mechanism involved in regulating Sprouty1 expression in prostate cancer we investigated the rolls of transcription factors and microRNA expression in Sprouty1 regulation. Methods: Gene knockdown studies were carried out in prostate cancer cell lines with shRNA or siRNA directed towards EGR1, EGR2, GATA2 and GATA4 transcription factors and mir-21 and the RNA expression level validated using quantitative RT-PCR. Protein expression was analyzed by western blotting. Results: Our results demonstrates that knockdown of transcription factors Egr1, Egr2, Gata2 or Gata4 by transfecting prostate cancer cell lines with the corresponding shRNAs resulted in downregulation of Sprouty1 protein expression suggesting that these transcription factors stimulate Sprouty1 expression. On the other hand, the knockdown of mir-21 expression in prostate cancer cell lines, PC3 and DU145 led to increase activation of Sprouty1 expression but we did not see such response in LNCaP cells which does not have detectable basal level of mir-21 expression. Conclusion: Our preliminary observation suggests multiple mechanisms of Sprouty1 regulation, including epigenetic DNA methylation changes and small RNA expression that may act differently depending on the cellular context.

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Mad2 is a Critical Mediator of the Chromosome Instability Observed upon p53 and Rb Pathway Inhibition. *P.H.G. Duijf, J.M. Schvartzman, R. Sotillo, C. Coker, R. Benezra.* Department of Cancer Biology & Genetics, Memorial Sloan-Kettering Cancer Center, New York, NY.

Rb and p53 pathway inhibition are key events in the development of chromosome instability and cancer, yet the mechanisms by which this instability arises are unclear. Rb pathway inhibition leads to overexpression of the mitotic checkpoint gene Mad2 via E2F activation. While Mad2 overexpression is sufficient to initiate aneuploid tumor formation, whether it is required to mediate the phenotypes of Rb or p53 pathway inhibition is unknown. We show that Mad2 is upregulated in response to p53 loss as a result of reduced p21-mediated inhibition of cyclin-dependent kinase activity and Rb inactivation. In the context of Rb pathway inhibition, Mad2 overexpression is required for the acquisition of chromosome instability and anaplastic mammary tumor formation. In addition, in a p53 mutant knock-in mouse model that develops tumors with stable, diploid genomes, the Mad2 upregulation and chromosome instability that is caused by p21 loss can be rescued by genetic reduction of Mad2 levels. These results demonstrate that Mad2 overexpression is an essential mediator of the chromosome instability and tumor phenotypes observed upon inactivation of two major tumor suppressor pathways.

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Estrogen receptor gene silencing in human breast cancer cells using siRNA. *M. El-Zawahri[1], Y. Luqmani[2], A. Al-Azmi[3].* 1) Faculty of Biotechnology, Misr University for Science & Technology (MUST), 6th October City, Egypt; 2) Pharmaceutical Chemistry Dept, Kuwait University, Kuwait; 3) Molecular Biology Dept, Kuwait University, Kuwait.

The role of estrogen receptor alpha (ERα) in breast cancer has been highlighted by numerous of studies. Consequently, inhibition of ERα has become one of the major strategies for the prevention and treatment of breast cancer. However, failure to overcome development of endocrine resistance limits this approach. Our aim was to produce a model system to investigate cellular consequences of loss-of-function of ERα gene by establishing a breast cancer cell line with a permanent source of small interference RNA (siRNA) to specifically inhibit the production of ERα protein. Three plasmid constructs (plasmid I, II and III) intended to produce intracellular double stranded hairpin RNA to be processed into siRNA targeting different regions of the human ERα mRNA, and a scrambled sequence, were cloned into the pRNA-U6.1/Neo GenScript vector for expression of anti-ERα siRNA from the U6 promoter. MCF-7 human breast cancer cells were transformed with 2 or 4 μg of each plasmid (6 and 24h exposure), using two different transfection reagent conjugates: lipofectin-plasmid-complex containing each of the three different intact plasmid constructs, and XtremeGENE complexed to each of the linearized plasmids. Transformants were rescued by growth in G418 selection medium. Stably transformed cells were propagated in long-term culture. Presence of plasmid DNA in transformants was verified with primers targeting various regions of the vector. ERα mRNA levels were determined by Real-Time RT-PCR, and ERα protein by Western blotting; normalization was achieved by simultaneous analysis of β-actin. Our results showed down regulation of expression of the ERα mRNA in MCF-7 cells transfected with the three anti-ERα siRNA intact plasmid construct compared with the wild-type MCF-7 control cells. Plasmid II showed greater down-regulation than plasmids I and III. Linearized anti-ERα-siRNA construct PII most effectively down-regulated ERα mRNA and protein, compared to G418 resistant transformants containing scrambled siRNA. Complete knockdown was not observed, whereas 4 μg of linearized anti-ERα-siRNA construct PII produced more transformants. 24h exposure did not increase transformation efficiency. In conclusion, we have successfully established a long-term culture of MCF-7 breast cancer cells that exhibit decreased expression of ERα. This is hoped to provide a model system in which to study aspects of endocrine resistance.

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CHD1L Induces Mitotic Defects and Chromosome Missegregation through Decreasing Cdk1 activity by Upregulating TCTP. *X.Y. Guan, T.H. Chan, L. Chen, M. Liu, L. Hu.* Dept Clinical Oncology, Univ Hong Kong, Hong Kong, Hong Kong.

Our previous research efforts have led to the identification of CHD1L as a target oncogene responsible for the 1q21 amplification event in hepatocellular carcinoma (HCC). Here, by utilizing a 2D-PAGE proteomic approach, the translationally controlled tumor protein (TCTP), a tubulin binding protein, was identified as one of CHD1L target genes. Using chromatin immunoprecipitaion (ChIP)-PCR and luciferase reporter assays, we found that CHD1L protein directly binds to the promoter region (-733/-1027) of TCTP and activates its transcription. Clinically, overexpression of TCTP was detected in 41.9% of human HCCs, which was significantly associated with CHD1L overexpression ($p<0.001$), and significantly associated with heavy liver cirrhosis ($p=0.024$) and advanced tumor stage ($p=0.049$). In vitro and in vivo functional studies showed that TCTP could strongly promote foci formation, accelerate mitotic phase progression and induce tumor formation in nude mice. Further mechanistic study indicated that during mitosis, the elevated expression of TCTP accelerated the ubiquitin-proteasome degradation of Cdc25C, which in turn led to the failure in the dephosphorylation of Cdk1 on Tyr15 and the decreased Cdk1 activity, finally causing a faster M phase exit and chromosome missegregation. In addition, silencing TCTP expression inhibits its tumorigenicity and reverses the faster M phase progression. Overall, our present study on TCTP defines a novel molecular pathway (CHD1L)-(TCTP)-(Cdc25C)-(Cdk1) during hepatocarcinogenesis by which CHD1L activates TCTP transcription via binding to the latter's promoter region and TCTP overexpression leads to the malignant transformation of hepatocytes with the phenotypes of accelerated mitotic progression and the production of aneuploidy.

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Variants on the promoter region of PTEN affect breast cancer progression and patient survival. *T. Heikkinen[1], LM. Pelttari[1], D. Greco[1], J. Tommiska[1], P. Vahteristo[1], P. Heikkila[4], K. Aittomaki[3], C. Blomqvist[2], H. Nevanlinna[1].* 1) Department of obstetrics and gynecology, Helsinki university central hospital, Helsinki, Finland; 2) Department of oncology, Helsinki university central hospital, Helsinki, Finland; 3) Department of clinical genetics, Helsinki university central hospital, Helsinki, Finland; 4) Department of pathology, Helsinki university central hospital, Helsinki, Finland.

The *PTEN* gene functions as a negative regulator of phosphatidylinositol 3-kinase (PI3K), and hence has a direct regulatory effect on PI3K oncogenic pathway activation. Inherited inactivating mutations in *PTEN* cause Cowden syndrome, in which the estimated risk for breast cancer is 25-50%. Low penetrance variation in *PTEN* gene or other members of the PI3K pathway has not been reported. We have screened the *PTEN* gene for germline variation from 84 patients with family history of breast or other Cowden-related cancers using CSGE heteroduplex analysis and direct sequencing, and further genotyped three rare promoter polymorphisms -903GA, -975GC, and -1026CA in 2233 breast cancer patients and 1272 healthy controls. None of the variants associated with breast cancer risk, but all variants associated with worse prognosis. The Cox's regression hazard ratio for 10 year breast cancer specific survival was 2.17 (95% CI 1.52-3.10) p=0.00002, and for 5 year breast cancer death or distant metastasis free survival 1.97 (95% CI 1.40-2.79) p=0.00011 for patients carrying any of the variants.

We evaluated the effects of the promoter variants on transcriptional activity with luciferase reporter assay by cloning the promoter elements carrying different variants into an expression vector and comparing the changes in expression in HeLa and MCF7 cell lines. The variants -975GC and -1026CA had slight, but significantly reduced expression in HeLa cells. All variants were predicted to change transcription factor binding sites when the wild type and variant sequences were compared using Genomatix SNPInspector software. We also compared the gene expression in breast cancer tumors of 10 variant carriers and 10 matched non-carriers and identified 160 differentially expressed genes clustering in distinguished signatures. All three germ line variants exhibited similar gene expression patterns in the tumors.

The genetic analyses together with the gene expression results suggest that inherited variation in the *PTEN* promoter region causing even subtle changes in *PTEN* expression levels or function will affect the tumor progression and gene expression profile in breast cancer, with also clinical implications for reduced survival of breast cancer patients.

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Detection of significant DNA copy number alterations in breast tumors from Mexican patients with high-resolution SNP arrays and GISTIC analysis. *A. Hidalgo-Miranda[1], S. Rodriguez-Cuevas[2], R. Rebollar-Vega[1], S. Romero-Cordoba[1], V. Quintanar-Jurado[1], V. Bautista-Pina[2], I. Ciceron-Arellano[1], K. Carrillo-Sanchez[1], S. March-Mifsut[1], G. Jimenez-Sanchez[1], L. Uribe-Figueroa[1].* 1) Laboratorio de Oncogenomica, Instituto Nacional de Medicina Genomica, Mexico; 2) Fundacion Mexicana de Cancer de Mama, Mexico.

Breast cancer represents the second cause of cancer related death in Mexican women, and also represents an important health problem worldwide. Specific patterns of DNA copy number changes in breast tumors have been associated with particular tumor types and clinical behaviors. Analysis of these DNA copy number alterations have been carried out using different methods, like comparative genomic hybridization (either on metaphase chromosomes or different microarray platforms), and more recently, with high density oligonucleotide and SNP arrays. Increased array resolution, as well as the introduction of statistical methods to evaluate the significance of the copy number aberrations, has allowed the detection of particular gene targets in several human tumors. In order to define the pattern of statistically significant DNA copy number changes in breast tumors from Mexican patients, we used a high resolution SNP array to analyze the DNA copy number profiles of 89 breast normal/tumor pairs from Mexican patients. Tumor tissue and peripheral blood lymphocyte DNA from each patient was analyzed with the Affymetrix SNP 6.0 array. All tumor samples contained more than 80% tumor cells. Array normalization and paired copy number analysis using the SNP probes in the array was done using DChip, followed by segmentation using the GLAD algorithm and significant regions were identified using the Genomic Identification of Significant Targets in Cancer (GISTIC) method, both implemented in the GenePattern platform. GISTIC analysis identified 49 significant regions with copy number changes in the tumor compared to the paired normal tissue, including 22 amplifications and 27 deletions. In the amplifications events, seven were considered as "broad" (average: 72 Mb), 11 were "focal" (average: 91 kb) and four were considered as "both". In the case of the deletions, 15 were broad, nine were focal and three were both. Some of these regions have not been previously reported as common areas of copy number change in breast cancer making the analysis of the genes inside these significant regions an interesting research task. To our knowledge, this is one of the first reports regarding high-resolution DNA copy number aberrations in breast cancer from a Latin American population, as well as one of the first analysis using the SNP 6.0 array in combination with a statistical method for the detection of significant regions with DNA copy number changes in breast cancer.

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Expression of miR-141 and miR-223 in ovarian carcinoma correlates with grade. *L.A. Keogh, C. Barret, C. d'Adhemar, P. Smyth, R. Flavin, J.J O' Leary, O. Sheils.* Histopathology, University of Dublin, Trinity College, Dublin, Ireland.

Ovarian cancer is the fifth most common cancer in women, and has the highest mortality rate among gynaecological cancers. This is largely due to the fact that it is difficult to detect at early stages. Each histological subtype of Epithelial Ovarian Cancer (EOC) also has a pattern of pathological progression, making it difficult to pinpoint specific diagnostic and prognostic markers. The primary focus of this study was an evaluation of miRNA expression in serous ovarian neoplasia. MicroRNAs are small, non- coding RNA molecules that are associated with many pathological processes and virtually all cancers. They are robust by nature and can be detected in archival material. The microRNA targets chosen in this study were hsa-miR-141 and hsa-miR-223. These targets were selected following a pilot study of global miRNA expression in ovarian neoplasia previously completed by our group. Two endogenous controls; let-7a and miR-16, were used for housekeeping purposes. N= 200 cases including benign, borderline, and carcinomas from both serous and mucinous subtypes were selected. 10μm sections were cut from formalin- fixed paraffin embedded (FFPE) tissue. These sections were then haematoxylin and eosin (H&E) stained and Laser Capture Microdissection (LCM) was used to harvest pure populations of epithelial cells. Total RNA was extracted using RecoverAll Total Nucleic Acid Isolation kit (Ambion). Total RNA reverse transcribed using Applied Biosystems Taqman MicroRNA Reverse Transcription (RT) kit, and qPCR was performed using specific Taqman MicroRNA assays. Results illustrated a clear trend in expression of miR-141 from benign through borderline to malignancy in mucinous neoplasias. The level of expression of miR-141 was relatively constant through grades of serous neoplasms. miR-223 expression also demonstrated a clear progression from benign through borderline to malignant in mucinous samples, and also in serous samples. There was significant ($p<0.05$) variation in expression of both miR-141 and miR-223 between mucinous and normal samples, and also between serous and normal samples indicating potential utility for these markers in the diagnostic setting.

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TP53 isoforms are regulated by the TEL/ETV6 transcriptional repressor in childhood pre-B acute lymphoblastic leukemia. *J. Larose[1], C. Malouf[1,2], B. Neveu[1,2], S. Langlois[1], D. Sinnett[1,3].* 1) Hematology-Oncology, Res Ctr CHU Sainte-Justine, Montreal, Canada; 2) Departement of Biochemistry, Faculty of Medicine, University of Montreal, Montreal, Canada; 3) Departement of Pediatrics, Faculty of Medicine, University of Montreal, Montreal, Canada.

Cancer is the second major cause of death in the pediatric population. The most frequent pediatric cancer, accounting for 25% of all cases, is acute lymphoblastic leukemia (ALL). Throughout the years, many genetic alterations were associated with childhood ALL, including the chromosomal translocation t(12;21) observed in 30-40% of childhood pre-ALL. In utero, this translocation creates the chimera ETV6-AML1 which is insufficient to the complete development of pre-B ALL. Interestingly, when children are diagnosed with pre-B ALL, they carried a deletion of the second allele of ETV6. This causes the complete inactivation of ETV6, a transcription repressor of the Ets family. In childhood leukemia, we believe that ETV6 acts as a tumor suppressor gene because its inactivation is required for the complete development of pre-B ALL. The leukemogenesis events would be fulfilled through the action of the transcriptional targets of ETV6. Using a combination of microarray expression analysis and qRT-PCR assays in leukemia patients, we identified TP53 as one putative transcriptional target of ETV6. The expression of TP53, a well-characterized tumor suppressor is driven by a classic and an alternative promoters. The classic promoter is responsible for the expression of the full-lenght as well as the Δ40 isoforms, while the alternative promoter leads to the expression of the Δ133 isoforms. Using gene reporter assays in HeLa and Jurkat cell lines, we demonstrated that ETV6 represses the activity of both TP53 promoters by targeting a region close to the basal promoter. The analysis of ETV6 constructs deleted either for the DNA-binding domain (ETS domain) or the protein-protein interaction domain (POINTED/PNT domain) indicated that ETV6 doesn't need a consensus EBS/Ets-binding site, unlike other transcription factors of the Ets family. To understand the role of TP53 isoforms (full-lenght, Δ40 and Δ133) in leukemogenesis, we are conducting expression studies in cells from pre-B ALL patients as well as phenotypic studies in leukemic cell lines. This study is a new step toward the understanding of the molecular mechanisms used by ETV6 to regulate transcription and the implication of its transcriptional targets in leukemogenesis.

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Characterization of new transcriptional targets of ETV6/TEL, a transcription factor implicated in childhood pre-B acute lymphoblastic leukemia. *C. Malouf[1,2], S. Langlois[1], J. Larose[1], P. Beaulieu[1], D. Sinnett[1,3].* 1) CHU Sainte-Justine, Montreal, Quebec, Canada, H3T 1C5; 2) Department of Biochemistry, Faculty of Medicine, University of Montreal, Montreal, Quebec, Canada, H3T 1J4; 3) Departement of Pediatrics, Faculty of Medicine, University of Montreal, Montreal, Quebec, Canada, H3T 1C5.

Cancer is the second major cause of death in the pediatric population. The most frequent pediatric cancer, accounting for 25% of all cases, is acute lymphoblastic leukemia (ALL). Throughout the years, many genetic alterations were associated with this type of leukemia, including the chromosomal translocation t(12;21) presents in 30-40% of childhood pre-B ALL. *In utero*, this translocation creates the chimera ETV6-AML1 which is insufficient to the complete development of pre-B ALL. Interestingly, when children are diagnosed with pre-B ALL (2-10 years old), they have a deletion of the second allele of ETV6. This causes the complete inactivation of ETV6, a transcription repressor of the Ets family. In childhood leukemia, we believe that ETV6 acts as a tumor suppressor gene because its inactivation is required to the complete development of pre-B ALL. The leukemogenesis events would be fulfilled through the action of the transcriptional targets of ETV6. Since very few of them have been identified, we previously conducted a genomic study combining microarray and qRT-PCR technologies in leukemia patients. This led to the identification of putative transcriptional targets of ETV6, including IL18/interleukin 18, LUM/lumican, SPHK1/sphingosine kinase 1 and PTGER4/prostaglandin E receptor 4 subtype EP4. Using gene reporter assays in HeLa and Jurkat cell lines, we showed that ETV6 represses the activity of the promoters of IL18, LUM, SPHK1 and PTGER4 in a region close to the basal promoter. The analysis of ETV6 constructs deleted either for the DNA-binding domain (ETS domain) or the protein-protein interaction domain (POINTED/PNT domain) indicated that ETV6 doesn't need a consensus EBS/Ets-binding site, unlike other transcription factors of the Ets family. Since chromatin immunoprecipitation experiments in HeLa cells showed the enrichment of ETV6 in the five promoters, we hypothesized that ETV6 binds an uncharacterized DNA sequence. The combination of bio-informatic studies (MEME and TRANSFAC) and functional experiments (gene reporter assays, DNA-protein interactions studies) will most likely answer this question. This study is a new step toward the understanding of the molecular mechanisms used by ETV6 to regulate transcription and the implication of its transcriptional targets in leukemogenesis.

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The Role of Hypoxia in the Response of Ovarian Cancer Cells to Chemotherapy. *L.M. McEvoy[1], S.A. O'Toole[1,2], C.D. Spillane[1,3], G. Kijanka[4], C.M. Martin[1,3], O. Sheils[1], J.J. O'Leary[1,3].* 1) Histopathology, Trinity College Dublin, Dublin, Ireland; 2) Obstetrics & Gynaecology, Trinity College Dublin, Dublin, Ireland; 3) Coombe Women and Infants University Hospital, Dublin, Ireland; 4) Biomedical Diagnostics Institute, Dublin City University, Dublin, Ireland.

Ovarian cancer is the fifth most common cancer in women and the leading cause of death from gynaecological malignancy in the Western world. Approximately 70% of patients present with late stage disease, which has already spread to other parts of the body and the long term survival for late stage ovarian cancer is only 30%. Although most patients respond well to chemotherapy initially, the tumour often recurs and is frequently chemoresistant, contributing to the poor long term survival. Tumour hypoxia is associated with resistance to both radiotherapy and chemotherapy. Hypoxia turns on gene pathways which promote aggressiveness, metastasis and chemoresistance and is a common feature of solid tumours such as ovarian cancer. This study aimed to examine the effect of hypoxia on the response to chemotherapy of two ovarian cancer cell lines, A2780 and A2780cis. The cells were grown in normal oxygen conditions (21% O_2, 5%CO_2) or exposed to hypoxia (0.5% O_2,5%CO_2) and treated with cytotoxic drugs cisplatin or paclitaxel in normoxia or hypoxia as part of a hypoxia design matrix. Following drug treatment for 72 hours, the cell viability was assessed using a standard MTT assay. A2780 cells were sensitive to cisplatin and resistant to paclitaxel, whereas A2780cis cells were resistant to cisplatin and sensitive to paclitaxel. Acute hypoxia exposure before treatment increased resistance to cisplatin in both cell lines by increasing the IC_{50}, however it increased sensitivity to paclitaxel in A2780s, which had a reduced IC_{50} value. Treatment of cells in hypoxia without any pre-exposure increased resistance to cisplatin and paclitaxel in A2780cis however A2780 cells did not follow a dose response pattern in these conditions. Several different gene pathways have been implicated in cisplatin and paclitaxel resistance, and understanding the mechanisms underlying these differential response patterns may provide novel therapeutic targets to improve the outcome for ovarian cancer patients with hypoxic tumours.

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***RAD51C* is a novel predisposing gene for hereditary breast and ovarian cancer.** *A. Meindl[1], H. Hellebrand[1], C. Wiek[2], V. Evren[2], B. Wappenschmidt[3], D. Niederacher[4], T. Kasparek[1], A. Becker[3], H. Schaal[5], D. Schindler[6], R. Schmutzler[3], H. Hanenberg[2].* 1) Dept OB/GYN, Klinikum Rechts der Isar, Munich, Germany; 2) Heinrich-Heine University, Department of Pediatric Hematology, Duesseldorf, Germany; 3) University Hospital, Center for Familial Breast and Ovarian Cancer, Cologne, Germany; 4) Heinrich-Heine University, Department of Obstetrics and Gynaecology, Duesseldorf, Germany; 5) Heinrich-Heine University, Institute for Virology, Duesseldorf, Germany; 6) Department of Human Genetics at the University, Wuerzburg, Germany.

Germ-line mutations in a number of genes involved in the recombinational repair of DNA double-strand breaks are associated with a predisposition to breast and ovarian cancer. While mutations in the two high penetrant genes *BRCA1* and *BRCA2* are associated with high disease risks for both entitites, subsequently identified genes involved in DNA repair like *ATM*, *CHEK2* or *PALB2* and mutated familial breast cancer cases, had been linked to more moderate risks. Recently we identified six monoallelic pathogenic mutations in the *RAD51C* gene of 480 breast and ovarian cancer (BC/OC) families that confer highly increased life time risks (Meindl *et al*., Nat Genet 42:410-414, 2010). Analysis of RAD51C pedigrees indicated that the clearly pathogenic mutations completely segregated with the disease (=84high penetrance"), and screening of some RAD51C-deficient tumors revealed loss of the wild type allele with exclusive retaining of the mutated one (=84tumor suppressor"). We also found a more frequent missense variant (16 out of 480) that confers at least moderate risks for BC/OC families. This more frequent mutation is characterized currently regarding its functional implications, clinical consequences and familial segregations. In addition, we have started to screen further BC/OC families in other RAD51 paralogs like XRCC3 and RAD51B, but identified there up to now only few substitutions. In summary, the results demonstrate firstly, that RAD51C is a novel predisposing tumor suppressor gene and support the hypothesis that most of the familial cases showing no mutations in *BRCA1/2*, are linked to yet unknown genes, which are also highly penetrant, but infrequently mutated. However, it seems that other RAD51 paralogs are not essentially involved in familial BC/OC cases. Nevertheless, our results implicate that more familial cases than formerly expected are sensitive to a treatment with PARP-inhibitors, a conclusion which is also supported by the observation that the penetrance of e.g. *CHEK2* or *PALB2* mutations is higher in familial than in sporadic cases.

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TRIM8 controls p53 oncosuppressor and ΔNp63α oncogenic activities. *L. Micale[1], M.F. Carattozzolo[2], C. Fusco[1], F. Galli[3], B. Augello[1], A.M. D'Erchia[2], L. Guerini[3], M.G. Turturo[1], E.V. D'Addetta[1], A. Calcagni[1], M.N. Loviglio[1], G. Pesole[2,4], E. Sbisà[5], A. Tullo[5], G. Merla[1].* 1) Laboratory of Medical Genetics, IRCCS "Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy; 2) Dipartimento di Biochimica e Biologia Molecolare "E.Quagliariello", via Orabona 4, 70126 Bari, Università degli Studi di Bari "A. Moro", Italy; 3) Department of Biomolecular Science and Biotechnology, University of Milan, 20133 Milan, Italy; 4) Istituto Biomembrane e Bioenergetica IBBE, CNR-Bari, Via G.Amendola 165/A, 70126 Bari, Italy; 5) Istituto Tecnologie Biomediche ITB, CNR-Bari, Via G.Amendola 122/D, 70125 Bari, Italy.

The p53 oncosuppressor protein plays a crucial role in the protection of genome integrity and the inactivation of its pathway appears to be a common, if not universal feature of human cancer. Although the contribution of its relative p63 to onset and tumor progression is less established it is known that TAp63 isoforms shows a p53-like anti-oncogenic activity, while N-terminal truncated ΔNp63α is characterized by pro-oncogenic potential. Here we describe a previously unknown function for the human E3 Ubiquitin ligase TRIM8 as a new key node for enhancing p53 oncosuppressor activity and, at the same time, for dropping oncogenic ΔNp63α activity. We found that TRIM8 overexpression induces MDM2 degradation leading to p53 stabilization and transcriptional activation, which in turn induces cell cycle arrest. Simultaneously, TRIM8 induces the degradation of the oncogenic ΔNp63aα isoform in a proteasome-dependent way. Remarkably, TRIM8 knockdown prevents p53 activation and ΔNp63α degradation after UV exposure and Nutlin-3 treatment. TRIM8 is located at 10q24.3, a region frequently associated to rearrangements and deletions in different human cancer, particularly gliomas. By qPCR we show that TRIM8 expression is strongly downregulated in glioblastomas cell lines. Our results reveal a previously unknown regulatory pathway of p53 and p63 activities, suggesting TRIM8 as a therapeutic target to enhance p53 oncosuppressor activity function and to impair ΔNp63α oncogenic activity.

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APC germinal mosaicism in a patient with Familial Adenomatous Polyposis carrying the c.4666del mutation. *C. Mongin[1, 2], C. Colas[1], F. Coulet[1], M. Eyries[1], Y. Lahely[1], JH. Lefevre[2], Y. Parc[2], F. Soubrier[2].* 1) Laboratoire d'Oncogenetique, GH Pitie-Salpetriere-APHP, PARIS, Ile de France, France; 2) Chirurgie digestive, Hopital Saint Antoine-APHP, PARIS, Ile de France, France.

We report a case of germinal APC mosaicism in a 36 years old male patient diagnosed forGardner syndrome with features of adenomatous polyposis and without family history of polyposis. No mutation was found after the initial screening for APC mutation. The APC gene of his daughter was sequenced because of the occurence of osteomas at five and nine years old, and a germline heterozygous mutation c.4666del, p.Thr1556LeufsX9 was detected. A second reading of the father's sequences, revealed that the germline mutation c.4666del was detectable but in a lower proportion than that predicted by a heterozygous mutation, suggesting the presence of somatic mosaicism in the father. The detection of the mutation in other tissues (buccal swab, normal colon tissue) and in the offspring suggests that it may have occurred early during embryogenesis, before the separation of the embryonic layers. As expected, the higher level of mutated allele was detected in adenomas. The c.4666del mutation is located in the region of APC at risk for Gardner syndrome and this patient had extracolonic features (osteomas). The mutation was missed by sequencing because of the low proportion of mutated cells in blood lymphocytes, but the High Fusion Resolution (HRM) method for pre-screening was tested and showed a better level of detection in this patient. Several dilutions of mutated DNA in wild type DNA show that the HRM technique allows a 5% proportion of the mutated allele to be detected. Sanger sequencing alone is not sensitive enough to detect low mosaicism levels and prescreening methods like HRM could be used for APC analysis because of the high rate of de novo mutations. Mosaicism is an important consequence of de novo APC mutations and must be considered in the diagnosis of apparently de novo cases. Mutation screening of offspring with the disease could be preferred when possible because of the risk of mosaicism. When no mutation is detected it can be envisioned to sequence adenomas DNA as a first step toward identifying APC mosaicism.

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I171V NBN germ-line mutation predisposing to cancer in adults cannot be considered as a risk factor for solid tumors in children. *J. Nowak[1], I. Ziolkowska[1], M. Mosor[1], M. Wierzbicka[2], D. Januszkiewicz[1,2].* 1) Institute of Human Genetics, Polish Academy Sciences, Poznan, Poland; 2) University of Medical Sciences, Poznan,Poland.

MRE11, RAD50 and NBN (MRN) complex is involved in DNA repair and cell cycle checking signaling. NBN being a part of MRN complex plays an important role in genome stabilization. Molecular variants of NBN gene may therefore constitue a cancer risk factor. Recent findings show that cells from NBN gene mutation carriers are characterised by spontaneous chromosome instability which may lead to the induction of malignant transformation. Heterozygous 657del5 mutation in the NBN gene have been described in various groups of cancer patients, like acute lymphoblastic leukemia , non-Hodgkin lymphoma, colon and rectum, prostate cancer and cancer cell lines .The aim of the study was to analyze the frequency of a panel mutations of NBN gene by screening all 16 exons of this gene along with polymorphisms examination. DNA was isolated from peripheral blood of 135 children with acute lymphoblastic leukemia, 270 women with breast cancer, 176 patients with larynx cancer, 93 with second primary tumors of head and neck, 131 with colorectal carcinoma and 1274 healthy individuals. I171V mutation of NBN gene was the most frequent and has been found in 23 patients compared to only 8 in healthy individuals. We observed the high incidence of germinal mutation I171V of NBN gene in breast, colorectal, larynx cancer and in multiple primary tumors. It can be concluded that I171V mutation of NBN gene is associated with predisposition to malignances and NBN allele I171V may be a general cancer susceptibility gene of low or middle risk. In the next step we would like to answer the question whether I171V germinal mutation of NBN gene may constitute risk factor for solid tumors in children. The frequencyof this mutation has been analysed in patients with neuroblastoma (n=66), Wilms' tumor (n=54),medulloblastoma (n=57) and rhabdomyosarcoma (n=82) hospitalized in Pediatric Oncology, Hematology and Bone Marrow Transplantation Department in the years 1987-2010. 2947anonymous blood samples collected on Guthrie cards drawn from the newborn screening program have been used as controls. All the patients and population controls came from the same geographical region. I171V mutation of the NBN gene has been observed in 5 control cases. Among children with solid tumors only in one child with medulloblastoma I171V variant has been found. In conclusion I171V NBN germ-line mutation in contrary to adults cannot be considered as a risk factor for solid tumors in children.

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miRNA profiling in normal and tumor bladder tissue samples. *P. Porter-Gill, A. Kaushiva, Y. Fu, L. Prokunina'Olsson.* LTG/DCEG, NCI/NIH, Bethesda, MD.

Urinary bladder cancer (UBC) is estimated as the 9th most common cancer and the 13th leading cause of cancer death worldwide. Environmental exposures such as cigarette smoking and specific occupational exposures are the most important risk factors for UBC. Familial aggregation and the significant racial differences in UBC incidence suggest genetic susceptibility to this disease. Understanding the association between specific genetic changes and the development of UBC may improve the screening program for early detection and treatment. Biomarkers measurable in accessible body fluids such as blood and urine and that can differentiate normal and tumor state, are of particular interest for UBC. microRNAs (miRNAs) that represent a new class of short non-coding regulatory RNA molecules stable in different conditions and easy to measure with quantitative PCR (qPCR) methods, are therefore attractive as potential biomarkers. Several possible miRNA with the potential for predicting disease progression have already been suggested for UBC. In this study, we used a genome-wide unbiased approach and quantified expression of 671 miRNAs with TaqMan Low Density miRNA arrays V2.0 (Applied Biosystems) supplemented with 130 additional miRNA expression assays not present on the arrays, in total quantifying expression of 801 miRNAs. We used RNA prepared with mirVana total RNA extraction kit (Ambion) that preserves miRNA fraction. In total, the miRNA profiling will be performed on 42 tissue samples of transitional urothelial carcinoma and 42 adjacent normal bladder samples with detailed clinical information. The analysis will include comparisons in miRNA expression patterns between normal and tumor tissues, and quantitative level of expression in relation to genotypes of single nucleotide polymorphisms (SNPs) found to be associated with UBC in genome-wide association studies (GWAS). The most interesting miRNAs will be individually tested in plasma samples of the same individuals. The combination of information on genetic susceptibility factors identified by GWAS studies and miRNA expression profiling might provide additional tools for early disease diagnostics and help to guide treatment options.

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Integration of miRNA and mRNA expression profiles in breast cancer identifies prognostic markers and associated pathways. *I. Ragoussis[1], C. Camps[1], L. Winchester[1], H. Gee[2], H. Sheldon[2], M. Taylor[2], A. Harris[2], F. Buffa[2].* 1) Genomic Research, Wellcome Trust Ctr Human Gen, Oxford, United Kingdom; 2) Molecular Oncology, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, OX3 9DS, United King-dom.

mRNA and microRNA expression profiling plays a key role in tumor classification and the identifi-cation of prognostic markers and therapeutic targets for cancer. Since microRNAs target mRNAs, it is important to explore potential benefits of their joint analysis for enhanced prognostic marker and therapeutic target discovery. Here we performed an integrated data-mining of microRNA and mRNA expression profiles across 219 breast cancers (BCs), including correction for clinico-pathological variability. The approach enabled us to discover microRNAs independently prognostic for distant-relapse free survival after correction for 8 pathway gene signatures (e.g. proliferation, hypoxia, invasion) and clinical covariates (e.g. age, nodal involvement, grade). Twelve microRNAs whose expression was prognostic in either ER+ (3 novel and 1 known - miR-128a) or ER- (7 novel and 1 known - miR-210) BCs were identified. Of the latter, miR-342, -27b and -150 were strongly prognostic in ER-/Her2-/PR- cases. To examine whether prognostic importance of specific microRNAs might be due to function on predicted targets in BC, associations between microRNAs, pathways and targets were examined by global expression analysis. miR-210 and miR-27b fulfilled the strict criteria of an intact microRNA-target-pathway relationship and were selected for further validation. Indeed, expression of miR-210 and -27b predicted targets, was inversely correlated with hypoxia and invasion respectively, and prognostic both in this and 2 large independent BC datasets. Following from the confirmation of the validated miR-210 target ISCU, we provide a number of additional target genes for miR-210 and -27b that give an insight in the mechanisms of miRNA action relevant to disease progression, have prognostic value and could offer new therapeutic approaches.

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A urine based microRNAs signature for prostate cancer and benign prostatic disease. *M. Rodriguez-Dorantes[1], I. Sálido-Guadarrama[1], H. Miranda-Ortiz[1], P. García-Tobilla[1], S. Solórzano-Rosales[1], D. Saavedra-Briones[2], G. Morales-Montor[2], M. Sánchez-Alarcón[1].* 1) Del Alvaro Obregon, Natl Inst Genomic Medicine, Mexico DF, Mexico; 2) General Hospital Dr. Manuel Gea Gonzalez, Mexico DF, Mexico.

Prostate Cancer (PCa) is the second cause of death in men in industrialized countries. Serum prostate specific antigen (PSA) remains the only molecular marker used as indicator in the detection and management of PCa, it suffers from low specificity. The most effective therapy is radical prostatectomy and androgen ablation, unfortunately many of these patients cross to androgen independence, developing very aggressive tumors with poor prognosis. The important role of microRNAs (miRNAs) and the consequences of their deregulation in the progression of human cancer have been well established. More recently, high-throughput studies in patients have revealed that altered miRNAs profiling can classify cancers and predict outcome with high accuracy. The aim of this study was to evaluate the potential use of urine miRNAs as possible indicators of prostate disease outcome. Thirty six urine samples were collected from patients diagnosed as either PCA or BPD (benign prostate disease) obtained after Digital Rectal Exam (DRE).Total miRNA extraction was performed using the RNAeasy kit according to manufacturer. RNA quality was verify and RT and preamplification reactions were performed. We measure expression of 667 miRNAs by qPCR. MiRNAs expression was determined using comparative Ct method and P-values <0.05 were considered statistically significant. Data Analysis using DataAssist™ Software revealed differential expression of 29 miRNAs associated with several cancer processes. To improve our analysis we use Bioconductor package. After filtering our initial data set, we found 21 miRNAs that were significatively altered in CaP. Most of these are upregulated and only 2 were found to be downregulated. Hsa-miR-184 and hsa-miR-100 was also up regulated in circulating blood. Hsa-let-7 family members are related with tumor suppressor functions in normal conditions and are down regulated in CaP, however we found over-expression of some let-7 miRNAs (let-7b-c-d-e). Hsa-miR-17 a known oncogene member of mir-17-92 cluster; other miRNAs related to oncogene activity associated with proliferation and growth were observed. hsa-miR-150 is related to oncogen activity and promotes the proliferation and growth of gastric cancer cells. Our data suggest that miRNAs obtained from urine samples could provide a powerful tool at aiding early identification of patients with suspected prostate cancer with a great impact in reducing the number of unnecessary biopsies.

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NATURAL ANTISENSE RNAs (NATs) IN THE EPIGENETIC MODULATION OF THE CHROMATIN STATUS. *S. Rossetti, N. Sacchi.* Cancer Genetics Program, Roswell Park Cancer Inst, Buffalo, NY.

It is emerging that natural antisense RNAs (NATs) can induce concerted epigenetic changes and heritable biological phenotypes typically induced by cancer genetic rearrangements. Here we show that stable expression of two NATs, one for the colony stimulating factor 1 receptor (CSF1R) and one for the retinoic acid receptor beta 2 (RARB2), in mammalian cells can induce transcriptional silencing of the respective sense transcripts. Analysis of the chromatin associated with the regulatory regions of both CSF1R and RARB2 shows the acquisition of heritable chromatin repressive marks. The observed loss of function in cells stably expressing the NATs indicates the occurrence of homozygous epigenetic silencing at CSF1R and RARB2 alleles. Interestingly, the same epigenetic changes and biological phenotypes can be induced by repressive chromosome rearrangements involving either AML1, one the CSF1R direct transcriptional regulators, or RARA, the upper regulator of RARB2. This suggests that gene specific NATs are mechanistically implicated in the genesis of epigenetic repression of transcription induced by cancer genetic rearrangements involving either AML1 or RARA.

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S100A4 regulates tumor growth, motility, and invasion in pancreatic cancer. *H. Sekine, T. Tabata, N. Tsukamoto, N. Chen, K. Sato, Y. Umetsu, K. Nakamura, G. Kim, A. Yamamura, Y. Yoshino, S. Fukushige, M. Sunamura, A. Horii.* Department of Pathology, Tohoku University School of Medicine, Sendai, Japan.

S100A4 protein belongs to the S100 subfamily, which has grown to be one of the large subfamilies of the EF-hand Ca^{2+}-binding proteins, and overexpression of *S100A4* is suggested to associate with cell proliferation, invasion, and metastasis. We observed frequent overexpression of *S100A4* in pancreatic cancer cell lines by quantitative real-time RT-PCR and Western blotting. Tissue microarray analysis validated S100A4 overexpression in primary pancreatic cancer tissues (48/83, 58%), and overexpression of S100A4 associated with perineural invasion (P = 0.029) and invasion pattern (P = 0.001). We further analyzed RNAi-mediated knockdown to address the possibility of its use as a therapeutic target for pancreatic cancer. After *S100A4*-knockdown, only *S100A4*-overexpressing cells showed induction of G2 arrest and eventual apoptosis, and suppression of cell motility and invasion. Microarray analyses revealed that knockdown of *S100A4* induced upregulation of the tumor suppressor genes *PRDM2* and *VASH1*. We then introduced the *S100A4* gene in pancreatic cancer cell lines with low level of *S100A4* expression and found remarkably accelerated cell growth and motility; no change was observed in the *S100A4*-overexpressing cells. Results of microarray analyses after *S100A4* expression vector-mediated induction in two low level *S100A4*-expressing cells showed 72 and 18 genes that were two fold or more commonly upregulated or downregulated, respectively. Among these, *IFI27* and *NOV* were selected as commonly upregulated genes for further characterization, and the results will be demonstrated and discussed. Our present results strongly suggested that *S100A4* is playing an important role in cell growth, motility, and invasiveness in pancreatic cancer, and that characterization of downstream target molecules will provide further information of progression of pancreatic carcinogenesis which can lead to better clinical management of pancreatic cancer patients.

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Polycomb repression of microRNAs in murine lymphoma. *C. Sugiana, A. Adisa, L. Happo, M. Wakefield, C.L. Scott.* Molecular Genetics of Cancer, Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria 3052, Australia.

Over-expression of polycomb group (PcG) proteins has been observed in a range of human cancers documented to have poor treatment outcomes. PcG genes are conserved from flies to humans, important for development and for stem cell function. Studies in mammalian systems have identified them in protein complexes that silence genes involved in many regulatory processes, such as apoptosis, cellular proliferation and senescence. More recently, PcG proteins have been shown to co-occupy microRNA (miRNA) promoters which are targets of crucial transcription factors during development.

Members of the Polycomb Repressive Complex 1 (PRC1), *Bmi1* and *Cbx7*, have been documented to have direct roles as oncogenes, capable of initiating and potentiating murine haemopoietic malignancies. Bmi1 and Cbx7 repress transcription from the *Cdkn2a* locus (encoding both $p16^{Ink4a}$ and $p19^{Arf}$) and act epistatically to the Arf-p53 pathway during tumorigenesis.

To mimic over-expression of PcG as a late hit in advance grade tumors, we introduced *Bmi1* into established Eμ-myc lymphoma cell lines. We are studying the effects of Bmi1 on response to DNA damaging drugs. We hypothesized that Bmi1 may target microRNA regulating important downstream targets.

However, little is known about miRNA regulation and targets and there is no gold standard method for miRNA expression assessment. Therefore, we trialed several of the latest methods for small RNA isolation, quality assessment and qPCR-based expression analysis to test our hypothesis. Analysis results and strengths/weaknesses of each method will be presented and essential considerations for robust miRNA expression measurement will be discussed.

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P53, p21, MDM2 and p16 protein expression and their relations with p21 polymorphisms and p16 Hypermethylation among the ESCC patients in northeastern Iran. *N. Taghavi[1,2,3], F. Biramijamal[1], MR. Abbaszadegan[4], M. Sotoudeh[3], H. Khademi[3], O. Moaven[4], R. Malekzadeh[3].* 1) Med Gen, NIGEB, Tehran, Iran; 2) Khatam-Al-Anbia hospital, Social Security Organization, Gonbad Kavous, Golestan, Iran; 3) DDRC, Tehran university of medical sciences, Tehran,Iran; 4) Bu-Ali Research Institute,MUMs, Mashhad, Iran.

Backgrounds and Objectives: High incidence and mortality rate of Esophageal Squamous Cell Carcinoma [ESCC] have been reported in the northeastern Iran; however the complex network of molecular alterations underlying the ESCC progression is not clearly elucidated in this reign. Tumor suppressor genes, P53, P21 (waf1/cip1) and P16 INK4a, and the proto-oncogene, MDM2, are considered as the essential G1 cell cycle regulatory genes that loss of their function is associated with ESCC carcinogenesis. Two polymorphisms of p21 gene, (rs1801270) and (rs1059234),as well as, p16 methylation may affect the protein expression and play a role in esophageal cancer susceptibility. The present study was aimed to investigate the expression of p53, p21, p16 and MDM2 proteins in relation to p21 polymorphisms, aberrant methylation of P16 gene, and some possible risk factors, such as cigarette smoking in ESCC patients of northeastern Iran. Methods: The expression of p53, p21, p16 and MDM2 proteins were investigated Immunohistochemically, on paired tumor tissues and available paraffin-embedded blocks of adjacent normal specimens of 126 ESCC patients, along with normal esophageal tissues of 100 healthy subjects. Genotyping of p21 polymorphisms were determined using PCR-RFLP method, and methylation status of the p16 promoter gene, was also assessed with Methylation-Specific-PCR. Result: The over-expression of p53, p21, but not MDM2, as well as p16 under-expression was associated with esophageal squamous cell carcinogenesis, however their expression was not associated with p21 polymorphisms in ESCC patients.On the other hand, P16 methylation was significantly associated with decreased p16 expression (P = 0.03), as well as, p53 over-expression (P = 0.02)in these patients. Furthermore, there was an synergistic interaction between cigarette smoking and either p53 over-expression or p21 polymorphisms in ESCC carcinogenesis. in addition, it has been shown that p21 polymorphisms was not associated with p16 methylation also. Conclusion: Among the population studied in northeaster Iran, our data showed a possible crosstalk between involved molecular mechanisms including p16 methylation and p53 over-expression, as well as a gene- enviroment interaction between cigarette smoking and p21 polymorphisms or p53 over-expression in susceptibility to esophageal squamous cell carcinoma in northeastern Iran.

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Cisplatin resistance in cancer: role of copper pathway genes. *B.D. Teitge, G. Macintyre, D.W. Cox.* Medical Genetics, University of Alberta, Edmonton, Alberta, Canada.

There is evidence that the copper transport system plays a role in tumour resistance to cisplatin, a platinum based chemotherapeutic drug. Several copper transport genes have been associated with cisplatin resistance, but the contribution of each is unknown. Five genes proposed to play a role in cisplatin resistance (*ATP7B*, *ATOX1*, *CTR1*, *CTR2*, *COMMD1*) were each cloned into a Chinese hamster ovary cell line at a single location, under the control of a tetracycline-inducible promoter. Each cell line was verified for sequence and integration. Using this controlled expression system, *ATP7B* expression alone was sufficient to confer resistance to copper, and *CTR1* (copper transporter1) expression enhanced cisplatin sensitivity. Surprisingly, overexpression of *CTR2* did not confer resistance to cisplatin, while *ATP7B* (copper ATPaseB, defective in Wilson disease) or *ATOX1* (anti-oxidant protein1) over-expression conferred a minimal increase in resistance to cisplatin. However, combined expression of *ATOX1* and *ATP7B* enhanced resistance to cisplatin, suggesting that these proteins have an additive, though subtle impact on cisplatin resistance. In support of other studies, we conclude that the cisplatin resistance of some tumours is likely determined by the aberrant, or reduced, expression of a single gene, *CTR1*. However, the detection of over-expression of other copper pathway genes, such as *ATP7B*, in a tumour may not fully explain the cisplatin resistance observed. Given this dissimilarity in the transport of cisplatin and copper, we investigated whether cisplatin affects the localization of the copper-dependent transcription factor, ATOX1. Using confocal microscopy and sub-cellular fractionation, ATOX1 was found to undergo nuclear relocalization when activated by copper. Cisplatin reduced the nuclear relocalization of ATOX1. This is the first demonstration that cisplatin might have an antagonistic effect on a copper chaperone, and demonstrates the dissimilarity in cisplatin and copper transport.

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NOVEL MLH1 DUPLICATION IDENTIFIED IN COLOMBIAN FAMILIES WITH LYNCH SYNDROME. *C. Trujillo[1], V. Alonso-Espinaco[2], M.A. Giráldez[2], H. van der Klift[3], L. Carvajal-Carmona[4], J. Muñoz[2], F. Balaguer[2], T. Ocaña[2], I. Madrigal[5], I. Tomlinson[4], M. Milà[5], J. Wijnen[3], A. Castells[2], S. Castellví-Bel[2].* 1) Genetics Section, Dr. Erfan & Bagedo General Hospital, Jeddah, Saudi Arabia; 2) Gastroenterology Department, Institut de Malalties Digestives i Metabòliques, Hospital Clínic, CIBEREHD, IDIBAPS, University of Barcelona, Barcelona, Catalonia, Spain; 3) Center for Human and Clinical Genetics, Leiden University Medical Center, The Netherlands; 4) Wellcome Trust Centre for Human Genetics, Nuffield Department of Medicine, University of Oxford, UK; 5) Biochemistry and Molecular Genetics Department, Hospital Clínic, CIBERER, IDIBAPS, Barcelona, Catalonia, Spain.

Lynch syndrome (LS) accounts for 2-3 % of all colorectal cancer (CRC) and it is the most commonly inherited CRC form. Germline mutations in the DNA mismatch repair (MMR) genes MLH1, MSH2, MSH6, PMS2 and EPCAM underlie the majority of cases of LS. Aim: To identify the genetic mutation responsible for LS in an extensive Colombian family and to study its prevalence in this population. Material and methods: A large LS family from Colombia complying Amsterdam criteria II was studied by immunohistochemistry (IHC), and molecular screening of the MMR genes was performed initially by MLPA (multiplex ligation-dependent probe amplification). Results were confirmed by additional independent MLPA, Southern blot and sequencing. Specific mutation screening was developed by a rapid PCR test. Results: Index case tumor IHC results (MLH1-, MSH2+, MSH6+, PMS1-) pointed to a molecular defect in the MLH1 gene. Indeed, the MLPA analysis detected a duplication of exons 12 and 13 of MLH1. Afterwards, this mutation was confirmed by and independent MLPA test and characterized precisely to span 4,219 base pairs (bp) from the 3' boundary of intron 11 to the 5' boundary of intron 13 by Southern blot and sequencing. Furthermore, specific screening for this MLH1 duplication in this family permitted to identify 6 additional carriers and 13 non-carriers of this MLH1 duplication. Also, when screening independent CRC Colombian cases, another carrier family of this same duplication was identified from the same geographical area. Conclusions: A novel duplication of exons 12 and 13 of the MLH1 gene was detected in 2 independent LS families from Colombia. A putative founder effect for this LS mutation in the Antioquia area could be suggested.

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Complex genetics in the original Gardner syndrome kindred 109. *T.M. Tuohy[1], M.W. Done[1], M.F. Leppert[2], R.W. Burt[3].* 1) High Risk Cancers Clinic, Huntsman Cancer Inst, University of Utah Salt Lake City, UT; 2) Department of Human Genetics, University of Utah,Salt Lake City, UT; 3) Department of Medicine, Huntsman Cancer Inst, University of Utah, Salt Lake City, UT.

Familial Adenomatous Polyposis (FAP) is an autosomal dominant colorectal cancer predisposition syndrome characterized by hundreds to thousands of colonic polyps and, if untreated by a combination of screening and/or surgical intervention, a ~99% lifetime risk of colorectal cancer. Additional cancers are also associated with the condition, albeit at lower frequencies. A variant of the condition, historically referred to as Gardner syndrome, is associated with extra-colonic features, notably desmoid tumors, multiple epidermoid cysts, osteomas of the mandible and skull, and dental abnormalities. Since the condition was mapped in the majority of clinically verified families to the *APC* gene, over 900 clinically pathogenic mutations have been identified, and several studies have addressed trends in the available data towards phenotype-genotype correlations. We report genetic lesions in both *APC* and *MUTYH* that segregate through kindred 109, one of the most severely affected FAP kindreds studied to date, originally reported by E.J. Gardner, whose description gave rise to the historically used term Gardner syndrome. Archived samples from the original linkage studies were studied in parallel with recently obtained samples from research participants recruited from clinical services at the Huntsman Cancer Institute, and analyzed for phenotype-genotype correlation. We have used sequence and STR analysis of the *MUTYH* locus, together with archived pedigree information to track the segregation pattern in affected branches of the kindred. In the oldest living generation, both individuals with the *APC* mutation alone died before the age of 30, while 3 out of 4 individuals, ages (70 - 73), with both the *APC* frameshift mutation and the *MUTYH* variant of uncertain significance are still living. However, among the next generation, 1 out of 8 individuals, ages (33 - 48), with the *APC* mutation alone died ate the age of 46, while all 4 individuals, ages (38 - 51), with both the *APC* mutation and the *MUTYH* variant of uncertain significance are still living. This observation contrasts with the observations of Sieber et al (2004) who found that deficiency of *MUTYH* enhanced the tumorigenesis phenotype in Min mice, which, analogous to K109, also harbor a chain-terminating mutation in exon 15 of *APC*. Although preliminary, our results may offer a useful avenue of exploration of a possible interaction between the *APC* and *MUTYH* pathways that differs from the mouse models.

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BRCA1/2 mutations in pancreatic cancer patients and their clinical characteristics. *E. Dagan[1,2], R. Epelbaum[3,4], R. Gershoni-Baruch[1,4].* 1) Inst Human Genetics, Rambam HealthCare Campus, Haifa, Israel; 2) Dept of Nursing, Faculty of Welfare and Health Sciences, University of Haifa, Haifa, Israel; 3) Inst of Oncology, Rambam HealthCare Campus, Haifa, Israel; 4) Ruth and Bruce rapoport, Faculty of Medicine, Technion-Inst of Technology, Haifa, Israel.

Background and aim: In Ashkenazi Jews three predominant mutations in BRCA1/2 genes were found, with a frequency of 2-3% in the healthy population, and 20% to 40% in breast and ovarian cancer patients, respectively. The present study aimed to evaluate the frequency and clinical relevance of BRCA1/2 predominant mutations in Ashkenazi pancreatic cancer (PC) patients. Methods: A cohort of 42 PC patients was recruited from the Institute of Oncology at Rambam HealthCare Campus. Of these, 38 [13 (34.2%) females and 25 (65.8%) males] who were of Ashkenazi origin, were included. Following informed consent form, sociodemographic, clinical and health behavior profile were collected at patient-researcher encounter. Data were complemented via the patients' oncology charts, when needed. A blood sample was drawn for the genetic testing. Results: Nine patients (23.7%) were found to carry one of the three predominant mutations in BRCA1/2 genes. Of these, seven carried the 6174delT mutation in BRCA2 gene and two had the 185delAG mutation in BRCA1 gene. Earlier age at diagnosis was noted among mutation carriers compared to non-carriers (58.7years±7.0 and 65.0years±9.8, respectively, p=0.049). Eight (88.9%) PC patients with BRCA1/2 mutations compared to nine (34.6%) non-carriers reported on positive family history of breast, ovarian or pancreatic cancer (p=0.05). Most of the PC patients disclaimed engagement in physical activity (n=25, 65.8%) and 26 (68.4%) were non-smokers. No differences between carriers and non-carriers were found as regards history of heart diseases and diabetes mellitus. Conclusions: Taken together, more than 20% of PC patients of Ashkenazi descent were found to carry a mutation in BRCA1/2 genes, mainly in BRCA2. Mutations in BRCA1/2 constitute a major cause for PC in Ashkenazi Jews and carriers are diagnosed about five years earlier than non-carriers. These findings should be applied to the clinical setting and genetic diagnosis for PC patients and family members at risk is warranted. Furthermore, BRCA2 mutation carriers should be monitored for early detection for PC.

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The development of the cutaneous neurofibroma. *E-M. Jouhilahti[1], S. Peltonen[2], T. Callens[3], E. Jokinen[1], A.M. Heape[4], L. Messiaen[3], J. Peltonen[1].* 1) Department of Cell Biology and Anatomy, Institute of Biomedicine, University of Turku, Finland; 2) Department of Dermatology, University of Turku and Turku University Hospital, Finland; 3) Department of Genetics, Medical Genomics Laboratory, University of Alabama at Birmingham, USA; 4) Department of Anatomy and Cell Biology, Institute of Biomedicine, University of Oulu, Finland.

Cutaneous neurofibromas are the hallmarks of neurofibromatosis type 1 (NF1). They are composed of multiple cell types, and are currently believed to arise from small nerve tributaries of the skin. A key finding in the context of this view has been that only subpopulations of tumor Schwann cells harbor bi-allelic inactivation of the NF1 gene (NF1-/-). Our aim was to further elucidate the pathogenesis of cutaneous neurofibromas. Cells expressing biomarkers associated with multipotency were detected in cutaneous neurofibromas. A method for isolating and expanding multipotent neurofibroma-derived precursor cells (NFPs) from dissociated human cutaneous neurofibromas was developed and employed to analyze their growth and differentiation potential. In analogy to solitary cells resident in neurofibromas, NFPs were found to express nestin, and had the potential to differentiate at least to Schwann cells, neurons, epithelial cells and adipocytes. Mutation analysis of the NFPs revealed that their genotype was NF1+/-. The results lead us to speculate that the development of cutaneous neurofibromas includes the recruitment of multipotent NF1+/- precursor cells. These cells may be derived from the multipotent cells of the hair roots, which are often intimately associated with microscopic neurofibromas.

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Transcription Independent Over-expression of Insulin-Like Growth Factor II in Adrenal Hyperplasias. *K.S. Nadella, C. Johnson, M.Q. Almeida, L. Isaac, S. Koliavasilis, K. Pfeifer, C. Stratakis.* NICHD, Bethesda, MD.

Insulin like growth factor-II (IGF2), a key regulator of cell growth is tightly regulated in its expression during embryogenesis and normal growth through imprinting. H19 transcript, an untranslated RNA regulates the expression of IGF2. IGF2 and H19 are reciprocally imprinted genes where IGF2 is expressed from paternal and H19 from maternal alleles. Loss of imprinting of IGF2 leading to biallelic expression was reported in many cancers including the adrenocortical tumors. IGF2 binding to IGF type I (IGF 1R) receptor mediates mitogenic and antiapoptotic effects while its binding to mannose 6-phosphate/IGF type 2 (M6-P/IGF 2R),a receptor with tumor suppressor function modulates the levels of IGF2 by targeting it to lysosomes for degradation. Since alterations of IGF2 axis is associated with the pathogenesis and progression of adrenal tumors, we have investigated the expression of IGF2 in three different subsets of human adrenal leisons, namely Massive Macronodular Adrenocortical Disease (MMAD), Cortisol Producing Adenomas (CPA) and Primary Pigmented Nodular Adrenocortical disease (PPNAD), to see if IGF2 is differentially regulated between adrenal hyperplasias (MMAD and PPNAD) and other lesions. Our aim is to determine the utility of IGF2 as a biomarker and a possible therapeutic target to distinguish and treat these disease subsets. Western Blotting analysis from 23 tumor samples revealed over expression of IGF2 protein in 4/6 MMAD, 4/6 CPA and 4/11 PPNAD tumors. Immunohistochemistry further confirmed the tumor specific accumulation of IGF2 protein in cytoplasmic compartment. To investigate the molecular mechanisms responsible for IGF2 over expression we screened samples for loss of imprinting by measuring biallelic expression of IGF2 and H19 transcript through previously reported single nucleotide polymorphisms in IGF2 and H19 genes. We also quantitated total IGF2 mRNA using qRTPCR. We did see loss of imprinting in some samples. However, no correlation was observed between loss of imprinting and total RNA levels and more importantly between IGF2 RNA and protein. Our data therefore suggest a post transcriptional mechanism for IGF2 protein stabilization either through loss of function mutations in IGF2R or in IGF2R binding sites in IGF2 that might have lead to observed accumulation of IGF2 and therefore its availability for enhanced proliferation of adrenal cells. Experiments are on to further address this possibility.

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Non-coding miRNA-141 distinguishes Hashimoto's Thyroiditis and papillary thyroid carcinoma. *E.R. Cantwell, P. Smyth, J.J. O'Leary, O. Sheils.* Department of Histopathology, Trinity College Dublin, Ireland.

MicroRNAs (miRNAs) are small non-coding RNAs approximately 22 nucleotides in length which function as regulators of gene expression. Disregulation of miRNAs has been associated with initiation and progression of oncogenesis in humans. Recently, our group described a unique miRNA expression signature distinguishing papillary thyroid carcinoma (PTC) cells from normal cell lines. The miR-200 family members' miR-141 and miR200a were found to be significantly up-regulated in PTC cells. An investigation into the expression of miRNA-141 in a series of archival thyroid neoplasms (n = 111; classic PTC, follicular variant PTC, follicular thyroid carcinoma (FTC), Hashimoto's thyroiditis, or normal thyrocytes) was performed. Each cohort had a minimum of 20 validated samples surgically excised within the period 1980 - 2009. All samples were derived from the Irish population thereby minimising potential geographical variation. 7μm formalin-fixed paraffin-embedded sections were cut, mounted on slides, deparaffinised and Haematoxylin and Eosin (HE) stained. Laser capture microdissection was used to specifically dissect neoplastic cells from the surrounding tissue. RNA was extracted from the harvested cells and quantitative RT-real time PCR was performed. Comparative CT analysis was applied using RNU6B as an endogenous control. Statistically significant ($p<0.01$) differential expression profiles of miR-141 were found between tissue types. Hashimoto's thyroiditis samples and follicular variant PTC displayed a statistically significant down-regulation compared to both classic PTC and normal thyrocytes. The rate of Hashimoto's thyroiditis in PTC cases has been reported to be between 0.5% and 38%. It is of interest that Hashimoto's thyroiditis thyrocytes, which are postulated by some to be in a stage between normal epithelium and classic PTC, have a significantly altered miR-141 profile compared to both classic PTC and normal thyrocytes. In conclusion, miRNA expression profiles differentiate Hashimoto's thyroiditis thyrocytes from normal and PTC thyrocytes. MiRNAs may act as potential biomarkers for fvPTC in the differential diagnosis of papillary-type thyroid carcinomas.

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Identification of the regulatory networks of miR-199a-2 in testicular germ cell tumors. *S. Gu[1], Y.K. Suen[1], H.H. Cheung[1], W.Y. Chan[1,2].* 1) School of Biomedical Sciences, Chinese University of Hong Kong, Shatin, Hong Kong; 2) Lab of Clinical and Developmental Genomics, NICHD, NIH, Bethesda, MD.

Previous studies showed that DNA hypermethylation silences the expression of miR-199a-2 in testicular germ cell tumors (TGCTs), resulting in the overexpression of its target PODXL (podocalyxin-like protein). miR-199a-2 knockin suppressed cell proliferation and migration in vitro and tumor growth and metastasis in vivo. High levels of PODXL are found in several types of tumors. Since it was reported that mammalian miRNAs have an average of 300 conserved targets per miRNA family, it is likely PODXL is not the only target of miR-199a-2 in TGCT. The role of the other targets of miR-199a-2 is not known. To understand the network of genes regulated by miR-199a-2 in TGCTs, proteomics and function genomics analyses of miR-199a-2 knockin TGCT cells (NT2 cells) were performed. miR-199a-2 knockin and vector control NT2 cells were cloned by limiting dilution. Total cellular proteins of the cloned cells were analyzed by two-dimensional gel electrophoresis. A number of proteins up- or down-regulated by miR-199a-2 were identified. These proteins were isolated and further analyzed by mass spectrometry. Differences in gene expression caused by miR-199a-2 knockin were also investigated using expression chips. Among the differentially expressed genes, those of two oncoproteins were found to be down-regulated and those of four tumor suppressors were up-regulated by miR-199a-2. The proteomic and functional genomic data will be collated and the networks comprised by the differentially expressed genes/proteins will be constructed using bioinformatics programs. Results obtained would lead us to understand the pathways modulated by miR-199a-2 that underscore the behavior of TGCT cells.

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Inherited polymorphisms in the RNA-mediated interference machinery and microRNA expression in lung cancer. *M. Rotunno[1], Y. Zhao[2], A.W. Bergen[3], J. Koshiol[1], L. Burdette[1], M. Rubagotti[4], R.I. Linnoila[5], F.M. Marincola[6], P.A. Bertazzi[4], N.E. Caporaso[1], L. McShane[2], E. Wang[6], M.T. Landi[1].* 1) Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, MD, USA; 2) Division of Cancer Treatment and Diagnosis, National Cancer Institute, NIH, MD, USA; 3) Stanford Research Institute, Stanford, CA, USA; 4) University of Milan, Italy; 5) Center for Cancer Research, National Cancer Institute, NIH, MD, USA; 6) National Institutes of Health Clinical Center, NIH, MD, USA.

MicroRNAs (miRs) are known to have an important role in lung cancer, the largest cause of cancer mortality worldwide. We investigated whether single nucleotide polymorphisms (SNPs) in the RNA-mediated interference machinery involved in miR maturation are associated with lung cancer risk and survival and with miR expression profiles in lung cancer tissue. We analyzed 12 SNPs in the *PolR2A*, *Drosha* and *Dicer* genes (*POLR2A*, *RNASEN* and *DICER1*, respectively) in DNA extracted from blood of 1,984 cases and 2,073 controls from the Environment And Genetics in Lung cancer Etiology (EAGLE) study. In addition, we investigated miR expression profiles in 165 lung adenocarcinoma (AD) and 125 squamous cell carcinoma (SQ) tissue samples from the same population using a custom oligo array. We assessed the association of individual genotypes and of haplotypes with lung cancer risk in the full cohort using unconditional logistic regression models, and with lung cancer-specific survival in patients using Cox regression models. For the lung cancer cases, the associations of the SNPs with miR expression profiles were assessed using two-sample t-tests and global permutation tests were performed to confirm statistical significance of associations with full miR expression profiles. There was no association between SNPs or haplotypes and lung cancer risk overall. However, we identified a haplotype in *RNASEN* that was significantly associated with shorter lung cancer-specific survival (hazard ratio for mortality = 1.86, 95% CI = 1.19-2.92, p-value = 0.007). In addition, a SNP within the same *RNASEN* haplotype was associated with alterations in miR expression profiles in lung adenocarcinoma (global p-value=0.001). The 56 miRs affected by this *RNASEN* variant included miRs known to have tumor suppressor or oncogenic potential or to be associated with survival, such as the let-7 family, miR-21, miR-25, miR-126, miR15a, and others. This study provides the first evidence that inherited variation in the miR-processing machinery might affect miR expression levels and lung cancer-specific survival.

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Elucidation of mRNA targets of miR-222 and miR-25 in thyroid cell lines. *P. Smyth[1], S. Aherne[1], L. Smith[2], J. O'Leary[1], O. Sheils[1].* 1) Histopathology, University of Dublin, Trinity College, Dublin, Ireland; 2) Fluidigm Corp., San Francisco, CA, USA.

Thyroid cancer is the most common endocrine malignancy and accounts for the majority of endocrine cancer deaths each year. The majority of thyroid tumours are papillary thyroid carcinomas (PTC) and are well differentiated, indolent and are associated with good prospects of survival. Anaplastic carcinomas (ATC), however, are very aggressive with survival rates of less than one year in most instances. Studies on miRNA expression in PTC have found an aberrant miRNA expression profile in PTCs compared with normal thyroid tissues and have identified certain miRNAs that have become associated with this disease, such as miR-221, miR-222, and miR-146. This study investigated the functional roles of two miRNAs; miR-222 & miR-25 in the context of ATC. To explore this, miRNA mimics and inhibitors were transfected into thyroid cell lines. An unbiased genome wide approach using Affymetrix™ microarrays was utilised to elucidate the mRNA targets of miR-222 & miR-25 in the thyroid cell lines. Statistical analysis of the data produced from these experiments identified almost 100 mRNAs of diverse functions that are that are either directly or indirectly targeted by miR-222 and miR-25. The down-regulation of 23 and 22 mRNA targets in response to miR-25 and miR-222 expression respectively were successfully validated using Fluidigm® real-time PCR technologies. Gene ontology analysis of the miR-25 target gene list showed it to be significantly enriched for genes involved in cell adhesion. The reduction of TRAIL and MEK4 protein expression in response to miR-25 expression was validated using western blots. The gene targets of these two miRNAs provide an interesting insight into their possible roles in thyroid cancer progression.

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Interaction of a BAR Adapter Protein, Bin1, with Ku Affects the Maintenance of Telomere Length and Cancer Development. *A. Ramalingam[1], J.B. DuHadaway[1], P.S. Donover[1], G.C. Prendergast[1,2,3].* 1) Lankenau Institute for Medical Research, Wynnewood, PA; 2) Department of Pathology, Anatomy & Cell Biology; 3) Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA.

Ku70 and Ku80 together form one of the essential complexes that cap telomere ends and are central to the nonhomologous end joining (NHEJ) DNA repair pathway. Recently we showed that a murine BAR adapter protein, Bin1, physically interacts with Ku70/Ku80 in an *in vitro* protein-protein interaction assay. Bin1 ablation in mouse mammary glands delayed tissue modeling and drove cancer progression, and mice mosaic for *Bin1* showed increased susceptibility, particularly to lung cancer. Keratinocyte epithelial cell lines deleted for *Bin1* showed increased tumorigenicity in syngeneic mice. Absence of *Bin1* rendered the cells less susceptible to etoposide, a topoisomerase II inhibitor, but did not affect the sensitivity of the cells to similar double strand break(DSB)-causing other chemical agents or γ-irradiation. This etoposide-resistance of *Bin1* deletion could not be explained by change in Ku localization, change in DSB formation nor by a change in drug uptake by endocytosis. However, presence of *Bin1* resulted in decreased NHEJ repair in a plasmid-based assay. More importantly, deletion of fission yeast homologue of *Bin1* resulted in telomere lengthening. It is well known that alterations in telomere length lead to chromosomal aberrations and eventually to the development of cancer. Using *Bin1* null keratinocytes and siRNA and overexpression experiments, we plan to look at the telomere length maintenance in a mammalian system to help explain the tumorigenic ability of *Bin1* null cells in the absence of altered telomerase activity. Also, there is evidence suggesting a link between telomere length and fecundity. One of the notable phenotypes of *Bin1* mosaic null mice was extended female fecundity. Altered telomere length due to the lack of Bin/Ku interaction might explain the extended period of fecundity in those mice.

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Germline *SDHC* whole-gene deletion in a patient with malignant extra-adrenal pheochromoctyoma: reconsideration of clinical testing strategy. *J. Moline[1,2], J.L. Chen[1,2], C. Eng[1-3].* 1) Genomic Medicine Institute, Cleveland Clinic, Cleveland, OH; 2) Lerner Research Institute, Cleveland Clinic, Cleveland, OH; 3) Taussig Cancer Institute, Cleveland Clinic, Cleveland, OH.

Approximately 25% of incident pheochromocytoma (PC) and/or paraganglioma (PGL) patients have been found, in a population-based study, to carry germline mutations in the genes responsible for multiple endocrine neoplasia type 2 (*RET*), von Hippel-Lindau disease (*VHL*) and hereditary PC-PGL syndrome (*SDHB, SDHC, SDHD*). *SDHB* and *SDHD* mutations account for the majority of those with heritable PGL. Those who are found to have an *SDHB* mutation typically have extra-adrenal PC, with the prevalence of malignancy in population-based studies being 14-35%. While *SDHD*-related PGL can be malignant, the rate is much lower than for *SDHB*. Because *SDHC* has never been associated with malignant PC, and typically characterized by benign unilateral head and neck PGL, *SDHC* testing is not clinically considered in the setting of malignant PC. A 49-year-old white female presented to the emergency room with severe abdominal pain, which may have been related to a viral illness. However, on further investigation, masses of the retroperitoneum, iliac wing, and sacrum were detected on CT scan. Pelvic bone biopsy revealed metastatic PGL. Plasma norepinephrine was elevated at 16,260 pg/ml (ref 80-520) and dopamine 52 pg/ml (ref 0-20). Past medical and surgical history was significant for hypertension, palpitations, sweating, and hypertensive crisis during total abdominal hysterectomy for uterine fibroids one year earlier. Family history was negative for PC and PGL. Given the finding of an extra-adrenal malignant pheochromocytoma in the patient, clinical *SDHB* sequencing was pursued, but only a SNP was found (c.765+29G>A). We performed mutation and large rearrangement analyses for *SDHB*, *SDHC*, and *SDHD*, as well as 2 newly identified PC/PGL predisposing genes, *SDHAF2*, and *TMEM127*. No sequence variations or large rearrangements were detected in *SDHD*, *SDHAF2*, or *TMEM127*. While we detected the *SDHB* IVS7 SNP, we also found a heterozygous *SDHC* deletion encompassing exons 1 through 6. While deletions of 1 or 2 *SDHC* exons have been described in patients with PC/PGL, this represents the first report of a patient with a whole gene deletion of *SDHC*. Notably, this patient is also the first known to have a malignant abdominal pheochromocytoma in the setting of a germline *SDHC* mutation. Our observations suggest that *SDHC* analysis, including for large deletions, may be considered following negative *SDHB* and *SDHD* testing for patients with malignant extra-adrenal pheochromocytoma.

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The familial association of multinodular thyroid goiter with ovarian Sertoli-Leydig cell tumors is attributable to germ-line DICER1 mutations. *M. Tischkowitz[1,2,3], A. Bahubeshi[1,2,3], M. Niedziela[4], N. Sabbaghian[1,2,3], T. Rio Frio[2, 5], N. Hamel[2,5], C. Pouchet[1,2,3], L. Gilbert[6], P.K. O'Brien[7], K. Serfas[8], P. Broderick[9], R. Houlston[9], F. Lesueur[10], E. Bonora[11], R.N. Schimke[12], D. Bouron-Dal Soglio[13], J. Arseneau[14], K. Schultz[15], J.R. Priest[16], W.D. Foulkes[1,2,3,5].* 1) Dept Med Gen, Jewish Gen Hosp,, Montreal, PQ, Canada; 2) Program in Cancer Genetics, Departments of Oncology and Human Genetics, McGill University, Montreal, Canada; 3) Segal Cancer Centre, Lady Davis Institute, Jewish General Hospital, Montreal, Canada; 4) Department of Pediatric Endocrinology and Diabetes, Poznan University of Medical Sciences, Poznan, Poland; 5) The Research Institute, McGill University Health Centre, Montreal, Quebec, Canada; 6) Department of Obstetrics and Gynecology, McGill University, Montreal, Quebec, Canada; 7) Department of Pathology, Etobicoke General Hospital, Toronto, Ontario, Canada; 8) Hereditary Breast Health Clinic, Health Sciences Centre Winnipeg, 820 Sherbrook Street, Winnipeg, Canada; 9) Section of Cancer Genetics, Institute of Cancer Research, Sutton, Surrey, UK; 10) Genetic Cancer Susceptibility Group, International Agency for Research in Cancer, Lyon, France; 11) U.O.Genetica Medica-Pad.11, Dipartimento di Scienze Ginecologiche, Ostetriche, Pediatriche, Policlinico S. Orsola-Malpighi, via Massarenti 9, 40138 Bologna, Italy; 12) Division of Endocrinology, Metabolism and Genetics, Internal Medicine Department, University of Kansas Medical Center, Kansas City, KS, USA; 13) Department of Pathology, CHU Sainte-Justine, 3175 Chemin de la Côte-Ste-Catherine, Montréal, Québec H3T 1C5, Canada; 14) Department of Pathology, McGill University, Montreal, Quebec, Canada; 15) Children's Hospital and Clinics of Minnesota, St. Paul, Minnesota, USA; 16) The International Pleuropulmonary Blastoma Registry, St. Paul, Minnesota, USA.

BACKGROUND Sertoli-Leydig Cell Tumors of the Ovary (SLCTO) are rare tumors which occur in the second and third decades. Familial cases have been described, usually in the context of multinodular goiter (MNG). Germline mutations in DICER1, a gene that codes for an RNase III endoribonuclease, have recently been identified in children with pleuropulmonary blastoma (PPB), some of whom have a family history of MNG and gonadal tumors including SLCTO. DICER1 is involved in the generation of small double-stranded microRNAs and endogenous small interfering RNAs from long double-stranded substrates. Given the tumor spectrum of PPB families and the heterogeneous functions of DICER1 we hypothesized that DICER1 mutations could be responsible for previously reported SLCTO/MNG families. METHODS We ascertained three cases of familial MNG/SLCTO and screened affected probands for mutations in the DICER1 gene by high-resolution melt analysis. We investigated the ovarian tumors from these probands for loss of heterozygosity and protein expression by immunohistochemistry (IHC). RESULTS We identified truncating DICER1 mutations in all three families. Analysis of the ovarian tumors showed no loss of heterozygosity. In all three SLCTO arising in DICER1 mutation carriers, IHC analysis showed increased expression of DICER1 in Sertoli cells, but not in Leydig cells. CONCLUSION The identification of DICER1 mutations in familial MNG in association with SLCTO explains an observation first made by Fraumeni and colleagues 36 years ago. In addition, this report shows that DICER1 mutations predispose to both MNG and SLCTO independently of PPB. The median age at diagnosis of SLCTO in three cases with mutations was 18 years, which falls between the average age of onset of 25 years in sporadic SCLTO and the median age at diagnosis of eight years in PPB-associated SLCTO. None of the DICER1-related tumors showed evidence of LOH, and even though the numbers are small it seems likely that DICER1 does not function as a classic tumor suppressor gene, but it may instead be that the tumors develop as a result of miRNA dysregulation through a possible haploinsuffiency effect.The IHC results underscore the seemingly complex staining pattern of DICER1 protein expression in DICER1-asssociated tumors seen in PPB tumors arising in DICER1 mutation carriers where DICER1 expression was lost in tumor-associated epithelium but was retained in the mesenchymal tumor cells.

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The incidence of breast cancer in women with Neurofibromatosis type 1: a cohort study in Detroit metropolitan area. *X. Wang[1], A.M. Levin[2], F.D. Vigneau[3], N.K. Levin[4], M.A. Tainsky[4].* 1) Dept Medical Genetics, Henry Ford Health System, Detroit, MI; 2) Department of Epidemiology and Biostatistics, Henry Ford Health System, Detroit, MI; 3) Epidemiology Unit Metropolitan Detroit Cancer Surveillance System, Surveillance, Epidemiology and End Results (SEER) Program, Wayne State University School of Medicine, Detroit, MI; 4) Program in Molecular Biology and Genetics, Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI.

Neurofibromatosis type 1 (NF1) is one of the most common cancer predisposition syndromes with an incidence of 1 in 3,500 worldwide. Its clinical expression and severity is highly variable. Certain neoplasms or malignancies are over-represented in NF1. They include gliomas, neurofibromas, malignant peripheral nerve sheath tumors, gastrointestinal stromal tumors, pheochromocytomas, rhabdomyosarcomas, and chronic juvenile myelocytic leukemia. The disease is caused by mutations in the NF1 gene, which encodes neurofibromin. Neurofibromin is a tumor suppressor which down-regulates the Ras signaling pathway. Previous studies with small sample size or death certificate-derived Multiple-Cause Mortality Files failed to reveal an increased risk of breast cancer. However, in 2007, Sharif investigated 304 women from the NF1 genetic registry in the U.K. and described a 4.9-fold increase in breast cancer among women with NF1 under age 50. Henry Ford Health System (HFHS) has a long history of providing specialty care for NF1 patients in the Detroit Metro area. We have collected information from 81 women with NF1 seen in HFHS from 1985 to 2009. These cases were linked to the Surveillance Epidemiology and End Results (SEER) registry covering the Metropolitan Detroit Area. Fifty six women (69%) were under age 50. Six women (7.4 %) developed invasive breast cancer before age 50. One of them was diagnosed with one local and one regional stage primary invasive breast cancers. Three women developed invasive breast cancer after age 50. One of these patients was diagnosed with one local and one regional stage primary cancers. In addition, one woman was diagnosed with three primary ductal carcinoma in situ (DCIS) cancers in her 40s. Two women over age 50 were also diagnosed with DCIS. The probability for women in the general population to develop breast cancer by age 50 in the U.S is only 1.9. Using standardized incidence ratios (SIRs) calculated based on the SEER age-adjusted invasive breast cancer incidence rates, our findings demonstrated a statistically significant increase of breast cancer incidence occurring in NF1 women under age 50, which is comparable to the Sharif study and supports the hypothesis that women with NF1 have an increased risk of developing breast cancer at a younger age. The higher incidence is most significant in the women between age 30 and 39 in our cohort.

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C-MYC and IGH Amplification In a Patient With Burkitt-Like Lymphoma. *A.L. Zaslav[1], S. Saferali[2], M. Bellone[3], F. Lan[2], B. Kiner-Strachan[2], Y. Hu[3], T. Ahmed[3], T. Mercado[1], R. Ponce[1], E. Knorr[1], H. Lee[1], D. Tully[1], G. Evans[1], M. Schuster[2].* 1) Department of Pathology, Cytogenetics Laboratory, Stony Brook University Medical Center, Stony Brook, NY; 2) Blood and Bone Marrow Stem Cell Transplantation Program, Stony Brook University Medical Center, Stony Brook, NY; 3) Department of Pathology, Stony Brook University Medical Center, Stony Brook, NY.

Gene amplification and the subsequent over expression of the amplified oncogene play an important role in tumor pathogenesis. Here, we present a 68 year old female patient with Burkitt-Like Lymphoma (BLL) with amplification of the C-MYC oncogene without the t(8;14)(q24;q32) or its variants and amplification of IGH. Standard chromosome analysis was performed on bone marrow (BM). FISH using the LSI IGH (14q32)/BCL2 (18q21) Dual Color probe and the LSI IGH/MYC/CEP 8 Tri Color probe was performed on BM and a lymph node biopsy. BM analysis revealed a normal 46,XX female karyotype in 19 metaphases. BM FISH demonstrated a normal signal pattern for both probes in 200 of 200 nuclei each. FISH results on the lymph node biopsy demonstrated a signal pattern consistent with a t(14;18)(q32;q21) in 91.5% of the nuclei, 3~4 copies of C-MYC (8q34) and 3 copies of IGH (14q32) in 24.5% of the nuclei. This patient had BLL with areas of the tumor that were consistent with follicular lymphoma (FL). The presence of the t(14;18)(q32;q21) confirmed the FL population. Our patient also had amplification of both C-MYC and IGH without the t(8;14)(q24;q32) or its variants. The significance of the IGH amplification to our knowledge is not known. Patients with BLL with a t(14;18)(q32;q21) and C-MYC amplification are rare and have been a poor prognosis. It is possible that this group of patients may represent a distinct morphologic subtype of BLL. FISH analysis for C-MYC amplification in these patients would be useful in the treatment and monitoring of their clinical course. Our patient will be carefully monitored and treated aggressively to prevent disease progression. Further studies are necessary to determine the significance of these findings.

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Hereditary diffuse gastric cancer (HDGC) associated with a novel truncating *CDH1* mutation: Possible association with non-Hodgkins lymphoma (NHL). *J. Horn[1], S. Modesitt[2].* 1) Cancer Center, University of Virginia Health System, Charlottesville, VA; 2) Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, University of Virginia Health System, Charlottesville, VA.

Background: Hereditary diffuse gastric cancer (HDGC) is associated with mutations in the *CDH1* gene. Outside of lobular breast carcinoma, the occurrence of other extra-gastric tumors associated with *CDH1* mutations is not well-established. We report here a family found to carry a novel truncating *CDH1* mutation in which an atypical constellation of malignancies has arisen. Case: The female proband had previous diagnoses of breast cancer (confirmed pleiomorphic lobular) and non-Hodgkins lymphoma (NHL); notably, her mother was also diagnosed with both breast cancer and NHL. In addition to the mother-daughter pair above, the family reported a nephew to the mother who was also diagnosed with NHL. The known tumor spectrum in the family includes gastric, breast, genitourinary, and skin cancers, as well as NHL. Methods/Results: Due to the strong family history of breast cancer, *BRCA* mutation testing was performed first via Myriad Genetic Laboratories and no mutation was identified; large rearrangement testing, bar the standard 5-site mutation panel in *BRCA1*, was not performed. Subsequently, *CDH1* mutation testing on the proband was carried out through the City of Hope Molecular Diagnostic Laboratory. A frameshift mutation was identified which should give rise to a truncated E-cadherin protein lacking the evolutionarily conserved cytoplasmic domain. Conclusions: In this family, two women (one with a confirmed *CDH1* mutation and one obligate carrier) and a man who is at 50% risk to have inherited the *CDH1* mutation all carried diagnoses of NHL. Other cancers atypical for HDGC occurring in the family in possible or obligate *CDH1* mutation carriers include a bladder cancer, as well as another bladder or kidney cancer. We therefore postulate an association between HDGC and NHL, and perhaps other extra-gastric cancers. NHL has been reported in at least one other HDGC family, though this was a solitary gastric lymphoma that occurred concurrently with diffuse gastric cancer; the authors attributed the NHL to *H. pylori* infection. The single occurrence within that family limits the clinical interpretation of whether the NHL occurred as a manifestation of HDGC. However, the presence of three cases (two in either confirmed or obligate mutation carriers) in our HDGC family supports the possibility of NHL as a component tumor of HDGC and merits further exploration.

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History of Cancer in Nineteen Consecutive Pedigrees with Schwannomatosis. *N. Shah[1], R.J. Spinner[2], D. Babovic-Vuksanovic[1].* 1) Medical Genetics, Mayo Clinic, Rochester, MN; 2) Neurosurgery, Mayo Clinic, Rochester, MN.

INTRODUCTION: Schwannomatosis is a rare genetic disorder that must be distinguished from neurofibromatosis type II (NF2), with which it shares its characteristic benign peripheral nerve sheath tumors. It is a tumor predisposition syndrome, in which candidate genes recognized to date implicate tumor suppressor pathways. It may be associated with hitherto unidentified risk of malignancy that may warrant specific surveillance or prophylactic interventions. However, risk for development of tumors other than schwannoma and meningioma in patients and their relatives is not known. METHODS: We reviewed the records of all patients with schwannoma evaluated in our department between May 1998 and April 2010. We excluded all patients with clinical features or family history of NF2. We found 31 unrelated individuals with at least one histologically confirmed schwannoma. NF2 mutation assay was performed on blood and/or tumor in 7 of these 31 with all results negative. None had a relative independently meeting schwannomatosis diagnostic criteria due to unknown vestibular imaging status. Of the 31, 26 had 2 or more non-intradermal schwannomas. 21 of these 26 had magnetic resonance imaging (MRI) confirming no vestibular involvement. 2 of these 21 were under age 30, and 4 of the remaining 5 without MRI were over age 45 but had no signs or symptoms of 8th nerve dysfunction. This left 19 'definite' (12/19 male, 63%) and 6 'possible' cases (2/6 male, 33%). We analyzed their pedigrees for the presence of cancer. RESULTS: Amongst the 19 definite cases, average age of onset of schwannomatosis symptoms was 36 years. 3 individuals had associated malignancy. 1 Ewing's sarcoma diagnosed age 12, 1 breast cancer diagnosed age 64 and 1 patient with several metachronous squamous and basal cell skin cancers at separate sites at unknown ages. Of the 6 possible cases, average age of onset of schwannomatosis symptoms was 31 years and there was no primary malignancy noted to the time of last follow-up. Family history of 1st or 2nd degree relatives of the definite cases revealed 15 primary malignancies in 14 individual relatives that occurred prior to 60 yrs. Similar evaluation of the possible cases revealed 8 primary malignancies in 8 individual relatives. CONCLUSION: Risk of malignancy in schwannomatosis patients and their relatives may be increased but further studies are needed to delineate the magnitude of this risk before recommending preventative management.

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Increased Prevalence of Non-Clear Cell Renal Cell Carcinoma in PTEN Hamartoma Tumor Syndrome. *J. Mester[1, 2], L. Rybicki[2, 3], C. Eng[1, 2].* 1) Genomic Med Inst, Cleveland Clinic, Cleveland, OH; 2) Taussig Cancer Institute, Cleveland Clinic, Cleveland, OH; 3) Quantitative Health Sciences, Cleveland Clinic, Cleveland, OH.

PTEN Hamartoma Tumor Syndrome (PHTS) is a multi-system autosomal dominant disorder causing increased risks for several benign and malignant neoplasias. PHTS includes patients with Cowden syndrome (CS) and Bannayan-Riley-Ruvalcaba syndrome (BRRS) found to have a germline mutation of the PTEN tumor suppressor gene. Diagnostic criteria for CS have been developed which includes renal cell carcinoma (RCC) as a minor feature. Yet, the National Comprehensive Cancer Network (NCCN) recently deleted awareness or screening for RCC in their most recent CS management guidelines. Somatic PTEN loss-of-function is an important step in sporadic renal carcinogenesis, and a few case reports reveal mostly clear cell RCC in PHTS patients. However, the prevalence of RCC and precise pathologic sub-histologies have not been formally studied. In our series of 155 PTEN mutation-positive subjects recruited by relaxed International Cowden Consortium criteria or presence of a known germline PTEN mutation, we identified 6 patients diagnosed with RCC: 2 males (ages 11 and 53) and 4 females (ages 44, 45, 58, and 64). Based on SEER data, 0.0164 RCC cases were expected for the total group, giving a Standardized Incidence Ratio of 365.9 (95% CI 148.3-760.9, $p<0.001$). Mutations included 2 frameshift, 3 nonsense (R130X, 2 R335X), and a missense (D24H). All RCCs were unifocal with no RCC-related metastases or deaths. Histology was variable and complex: 3 patients had papillary lesions, one with focal clear cell features; 2 had clear cell RCC, 1 one of which also had granular histology and the other with tubular, trabecular, and papillary features; and the last had a concurrent mucinous and spindle cell RCC. Family history was negative for other relatives with RCC. Given that papillary RCC makes up only 10-15% of all RCC cases in the general population, the relative prevalence of this histology in this patient group is notable and should prompt clinicians to place PHTS on the differential diagnosis list for patients with a personal or family history of papillary RCC. We recommend that physicians caring for patients with PHTS remain mindful of the >350-fold increased risk for RCC and have a low threshold for investigating possible RCC in PHTS patients with relevant complaints. Renal ultrasound is not sensitive for detecting papillary RCC compared to clear cell RCC and so PHTS patients should have alternate renal imaging (CT or MRI).

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Characterization of *BRCA1*-deficient breast cancer transcriptomes by RNA-Seq reveals novel transcript isoforms. *E. Lalonde[1,2,3], K.C.H. Ha[1,2], L. Li[1,3,4], W.D. Foulkes[1,3,4,5], J. Majewski[1,2].* 1) Department of Human Genetics, McGill University, Montreal, Quebec, Canada; 2) Genome Quebec Innovation Center, McGill University, Montreal, Quebec, Canada; 3) Program in Cancer Genetics, Departments of Human Genetics and Oncology, McGill University, Montreal, Quebec, Canada; 4) Lady David Institute for Medical Research and Jewish General Hospital, Montreal, Quebec, Canada; 5) Research Institute of the McGill University Health Center, Montreal, Quebec, Canada.

Next-generation RNA sequencing (RNA-Seq) characterizes the transcriptome at an unprecedented single-base resolution. Producing massive amounts of sequencing data, RNA-Seq presents significant computational challenges and requires novel strategies to efficiently extract information from the data. In particular, RNA-Seq can reveal novel transcript isoforms arising from aberrant alternative splicing which is known to contribute to cancer pathogenesis. Here, we use RNA-Seq (Illumina Genome Analyzer II) to characterize the transcriptomes of five *BRCA1*-deficient breast cancer samples. A matched lymphoblastoid cell line and a normal breast epithelial cell line are used as controls. We present examples of novel alternative splicing events (ASEs) distinguishing the *BRCA1* breast cancer transcriptome.

We detected ~1500 ASEs that are not annotated in the RefSeq or UCSC databases and that are expressed 10-fold higher in at least two of our breast cancer samples compared to the control samples. Over half of these ASEs are exon skipping events and over a third provide evidence for new exons. A promising example found in four of the samples involves skipping of exon 12 in *PALB2* leading to a frameshift, a premature termination codon and obliteration of a WD40 domain. Of interest, the WD40 domain is essential for binding BRCA2, cell cycle control and apoptosis. We have also identified an unannotated exon in *POLB*, an important gene in base excision repair. This ASE has previously been described by others as an infrequent event yet it is highly expressed in four of our *BRCA1*-deficient samples and in some cases has higher expression than the wildtype isoform.

We have described the transcriptomic landscape of *BRCA1* breast cancers. In addition to an in-depth analysis of alternative splicing, our group has identified various recurrent, unannotated genomic variations, such as SNVs and indels, as well as several gene fusions, the result of genomic rearrangements. These results provide a comprehensive overview of breast cancer transcriptomes and shed light into characterizing important pathways involved in its tumorigenesis. Furthermore, our findings will help increase our knowledge of *BRCA1* related mechanisms as we shift towards personalized medicine diagnosis and treatment. Finally, we demonstrate that RNA-Seq is effective in revealing various transcript abnormalities in cancer.

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Identifying Novel Expressed Gene Fusions in MCF-7 Cell Line Using Next Generation Sequencing. *O. Sakarya[1], H. Breu[1], L. Popescu[1], M.W. Muller[1], P. Vatta[1], B. Krishnaswami[1], G. Ojewole[1], A. Wong[1], N. Garg[1], Y.N. Wang[1], J.P. Bodeu[1], R.C. Nutter[1], M. Mooney[1], J.P. Brockman[2], P.P. Whitley[2], K.S. Bramlett[2], M. Radovich[3], A.S. Siddiqui[1], F.C. Hyland[1].* 1) Biological Information Systems, Life Technologies, Foster City, CA; 2) Ambion R&D, Life Technologies, Austin, TX; 3) Indiana University School of Medicine, Indianapolis, IN.

Chromosome aberrations, especially gene fusions, are implicated in the initiation of tumorigenesis. Various gene fusions are important diagnostic and prognostic indicators in leukemia, sarcomas, and other solid tumors. High throughput RNA sequencing (RNA-Seq) allows characterization of gene expression and of exon splicing patterns of expressed transcripts. We developed a new junction finder algorithm in order to identify and quantify exon splicing events using paired end RNA-Seq. With this algorithm, the single read and paired end splice junction evidence are stored in directed splicing graphs. Using these splicing graphs, we investigate the splicing patterns in two MicroArray Quality Control (MAQC) samples, and in the breast cancer cell line MCF-7. We sequenced 129,950,066 and 113,147,501 uniquely mapped pairs of RNA-seq reads from the MAQC samples Human Brain Reference (HBR) and Universal Human Reference (UHR). Compared to RefSeq annotations, we found 136,671/14,271 and 138,536/14,083 known/putative exon junctions in UHR and HBR, respectively. We further compared the sequencing depth required to distinguish low-expressed variants by progressively mapping barcoded libraries and report that ~70% of alternative splicing events were accessible with ~20,000,000 and ~82% with ~40,000,000 qualified read pairs. The remainder of the junctions were detectable only at higher depth. Of the 36 fusion junctions called for UHR, we verified 16 with TaqMan assays including the known BCR-ABL and GAS6-RASA3 fusions. Next, we sequenced the breast cancer cell line MCF-7, obtaining 192,246,750 uniquely mapped pairs of RNA-Seq reads. We identified 123,386 known and 17,317 putative junctions, and we called 60 fusions, of which 10 were between genes from different strands or chromosomes. These included the recently discovered MCF-7 fusions BCAS4-BCAS3, ARFGEF2-SULF2, AHCYL1-RAD51C, ABCA5-PPP4R1L and TBL1XR1-RGS117. We detected five other novel fusions at lower expression levels which are undergoing validation. These novel fusions suggest enrichment in the number of translocation events between Chromosomes 1, 17 and 20 during the evolution of the MCF-7 genome. We report thousands of previously unknown splicing events including five novel gene fusions in a breast cancer cell line. Easy and low-cost genome-wide detection of novel gene fusions allows interrogation of large numbers of tumor samples and discovery of biologically important gene fusions.

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Identifying determinants of tumorigenesis and drug resistance from comprehensive exome DNA and RNA sequence of major breast cancer cell lines. *R.J. Cho[1,2], S. Durinck[2], N.J. Wang[2], J.W. Gray[2], P.T. Spellman[2].* 1) Dermatology, UCSF, San Francisco, CA; 2) Division of Life Sciences, Lawrence Berkeley National Laboratories, Berkeley, CA.

Individual cell lines have long provided stable, ontogenic models of tumorigenesis and drug resistance for diverse cancer types. To date, the lack of comprehensive, detailed sequence information has greatly limited the ability to link specific mutations and pathways to phenotype. We have completed both DNA sequencing of all coding sequence and digital RNA sequencing of transcripts in 40 of the most commonly studied breast cancer cell lines. Our results clarify the relationships between established oncogenes and molecular subclasses of breast cancer. Through structural and gene expression analysis of mutations, we propose a hierarchy of candidate drivers validated in sequencing of primary breast cancers. Finally, we report on silencing of a subset of these candidates, toward functionally validating their roles in tumorigenesis.

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BRCA2 mutation analysis in familial and early onset prostate cancer. *C. Maier[1,2], I. Wiest[2], M. Luedeke[1,2], K. Herkommer[3], A. Rinckleb[1,2], H. Surowy[2], M. Schrader[1], W. Vogel[1,2].* 1) Urologische Klinik, Univ Ulm, Ulm, Germany; 2) Istitut fuer Humangenentik, Univ Ulm, Ulm, Germay; 3) Urologische Klinik, Klinikum rechts der Isar, Munich, Germany.

Although prostate cancer (PCa) is known for the highest heritability (42%) among common cancers, its genetic background is the least clarified and thus, the practice in genetic screening is the most backward. To date, the strongest known PCa risk is caused by the BRCA2 gene, as described for male mutation carriers within breast and ovarian cancer families. Studies in prostate cancer cohorts have further demonstrated more aggressive forms of PCa with an earlier onset and shorter survival for BRCA2 mutation carriers. In order to assess the role of BRCA2 in PCa cases from families without the strong hereditary breast and ovarian cancer history, we have started mutation analysis on probands that were recruited solely on the criterium of PCa familial clustering. The full family sample comprises 379 pedigrees with a total number of 1073 affected men (2.8 per family), for which at least one affected individual has donated blood. Presently, 284 probands have been screened, each as the youngest diseased case of 284 pedigrees. As an early onset group, 96 sporadic PCa cases were included with an age of 60 years or younger at the time of diagnosis (mean: 55.7; range: 29 - 60 years). In the total number of 380 cases sequenced for mutations, five (1.32%) were found to carry truncating BRCA2 variants, three frameshift (c.1813insA, c.3847insGT and c.4449delA) and two nonsense (K2013X and Q2499X) mutations. Further six rare alleles, which could influence splicing (IVS2-7T>A), or functionally alter the peptide sequence (P655R, K1025E, S2697N, E2981K and I3412V), remain as variants of unknown significance after literature and database search. The mutation frequency, as compared to the estimates of 0,1 - 0,7% for the general population, seems elevated in our probands with familial PCa (5 out of 284; 1.76%) and in probands with aggressive disease (4 out of 184; 2.17%). The carrier frequency could be enriched to 4.0% (3 out of 75) in a subgroup of probands where at least two relatives exhibited aggressive PCa. Early age of onset seemed not relevant in our cohort. Although BRCA2 turned out to be responsible for PCa clustering in particular families, and probably for a more severe manifestation, predictive testing would require additional inclusion criteria (e.g. family history of breast and ovarian cancer) in order to identify a significant number of BRCA2 mutation carriers at PCa risk.

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Understanding the role of the JAK2 susceptibility haplotype in myeloproliferative neoplasms (MPN) phenotype and predisposition using targeted next generation sequencing. *S. Mukherjee[1,2], O. Kilpivaara[3], H. Hunter-Zinck[6], A. Viale[5], N.D. Socci[4], R.L. Levine[3], R.J. Klein[1].* 1) Cancer Biology and Genetics Program, Memorial Sloan-Kettering Cancer Cancer Center, New York, NY; 2) Gerstner Sloan Kettering Graduate School of Biomedical Sciences, New York, NY; 3) Human Oncology and Pathogenesis Program, Memorial Sloan-Kettering Cancer Center, New York, NY; 4) Computational Biology Center, Memorial Sloan-Kettering Cancer Center, New York, NY; 5) Molecular Biology Program, Memorial Sloan-Kettering Cancer Center, New York, New York; 6) Program in Computational Biology and Medicine, Cornell University, Ithaca, NY.

Myeloproliferative neoplasms (MPN) is a clonal disorder of hematopoietic lineage. There are three types of MPN- polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). The somatic mutation JAK2 (V617F) is present in hematopoietic cells of many MPN patients. In our previous genome-wide study, we (and others) have identified an allele at the JAK2 locus (rs10974944) that predisposes to the development of JAK2 (V617F)-positive MPN. We also found that JAK2 (V617F) is preferentially acquired in cis with the predisposition allele (G). This led to several possible hypotheses including haplotype-specific variation in a regulatory motif, genotype-specific splicing, or an increased mutation rate at this locus. To dissect the functional variant(s) and to understand the haplotype-specific acquisition of somatic mutation, we carried out targeted sequencing of 300KB haplotype block harboring JAK2 using next generation sequencing technology (RainDance and SOLiD sequencing). We compared MPN cases that are homozygous for risk allele (GG-MPN cases) with the ones that are homozygous for wild type allele (CC- MPN cases). We found that GG-MPN cases have significantly higher number of single nucleotide variants in the JAK2 locus compared to CC-MPN cases. We observed the same pattern in healthy individuals with whole genome sequencing data published by 1000 genomes project and others. We looked into the selection pressure at JAK2 locus to determine if purifying or positive selection is influencing the observed haplotypic differences of single nucleotide variants. To identify the functional variant(s) that results in haplotype-specific mutation acquisition in MPN cases, we are analyzing sequence specific differences (such as motif / regulatory elements) between these two groups of patients. Our study is helpful to understand the etiology of MPN phenotype and predisposition.

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Targeted Resequencing for SNP Discovery using Massive Single-Plex PCR and Next Generation Sequencing. *C.M. Robbins[1], E.M. Lange[2], W. Tembe[3], A.M. Ray[4], K.A. Zuhlke[4], W. Isaacs[5], K.A. Cooney[4], J.D. Carpten[1].* 1) Integrated Cancer Genomics Div, TGen, Phoenix, AZ; 2) Department of Genetics, University of North Carolina, Chapel Hill, NC; 3) High-Performance Bio-Computing Division, TGen, Phoenix, AZ; 4) Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI; 5) Johns Hopkins University School of Medicine, Baltimore, MD.

Recent advances in Next Generation Sequencing (NGS) afford new opportunities for large scale SNP discovery across the entire human genome. However, there is also a need to search for variants within specific intervals of the genome. Several methodologies have been developed for reducing genomic complexity to allow for more targeted resequencing. Among these methods is the RainDance picoliter massive single-plex PCR assay, which provides the capability to assess up to 10,000 independent amplicons in a single reaction. To test the feasibility of utilizing this method for variant discovery, we designed a RainDance PCR assay containing 3,148 amplicons, which encompass a total of 2,328 exons from 221 genes predominantly across the 17q21-22 region of the human genome that showed evidence of being associated with hereditary prostate cancer in previous genome-wide linkage scans (GWS). This custom primer library pool was used to generate microdroplets of each unique amplicon with one DNA sample in one single PCR reaction. Individual pools of amplicons from each sample were then used as template for NGS using the Life Technologies SOLiD version 3-Plus system. To increase throughput and provide for more efficient sequencing, we employed a series of sixteen unique molecular barcodes for pooled sequencing on the SOLiD system. To date, a total of 94 individuals have been sequenced using our approach. On average 2230 variants were identified per DNA sample. Here we will provide data on the number of both novel and known Single Nucleotide Polymorphisms (SNPs) as well as quality assessment and overall performance of the RainDance method and performance metrics for SOliD NGS. Our experience has provided the opportunity for building out an efficient process and pipeline for targeted resequencing using NGS.

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Discovery of New Genes for Inherited Predisposition to Breast Cancer by Exome Sequencing. *C. Spurrell, T. Walsh, M. Lee, MC. King.* University of Washington, Seattle, WA.

BRCA1, BRCA2, and multiple other genes harbor mutations that significantly increase risk of breast cancer, but the genetic bases of breast cancer in most severely affected breast cancer families remain unresolved. We hypothesize that the remaining familial risk of breast cancer is due to individually rare alleles of moderate to high penetrance, some in known genes and many in as-yet-undiscovered genes. In order to discover these new breast cancer genes we are performing exome sequencing on a large number of families excluded for all known genes. We select two cases per family for exome sequencing. Exome libraries are sequenced to at least 50x coverage and then filtered for rare variants likely to truncate proteins. Approximately 10-15 events per family survive this filter. We validate these variants by Sanger sequencing, then genotype each validated variant in all family members to evaluate co-segregation with breast cancer. In the proband of one high-risk family, the only variant to pass our filtering steps was a nonsense mutation in CDH1, p.W156X. Mutations in CDH1 have been reported to cause inherited predisposition to gastric cancer and lobular breast cancer. In this family, two individuals with lobular breast cancer and one with gastric cancer are heterozygous for CDH1 p.W156X. In the pedigree there remain three unexplained cases of ductal breast cancer. We are currently sequencing a second proband in the family to identify a gene responsible for the ductal breast cancers. Exome sequencing will clarify within family genetic heterogeneity and reveal novel mutations and new gene for breast cancer susceptibility.

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International Cancer Genome Consortium Data Portal. *J. Zhang[1], S. Haider[2], A. Cros[1], S. Gnaneshan[1], J. Guberman[1], J. Hsu[1], Y. Liang[1], J. Wang[1], C. Yung[1], A. Kasprzyk[1].* 1) Ontario Institute for Cancer Research, Toronto, Ontario, Canada; 2) University of Cambridge, Computer Laboratory, Cambridge, UK.

The International Cancer Genome Consortium (ICGC) (http://www.icgc.org) orchestrates a multi-national effort to catalogue genomic abnormalities in 50 different tumour types and subtypes. For each type, 500 pairs of matched tumour and normal tissues will be studied using multiple technological platforms. ICGC's data, which will be generated independently by each of its 50 member institutions, will be linked to cancer etiology, drug response, and patient survival. As they are generated the data sets are integrated and made available to the public via the ICGC data portal (http://dcc.icgc.org). The first version of the portal includes four different data types: simple mutations, copy number mutations, structural rearrangements, and gene expression. In addition to the ICGC data, several external data sets, such as Ensembl Gene, KEGG Pathway, and Pancreatic Expression Database, have also been federated to provide support for more sophisticated queries such as 'find all non-synonymous coding mutations identified in PIK3R1 for all cancers' or 'find all members of the Toll-receptor pathway having deletions in stage III breast cancer'. As the ICGC data increases and the portal's functionality is further improved it is expected that this will become an increasingly important resource for cancer researchers with diverse user requirements.

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MSI detection by next-generation sequencing in Lynch syndrome patients. *H. Westers, J. Lops, P. van der Vlies, G.J. te Meerman, R.M.W. Hofstra.* Genetics, Univ Med Ctr Groningen, Groningen, Netherlands.

Lynch syndrome, the most common cause of hereditary colorectal cancer is an autosomal dominantly inherited disorder. It is well-established that germline mutations in the mismatch repair (MMR) genes MLH1, MSH2, MSH6, and PMS2 cause Lynch syndrome. A hallmark of MMR deficiency is microsatellite instability (MSI) and this instability occurs in more than 90% of the tumours of Lynch syndrome patients. Therefore, MSI is a very useful diagnostic marker of MMR deficiency. MSI is determined by analysing, on length, an international consensus panel of five microsatellite markers, using DNA from normal and tumour tissue of the same patient. It has been proposed that carriers of a heterozygous MMR gene mutation show a low degree of MSI in blood lymphocytes before tumour diagnosis (up to a few percent). This instability was detected by a PCR cloning and sequencing approach. However the current diagnostic methods are not sensitive enough to detect these very low frequency mutations in presymptomatic mutation carriers. As this would be a perfect test to identify carriers of MMR gene mutations we have set up an analysis using next-generation sequence technology that is able to detect these low levels of instability in a single run. Two MSI mononucleotide markers, NR21 and BAT26, were PCR-amplified with DNA from MSI-high tumors, MMR mutation carriers and DNA from controls as templates. The MSI profile of the tumour DNAs obtained by sequencing exactly matched the MSI profile obtained by marker length analyses indicating that MSI profiling using next-generation sequencing is well possible. In fact using bar-coding we were able to analyze easily 10 individuals in a single lane. The previously reported low MSI frequency in mutation carriers we could prove. However the instability detected did not differ from the instability found in control samples making this type of MSI analysis not predictive for MMR mutation carriers.

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Are rare copy number variants increased in breast-ovarian cancer predisposition families? *A.C.V. Krepischi[1], E.M.M. Santos[1], M.I.W. Achatz[1], S.S. Costa[2], D. Carraro[1], B. Lisboa[1], L.P. Kowalsky[1], C. Rosenberg[2].* 1) AC Camargo Cancer Hospital, São Paulo, SP, Brazil; 2) Institute of Biosciences, University of São Paulo, Brazil.

The impact of the genome structural variations known as CNV (copy number variation) on human diseases is increasingly evident, mainly due to the wide spread use of array-based comparative genomic hybridization (array-CGH). These large scale copy number variations are related to human variability, and may influence susceptibility to complex diseases, including cancer. Although a few major genes of cancer susceptibility have been identified, pathogenic mutations on these genes do not account for most of the predisposition in high-risk cancer families. We selected 52 unrelated cancer patients, members of breast-ovarian cancer predisposition families for array-CGH investigation. The goal was disclosing rare constitutional genomic imbalances that could harbour yet not described predisposition genes, defining novel risk or susceptibility cancer markers. The experiments were conducted on a 180K whole-genome Agilent oligoarray platform. The detected DNA copy number changes were compared both to our reference dataset (100 healthy Brazilian individuals), and to the Database of Genomic Variants (DGV); we defined as "rare copy number variants" (RCNV) those containing genes and covered by £3 individual CNVs documented in DGV. A total of 416 CNVs were identified in the cancer sample (average of 8.2±4.4 CNV per individual). The initial results point to a slight increase in the average of CNVs per individual in the breast cancer patients (8.2) when compared to the control sample (6.8), although larger numbers are needed to statistically confirm this trend. Regarding the RCNVs, a total of 33 were detected in 22 (~42%) patients, while the remaining 30 did not show any RCNV, although one of them exhibited a whole extra chromosome X. The corresponding frequency among controls were 17 RCNV detected among 14 (35%) of the controls. Several of the identified genomic imbalances either comprise entire genes or are intragenic rearrangements, many of which may play a role in cancer.

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Identification of 13 germline *APC* mutations in Familial Adenomatous Polyposis Saudi families. *H. Abalkhail[1], M. Faiyaz-Ul-Haque[1], N. Al-Sanea[2], A. Abduljabbar[2], L. Ashari[2], S. Al-Homoud[2], F. Al-Dayel[1].* 1) Department of Pathology & Lab Med (MBC 10), King Faisal Special Hosp, Riyadh, Saudi Arabia; 2) Department of Surgery, King Faisal Specialist Hospital, Riyadh, Kingdom of Saudi Arabia.

Familial adenomatous polyposis (FAP) is an autosomal dominant inherited form of colorectal cancer caused by germline mutations in the tumor suppressor APC gene (5q21-q22). The Classical form of FAP is characterized by the development of 100's-1000's adenomatous polyps in the colon and rectum at an early age in life. The APC gene encodes a 2843 amino acids distributed over 15 coding exons. To date, the majority of mutations reported are nonsense, deletion or insertion mutations that result eventually in premature protein truncation. Materials & Method: We analyzed the entire coding region of the APC gene using polymerase chain reaction (PCR) and direct sequencing with primers specifically designed to amplify the coding region of exon 1-15 and exon /Intron boundaries. Results: Mutational screening of the APC gene was carried out in 40 individuals (probands and family members) of Saudi's referred from the Colorectal Surgery Clinic at King Faisal Specialist Hospital and Research Centre with the aim to confirm the diagnosis and to detect the causative pathogenic mutations, to enable the presymptomatic diagnosis in at risk family members. In this study we report 13 germline mutations, the changes detected are predicted to result in protein truncation or splice alterations including: 4 nonsense, 4 small deletion (1-5 base pairs), 1 insertion, 1 splice alterations and 3 missense mutations (I1307K, E1317Q and D1822V) known to either be associated or indirectly cause cancer predisposition. Four of the detected mutations, including nonsense, small deletions and a splice site variant were novel. Furthermore, 12 novel unclassified variants (possibly damaging) of unknown clinical significance were detected. Conclusion: The result of this study represents the first Molecular analysis of APC gene in Saudi Arabia and displays the existence of novel pathogenic mutation in Saudi families. The molecular testing of APC gene is of great importance not only in the diagnosis and the management of FAP but also in the implications of screening regimens in at risk family members, thus modifies management, decrease cost, and reduce the psychological trauma for the tested individual.

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Novel germline *PALB2* truncating mutations in African-American breast cancer patients. *Y. Zheng[1], J. Zhang[1], Q. Niu[1], D. Huo[2], O. Olopade[1].* 1) Center for Clinical Cancer Genetics and Global Health, Department of Medicine, The University of Chicago, Chicago, IL 60637, USA; 2) Department of Health Studies, The University of Chicago, Chicago, IL 60637, USA.

The contributions of germline mutations in *BRCA1* and *BRCA2* to inherited susceptibility to breast and/or ovarian cancers have been well documented over the past two decades. Mutation screening of genes functionally related to *BRCA1* and/or *BRCA2* has revealed mutations in genes such as *CHEK2*, *ATM*, *BRIP1*, and *PALB2*. It has been demonstrated that PALB2 acts as a bridging molecule that connects BRCA1 and BRCA2 and thus facilitates BRCA2-mediated DNA repair. Truncating mutations in *PALB2* were reported to be enriched in Fanconi anemia, breast and pancreatic cancer patients in various populations. To date, there are no studies in African-Americans. We evaluated the contribution of *PALB2* germline mutations in familial and sporadic African-American breast cancer patients. After excluding 19 breast cancer cases that previously tested positive for *BRCA1/2*, our cohort contains 260 breast cancer patients, including 20 patients with strong family history (first- and/or second-degree relatives affected by breast and/or ovarian cancer, FDR + SDR $n \geq 3$; age of onset = 45.2 ± 10.9 yr [mean ± SD]); 25 patients with moderate family history (FDR + SDR n = 2; age of onset = 44.6 ± 7.4 yr); 69 patients with weak family history (FDR + SDR n = 1; age of onset = 44.7 ± 9.6 yr); and 146 non-familial or sporadic breast cancer cases (age of onset = 43.6 ± 10.0 yr). After direct sequencing of all the coding exons, exon/intron boundaries, 5'UTR and 3'UTR of *PALB2*, three (1.15%; 3 in 260) novel monoallelic truncating mutations were identified: c.758dupT (exon4), c.1479delC (exon4) and c.3048delT (exon 10); together with 50 sequence variants, 35 of them are novel. None of the truncating mutations were found in 262 controls from the same population. Interestingly, these three deleterious mutations were detectable in both familial and non-familial groups: the patient with c.758dupT had a weak family history, the patient with c.1479delC had a strong family history, whereas the patient with c.3048delT reported no family history of breast and/or ovarian cancer. These data suggest that *PALB2* mutations may have variable penetrance and be causative for both familial and non-familial breast cancer among African-Americans. To our knowledge, this is the first report of *PALB2* truncating mutations in African-American breast cancer patients, underscoring the need for further studies to better understand the mutation spectrum of *PALB2* in diverse populations.

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Systems biology approach to genome-wide survival analysis of ovarian cancer. *H. Dong[1], S. Hong[1], R. Cheng[3], L. Jin[1], M. Xiong[1,2].* 1) Sch Life Sci, Fudan Univ, Shanghai, China; 2) Human Genetics Center, University of Texas School of Public Health, Houston, Texas, U.S.A; 3) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas USA.

Genetic and epigenetic alternations that are likely to cause tumor formation are often organized into complex biological networks. It is the whole system and the system dynamics that play an essential role in giving rise to cellular function/dysfunction. Here we report to use system biology and network approaches to develop novel analytic strategies for genome-wide survival analysis of ovarian cancer using The Cancer Genome Atlas (TCGA) data. Expressions of 12,042 genes were measured in 408 tumor samples by Affymetrix U133A, expressions of 799 miRNAs were measured in 485 tumor samples by Agilenth 8x15Kv2 and somatic copy number variations (CNVs) were measured in 442 tumor samples by Agilenth 8x15Kv2. The Cox Hazards regression models were used to test for association of CNVs, genes and miRNAs with the survival times of the patients and sparse Gaussian graphical models were used to construct gene or miRNA co-expression networks with response and no response to chemotherapy. We identified 1,083 CNV regions, 259 genes and 28 miRNA including miR934, miR302a, miR551b, miR652 etc, which were significantly associated with the survival time of the patients with the P-values < 0.01. Some of the CNV regions contain known oncogenes in Epithelial cancer (ERBB2, AKT2), tumor suppressor genes in Epithelial cancer (MEN1), and other cancers (SUPT5H), and cancer related genes in Colorectal cancer (SFRS6) and in Breast cancer (ASL, FLNA). We observed that the genes VEGF, ITGB1, MEN1, TRIP13 with CNV gain and genes ERBB2, VASP, FANC1, POLG, ERCC1, TCAP and GRB7 with CNV loss were associated with patients with survival time larger than 5 years. We identified a largest connected subnetwork with response to chemotherapy where a total of 2,911 genes were connected and 56 genes with CNVs significantly associated with survival time, and a largest connected subnetwork with no response where 2,158 genes and 54 genes with CNVs. We also identified a largest connected miRNA co-expression subnetwork with response to chemotherapy where 497 miRNAs were connected and 49 miRNAs were significantly associated with survival times and a largest connected miRNA subnetwork with no response to chemotherapy where 393 miRNAs were connected and 31 miRNAs were significantly associated with survival time. Finally, we identified 19 CNV regions associated with survival times contain the cis-eQTL genes.

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Whole-Genome Sequencing of Non-Small-Cell Lung Cancer Samples. *Z. Zhang[1], D. Ballinger[2].* 1) Dept Bioinformatics, Genentech Inc, S San Francisco, CA; 2) Complete Genomics, Mountain View, CA.

Genentech, together with their collaborators at Complete Genomics, sequenced and compared the tumor and normal tissue of a 51-year-old Caucasian male with non-small-cell lung cancer. This was the first time that such a comparison has been made across the whole genome for a lung cancer patient. For the project, Complete Genomics sequenced the patient's primary lung tumor to 60x coverage and adjacent normal tissue to 46x coverage. More than 50,000 single nucleotide variations (SNVs) were discovered in the tumor which yielded about 17.7 somatic mutations per megabase of DNA. In addition, we observed a distinct pattern of selection against mutations within expressed genes compared to non-expressed genes and in promoter regions up to 5 kb upstream of all protein-coding genes, clearly identifying selection pressures within a tumor environment. We will also discuss the identification of somatic structural and copy number variants.

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***KRAS* Variant Limit of Detection on the Applied Biosystems 3500xL Genetic Analyzer.** *C. Davidson[1], K. Champion[3], M-P. Gauthier[1], F. Wang[1], N. Koch[1], J. Boonyaratanakornkit[2], A. Pradhan[1], J. Walker[1], J. Jones[3], A. Felton[1].* 1) Applied Biosystems, Foster City, CA; 2) AcroMetrix, Benicia, CA, United States; 3) Greenwood Genetic Center, Greenwood, SC, United States.

The *KRAS* (v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog) gene encodes one of the proteins in the RAS-MAPK pathway, one of several signaling cascades downstream of epidermal growth factor receptor (EGFR) activation. EGFR signaling pathways are important in the development and progression of several aggressive cancers, with *KRAS* gene mutations common in pancreatic, lung, and colorectal cancers. Activating *KRAS* mutations have been described most frequently in codons 12 and 13, with a minor proportion of activating mutations described in codons 61 and 146. These mutations result in constitutively active form of KRAS causing EGFR pathway signaling independent of EGFR activation, thus making therapeutic agents that block EGFR, such as cetuximab or panitumumab, ineffective. Here we describe limit of detection experiments for the detection of sequence variants in codons 12 and 13 of the *KRAS* gene. Methodologies investigated include capillary electrophoresis (CE) DNA sequencing, Shifted Termination Assay (STA) detection, single-base extension, pyrosequencing, high resolution melt (HRM), and real-time PCR. Two sample types were used to assess the limit of detection for *KRAS* variants: 1) mixtures at various percentages of gDNA extracted from *KRAS* mutant and wild-type cancer cell-lines; and 2) mixtures at various percentages of *KRAS* mutant and wild-type cells that were formaldehyde fixed and paraffin embedded to create a cell block and sections from which gDNA was extracted. The analytic sensitivity of the above methodologies is described with a detection of *KRAS* variants in codons 12 and 13 possible at a level of 1% or greater. Further, copy number variation (CNV) assessment of *KRAS* mutant cell line gDNA used for both sample types was assessed since it is known that cancer cell lines have CNV of the *KRAS* gene. The 3500xL Genetic Analyzer, the latest capillary electrophoresis platform from Applied Biosystems®, demonstrated an analytic sensitivity of ≥1% for the detection of *KRAS* variants in codons 12 and 13 by means of the single-base extension and shifted termination assay applications. The limit of detection of these applications is equivalent or better than the pyrosequencing, HRM, and real-time PCR approaches. In addition, data is shown that demonstrates it is important to consider the influence of the sample (FFPE versus purified gDNA), copy number, and zygosity when determining the limit of detection for a method or assay.

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The role of genomic heterogeneity in primary tumors from colon cancer patients. *S. Levy[1,2], K. Bethel[3,2], G. Oliveira[1,2], A. Carson[1,2], A. Torkamani[1,2], P. Kuhn[2].* 1) Scripps Genomic Medicine, Scripps Translational Science Institute, La Jolla, CA; 2) The Scripps Research Institute, La Jolla, CA; 3) Scripps Clinic Medical Group, La Jolla, CA.

Accurate genomics-based molecular characterization of genetic lesions occurring in tumor cells will provide better precision in clinical interpretation of cancer tissues. We adopted a genomics based approach to characterize gene based mutational events, in concert with changes in gene expression patterns, in different anatomic areas from the same primary tumor sample. In this manner we hope to understand how tumor heterogeneity impacts the ability to provide a precise molecular characterization of events within tumor tissues. Correspondingly, we have developed a methodology that will permit the association of correlated changes in the genome and transcriptome in relation to tumor locus, i.e. epicenter, invading edge, tumor-normal transition, thus enabling the identification of activating mutational events that are the hallmark of cancer cell progression within the tumor mass. In one particular application of this approach we assayed discrete anatomical loci isolated from a colon tumor and sequenced the exome compartment as enriched by Agilent SureSelect hybridization probes. Somatic mutations were detected by comparison of the DNA variants identified in the exome sequence derived from each tumor locus with orthologous exomic positions from adjacent normal colon tissue and whole blood. Mutational trends derived from such a somatic mutation comparison were correlated with gene expression to identify significantly over- and under-expressed mutational events.

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A novel FLT3 in frame Indel mutation (p.Met837delins Ser Arg Ala Pro Pro) in the TK2 domain of an AML patient with t(8;21). *A. Shrimpton, V. Arguello-Guerra, R. Hutchison.* SUNY Upstate Medical University, Clinical Pathology, 750 E Adams St., Syracuse NY13210.

During routine testing for the D835/I836 mutations in the second tyrosine kinase domain of FLT3 in a patient suspected to have AML, a novel additional larger band of equal intensity to the expected 114 bp band was noted. Post Eco R V digestion the additional band was still present and mapped to within the shorter 46 bp fragment. A comparison to wild type sequence revealed that the novel mutation as a deletion of a single T and an insertion of 13 nucleotides (GCCGAGCTCCCCC) which results in an in the frame mutation, pMet837delinsSerArgAlaProPro. The patient also had the t(8;21) translocation consistent with AML-M2. A family history raised the possibility that the novel FLT3 mutation was inherited rather than acquired, however both a cheek scrape sample and post treatment blood sample showed an absence of the novel mutation.

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Analysis of oncogenic mutations in the KIT and PDGFRA genes and characterization of gastrointestinal stromal tumors (GISTs) in Arab patients. *M. Ul Haque[1,2], A. Tulba[1,2], F. Al-Dayel[1, 2], H. Abalkhail[1,2], M. Toulimat[1], H. Alhussaini[3], M. Memon[4], S. Bazarbashi[4], A. Al-Abdullatif[1], T. Amin[4], M.B. Satti[5], I. Peltekova[6], S.H.E. Zaidi[7]*. 1) Molec Gen Lab, DPLM, King Faisal Specialist Hosp, Riyadh, Saudi Arabia; 2) Dept. of Genetics & Molecular Pathology, College of Medicine, Alfaisal University, Riyadh, Saudi Arabia; 3) Dept of Pathology, Riyadh Military Hospital, Riyadh, Saudi Arabia; 4) King Faisal Cancer Centre, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia; 5) Dept of Pathology, King Abdulaziz Medical City, Jeddah, Saudi Arabia; 6) Department of Medicine, Queen's University, Kingston, Canada; 7) Department of Medicine, University Health Network and University of Toronto, Canada.

Gastrointestinal stromal tumors (GISTs) are mesenchymal tumors found in the gastrointestinal tract, which are believed to originate from the interstitial cells of cajal. In GISTs, several mutations have been described in the KIT and PDGFRA genes, both of which encode for type 3 receptor tyrosine kinases. Specific mutations in these genes cause ligand independent activation of these receptors, which result in cell proliferation and progression towards tumor formation. GISTs with certain mutations in the KIT and PDGFRA genes are effectively treated with the specific tyrosine kinase inhibitor, imatinib mesylate (aka Gleevec). Therefore, it is essential to identify the spectrum of oncogenic mutations in GISTs in different ethnic and geographic populations. The present study was conducted to characterize the GISTs in Arab patients. In this cohort of 72 patients, most GISTs occurred in the stomach, followed by the rest of the digestive tract (intestine, colon, rectum, and esophagus). The majority of the tumors expressed CD117 and CD34 antigens, which are diagnostic markers for GISTs. Sequencing of the exons and exon/introns boundaries of the KIT and PDGFRA genes, in which GIST-causing mutations have been described, identified several non-synonymous mutations and unique deletions in the KIT gene. Among single amino acid substitutions, the p.Trp557Arg change was more common than the mutations affecting the p.Val559 and p.Val560 amino acids. All mutations and deletions are located in exon 11 of the KIT gene. This exon encodes for the juxtamembrane domain which inhibits KIT kinase activity in the absence of a KIT ligand. Mutations in this domain are known to cause constitutive kinase activity of the KIT receptor. Histological examination of the GISTs show higher mitotic index in tumors with exon 11 KIT deletions compared to single amino acid substitutions. This is the first report of presentation, characterization and molecular analysis of GISTs in Arab patients. Together, these data are important for the diagnosis of GISTs and the management of patients in this population of distinct ethnicity.

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Systematic identification of somatic mutations and virus integrations in liver cancer genomes by target capture sequencing. *K. Tatsuno[1], G. Nagae[1], S. Yamamoto[1], K. Sonoda[1], L. Wang[1], S. Ishikawa[1,2], S. Tsutsumi[1], Y. Midorikawa[1,4], N. Kokudo[3], T. Shibata[5], T. Yoshida[6], H. Aburatani[1]*. 1) Genome Science Div., RCAST, University of Tokyo, Tokyo, Japan; 2) Department of Pathology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; 3) Hepato-Biliary-Pancreatic Surgery Division, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; 4) Department of Surgery, Teikyo University School of Medicine University Hospital, Mizonokuchi, Kanagawa, Japan; 5) Cancer Genome Project, National Cancer Center Research Institute, Tokyo, Japan; 6) Genetics Division, National Cancer Center Research Institute, Tokyo, Japan.

Cancer genomes have extensive variations from single nucleotide mutations to large structural variations. And virus integrations into human host chromosomes also associate with genetic alternations in cancer cells. Systematic identification of these mutations and virus integrations would lead to a better understanding of cancer biology. Recently, exon capture methods for massively parallel sequencing have been developed and targeted sequence for thousands of genomic regions becomes possible with shorter time at reasonable cost. With this technology, we sequenced cancer-related exome subsets for 25 pairs of clinical virus positive hepatocellular carcinoma (HCC) and non-tumoral livers of the same patient. Genomic DNAs from clinical samples were sheared using Covaris™ and ligated with illumina GA paired-end adapters to prepare sequencing library. Exons of 1,248 cancer related genes (4.65 Mbase in total length) and HBV genome (3,215 base) were selected for capture targets. DNA fragments with target sequences were captured and enriched using SureSelect™ (Agilent) target enrichment system. Enriched DNA fragments were sequenced using illumina GAIIx sequencer according to manufacturer's protocol. Data analysis was done using illumina's pipeline and sequence data was mapped to hg18 using ELAND. Sequence statistics and mutation analysis were done by in-house programs.

On average we obtained approximately 1.6 giga bases of sequence data using one lane flow cell of illumina GAIIx with 51-cycle paired-end sequencing. About 60% of read sequences were mapped to targeted exons and average read depths for target regions were calculated more than 200X for each sample. About 95% of targeted bases were sequenced at least once and about 80% targeted bases were sequenced at least 20 times. With higher read depth data, we identified more than 300 somatic nonsynonymous single nucleotide substitutions and some of small insertion or deletion mutations. Most of these mutations were novel, while 14 genes were recurrently mutated among 25 samples, e.g. beta-catenin in 6 cases. We also identified many HBV-host human chromosome fusion DNA fragments both in HCCs and non-tumoral livers of HBV positive samples. Details of gene mutation profile of 25 liver cancers and virus integrations of HBV positive samples will be presented.

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Mitochondrial DNA haplogroups and susceptibility to prostate cancer in Colombian populations. *C. David[1], C.F Gomez[2,4], N. Ospina[1], J.A Cajigas[5], R.E Andrade[3,4], H. Groot[1], M.M Torres[1].* 1) Facultad de Ciencias , Universidad de los Andes, Bogota, Colombia; 2) Clínica de la Próstata. Fundación Santa Fe de Bogotá, Bogotá, Colombia; 3) Departamento de Patologia y Laboratorios. Fundación Santa Fe de Bogotá, Bogotá, Colombia; 4) Facultad de Medicina,Universidad de los Andes, Bogotá, Colombia; 5) Departamento de Urología.Hospital Militar Central. Bogota,Colombia.

Prostate cancer (PC) is one of the most common cancers and the second leading cause of cancer mortality in Colombian men. Mitochondrial respiratory activity is associated with the generation of reactive oxygen species (ROS), which may contribute to prostate cancer risk. Mitochondrial haplogroups (mtDNA) defined by common polymorphisms has been associated with risk of renal, breast and prostate cancer. Several studies have demonstrated dramatic differences in the risk of prostate cancer among men from different ethnic background. The Colombian population is culturally and genetically very diverse and is part of a complex admixed genetic ancestry. The aim of the present study was to assess the relationship between mtDNA haplogroups and prostate cancer. We sequenced the mitochondrial DNA hipervariable segment I (HSV1) in a population-based study that comprised 168 cases (CA) and 90 unrelated healthy individuals as a control group (CG).The mean age at onset was 68 years for the PC patients and 61 years for the control group. A total of 93 different mtDNA sequences were found in the patients and 58 in the control group. According to the geographical origin attributed to each mtDNA haplogroup, 82% of the mtDNA sequences found in both groups were Native American (A, B, C, and D). The most frequent was A (41.1%CA-43.3%CG), followed by B (22.0%CA-25.6%CG), C (12.0%CA-5.6%CG) and D (6%CA-7.8%CG). European haplogroups (U, H, K, J, M, T and HV) were also found in a lower proportion (12.5%CA-15.6%CG), likewise for African (L0, L1, L2, L3) (6.5%CA-2.2%CG). In this study there was no statistically significant differences between the distribution of mtDNA haplogroups in patients with prostate cancer and the control group, however, haplogroups C and L were more frequent in patients with cancer. In agreement with previous studies, our results showed no comparable association between any of the haplogroups studied and the susceptibility to the disease.

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MTHFR Polymorphisms and Breast Cancer Risk. *M. Hosseini[1], M. Houshmand[2,3], E. Ebrahimi[2].* 1) Dept Sci, Islamshahr Branch, Islamic Azad Univ, Tehran, Iran; 2) National Institute for Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran; 3) Special Medical Center, Tehran, Iran.

Folate plays an important role in DNA methylation, synthesis, and repair; intake has been associated with breast cancer.Two functional single nucleotide polymorphisms (SNPs) in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene, C677T and A1298C, lead to decreased enzyme activity and affect chemosensitivity of tumor cells.We evaluated these two common polymorphisms and their effects on the folate intake and breast cancer risk association in a Iranian sporadic breast cancer population-based case-control study of 294 breast cancer cases and 120 controls using a PCR-RFLP-based assay. Analyses of affected and controls show that homozygote genotype MTHFR 677CC has the highest frequency in both groups (40.6 in patients and 15.2 in control group). Genotype MTHFR 677 CC most risk factor were in our population: [CC / TT odds ratio, 1.524 (95% confidence interval; CI, 0.854-2.719) p=0.152], CC / CT odds ratio, 1.048 (95% CI, 0.638-1.720) p=0.854], TT / CT odds ratio, 1.455 (95% CI, 0.765-2.767) p= 0.252]. Genotype MTHFR 1298 AA most risk factor were in our population: [AA / CC odds ratio, 2.204 (95% confidence interval; CI, 1.203-4.038) p= 0.01], AA / AC odds ratio, 2.314 (95% CI, 1.434-3.735) p=0.001], CC / AC odds ratio, 0.952 (95% CI, 0.52-1.743) p=0.874]. There was a significant association of breast cancer risk with folate intake was observed in C677T and A1298C polymorphism.

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Cancer risk management strategies and perceptions in BRCA1/2 mutation-carrier families five years after test result disclosure. *C. Julian-Reynier[1], J. Mancini[1, 2], E. Mouret-Fourme[3], M. Gauthier-Villars[4], V. Bonadona[5], P. Berthet[6], J-P. Fricker[7], O. Caron[8], E. Luporsi[9], C. Noguès[3].* 1) INSERM UMR 912, Inst Paoli-Calmettes, BP156, Marseille CDX 9, France; 2) Lertim, Aix-Marseille Université, Faculté de Médecine, Marseille; 3) Centre René Huguenin, Saint Cloud; 4) Institut Curie, Paris; 5) Centre Léon Bérard, Lyon; 6) Centre François Baclesse, Caen; 7) Centre Paul Strauss, Strasbourg; 8) Institut Gustave Roussy, Villejuif; 9) Centre Alexis Vautrin, Nancy.

Background: Long term preventive strategies and breast/ovarian cancer risk perceptions of BRCA1/2 mutation carriers/non carriers were studied prospectively. Methods: A French cohort of unaffected female from BRCA1/2 mutated families was followed up for 5 years after test result disclosure, using self-administered questionnaires. Results: Response rate was 74%. Carriers (N=101) were younger (average age ± SD = 37 ± 10) than non carriers (N=145; 42 ± 12). Four strategies accounted for 88% of the carriers' decisions: 50% opted for breast surveillance alone, based on either Magnetic Resonance Imaging (MRI) and other imaging (31%) or mammography alone (19%); 38% opted for either Risk Reducing Salpingo-Oophorectomy (RRSO) and breast surveillance, based on MRI and other imaging (28%) or mammography alone (10%). The other three strategies were: Risk Reducing Mastectomy (RRM) and RRSO (5%), RRM alone (2%), and neither RRM/RRSO nor surveillance (6%). The results obtained on various age-groups are presented here. Over-screening practices were observed among non carriers. Test result disclosure increased short term carriers' high breast/ovarian cancer risk perceptions (p£0.01) and decreased non-carriers' short- and long- term perceptions (p<0.001). During follow-up, high breast cancer risk perceptions increased with time among those who had no RRM and decreased in the opposite case; high ovarian cancer risk perceptions increased further with time among those who had no RRSO and decreased in the opposite case; RRSO did not affect breast cancer risk perceptions. Conclusions: Informed decision-making involves letting women know whether opting for RRSO and breast MRI surveillance is as effective in terms of survival as RRM and RRSO.

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The patterns of BRCA1 and BRCA2 mutations in hereditary breast cancer in Iranian population. *F. keshavarzi[1], S. Zeinali[2].* 1) Islamic Azad University-Sanandaj branch, Sanandaj, Kurdistan, Iran; 2) Kawsar Human Genetics Reasearch Center,Tehran,Iran.

Introduction: Mutations in BRCA1 are present in approximately 50% of the early-onset breast cancer families and 80% of the early-onset breast and ovarian cancer families, whereas BRCA2 mutations are believed to account for a comparable percentage of inherited breast cancer cases. Therefore, the demand for BRCA1 and BRCA2 mutation screening is increasing as their identification will affect medical management of people at increased risk for breast and ovarian cancers. In this study we define point mutations, deletions and genomic rearrangements in BRCA genes in many early-onset and familial breast cancer. Material and method: All study subjects) 63 breast cancer patients (at age £ 40 years) or family history of breast or ovarian cancer (were recruited from Feb.2009 to Apr. 2010 at kawsar Human Genetics Research Center, Iran. After collecting blood samples and extracting DNA, their BRCA1 and BRCA2 genes were fully sequenced. Results and Discussion: Many missense substitutions in BRCA1 and BRCA2 genes were identified (Table 1). Here novel mutations are reported (Gly1140Ser, Ileu15Ieu, Ileu26Ieu, Leu3opal (stop codon), INS CD26 Small ins GTCCC^ATCTGcatctgGTAAGTCAGC, IVS7+83(-TT), IVS8 -70(-CATT), IVS2+9(-GC), IVS1-20(-GA), IVS1-8(-AG), IVS2+9(-GC), IVS2+24(-AG) in BRCA1 and Glu1391Gly in BRCA2). The missense substitutions Glu1038Pro, Gly1140Ser were found in large series of breast and ovarian cancer patient and 20% < of matched controls. The missense substitution Gly1738Glu in BRCA1 is pathogenic. Based on our preliminary results some haplotypes may have a pathogenic role in breast cancer development ,the haplotype at the BRCA1 locus defined by alleles Leu871Pro, GLu1038Gly, Ser1613Gly, Gly1140Ser was found in 10 affected families. Further studies are required to confirm the hypothesis that genetic polymorphisms are associated with breast cancer. Key words: BRCA1 gene, BRCA2 gene, genetic polymorphisms, breast cancer, Familial cancer.

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Staining for FGFR2 in breast cancers shows variable nuclear expression that correlates with patient genotype. *A.J. Martin[1], A. Grant[2], A. Thompson[3], C.A. Purdie[2], L. Jordan[2], J.N. Berg[1].* 1) Division of Pathology and Neuroscience, University of Dundee, Dundee; 2) Department of Clinical Pathology, Ninewells Hospital and Medical School, Dundee; 3) Division of Surgery and Molecular Oncology, University of Dundee, Ninewells Hospital and Medical School, Dundee.

Single Nucleotide Polymorphism (SNP) rs2981582 in intron 2 of the tyrosine kinase receptor Fibroblast Growth Factor Receptor type 2 (FGFR2) has been associated with increased risk of breast cancer. The high risk 'A' allele for rs2981582 has been associated with increased mRNA transcription and altered transcription factor binding. However, the effect that this has on FGFR2 protein expression is, as yet, unknown. We used immunohistochemistry to investigate the relationship between rs2981582 genotype and FGFR2 protein expression. 41 samples of invasive ductal carcinoma of no specific type were selected from women of known rs2981582 genotype. Patients were selected as homozygous for the low risk (G) allele (16 patients), heterozygous (A/G) (9 patients), or homozygous for the high risk (A) allele (16 patients). Samples were stained using rabbit polyclonal anti-FGFR2 antibody assessed using the Quickscore method. No significant association between genotype and cytoplasmic staining was identified but a significant association between prominent nuclear staining and the homozygous low risk (G) genotype was demonstrated (X2 for trend=4.1, p= 0.0429). These results are in contrast to those expected, given that increased mRNA transcription and altered transcription binding have been associated with the high risk (A) allele. FGFR2 is a membrane-bound tyrosine kinase receptor, so the nuclear position of protein expression is also surprising. Association between nuclear FGFR2 protein expression and rs2981582 genotype suggests that the mechanism of action of this polymorphism may be more complex than a direct effect on mRNA expression levels, and may relate to FGFR2 function or localisation during breast development or tumourigenesis.

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An Online Database of Cancer Genetic Associations from Candidate Gene and Genome Wide Association Studies. *S.D. Schully[1], V. McCallum[2], C.B. Benedicto[1], L.M. Dong[3], W. Yu[4], M.J. Khoury[4,1].* 1) DCCPS, National Cancer Inst, Rockville, MD; 2) OWD, National Cancer Inst, Rockville, MD; 3) DCEG, National Cancer Inst, Rockville, MD; 4) OPHG, CDC, Atlanta, GA.

Since 2001, the Human Genome Epidemiology (HuGE) Literature Finder has been tracking published literature of human genome epidemiologic studies. The HuGE Literature Finder provides access to a continuously updated knowledge base in human genome epidemiology, including information on population prevalence of genetic variants, gene-disease associations, gene-gene and gene- environment interactions, and evaluation of genetic tests. Historically, genetic susceptibility to complex disease has been uncovered through candidate gene studies. However, in recent years, the advent of Genome Wide Association Studies (GWAS) have greatly accelerated the pace of genetic association discovery. Building upon an evaluation of candidate gene studies published by Dong et al in 2008, and in an effort to consolidate the vast amounts of information coming from both candidate gene and GWAS, we have developed an online knowledge base and repository of all cancer genetic associations from GWAS and meta-analyses of candidate gene studies.

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Common leukemia- and lymphoma-associated genetic rearrangements and constitutional t(11;22) in healthy individuals. *J. Song[1], X. Hu[1, 2], H. Liu[3], D. Mercer[1], M. Li[1,2,4].* 1) Hayward Genetics Center, Tulane University Medical Center, New Orleans, LA; 2) Tulane Cancer Center, Tulane University Medical Center, New Orleans, LA; 3) Department of Anesthesiology, Tulane University Medical Center, New Orleans, LA; 4) Department of Pediatrics, Tulane University Medical Center, New Orleans, LA.

Leukemia and lymphoma-associated (LLA-) chromosomal rearrangements play critical roles in the process of tumorigenesis. These genetic aberrations are also important biological markers in the diagnosis, prognosis, and treatment of hematopoietic malignancies. The constitutional t(11;22) is the only recurrent, non-Robertsonian translocation in humans. Balanced carriers usually have no clinical symptoms; whereas affected offspring often demonstrate the supernumerary derivative 22 syndrome (Emanuel syndrome). We hypothesize that both LLA-chromosomal rearrangements and non-LLA-chromosomal rearrangements can occur randomly in normal individuals. To test our hypothesis, we performed sensitive nested RT-PCR on a large number of peripheral blood samples from healthy individuals for selected markers of LLA-chromosomal rearrangements including MLL-PTD, BCR-ABL (p190 and p210), MLL-AF4, AML1-ETO, PML-RARA, and CBFB-MYH11. We found the presence of all of these selected markers in healthy individuals at various rates of incidence. We found no correlation between the incidence and age except for the BCR-ABL p210 fusion, the incidence of which rises with increasing age. To detect the presence of t(11;22) in normal individuals, we performed nested PCR using genomic DNA from peripheral blood samples and found t(11;22) translocation fragments from both der(11) and der(22). Direct sequencing of the PCR products verified the breakpoints. In summary, our data lend strong support to the hypothesis that both LLA-chromosomal rearrangements and non LLA-chromosomal rearrangements occur randomly in healthy individuals, suggesting that these genetic aberrations are random events and are not themselves sufficient for malignant transformation.

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An Enhanced International Fanconi Anemia Registry (IFAR). *A.D. Auerbach[1], A. Smogorzewska[2], F.P. Lach[2], M.J. Wrobel[3], M. Sengupta[3], E. Barbour[3].* 1) Human Genetics & Hematology, Rockefeller Univ, New York, NY; 2) Laboratory of Genome Maintenance, Rockefeller Univ, New York, NY; 3) Hospital Informatics, Rockefeller Univ, New York, NY.

Fanconi anemia (FA) is a rare recessively inherited disorder characterized by genome instability, DNA crosslink hypersensitivity, congenital malformations, bone marrow failure, and predisposition to malignancy. The International Fanconi Anemia Registry (IFAR) was established at The Rockefeller Univ. in 1982 in order to collect data regarding patients with FA and their families (1). In addition, the Fanconi Anemia Mutation Database, http://www.rockefeller.edu/fanconi/mutate/, was established in 1998 to accelerate the availability of information on mutations in the 13 FA genes. The new IFAR project is developing a comprehensive, ontology-driven Phenotype Recording Instrument (PRI) and database for FA. The PRI integrates the existing IFAR data held in multiple disparate data sources for a unique look at the FA patient population, which includes the patient data available in the current IFAR database, the polymorphic (SNP) and mutation data available for patients representing the known FA genes. Furthermore, the NIH's Genomics Center (NHGRI) is currently sequencing certain patient tissue samples using Next Generation technology, which will further enhance the current IFAR patient data. The new development effort will also include "deep phenotyping" from new patient history questionnaires. We are hypothesizing the addition of these two data stores, full gene sequencing and deep phenotyping, will add significantly to the understanding of FA while providing a deeper basic scientific understanding of DNA repair mechanisms, and more prognostic ability for the current patient population. The enhanced data model resulting from the aforementioned data sources is being modeled using an OWL ontology defined with Protégé. We are building data mining and visualization tools that will be required to maximize the research view of this integrated patient data. As the name of the existing IFAR system implies, this registry is an International collaboration. The new system will enable worldwide international collaboration between FA researchers. To accomplish this, we are building a web-based system utilizing the Java JSP front-end coupled with an Oracle 10g database backend for performance and data integrity. This is truly a unique medical informatics opportunity made possible because of the 28 year collection of FA data combined with next generation sequencing and deep phenotyping data. (1) Kutler DI et al., Blood 101:1249-1256,2003.

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Integration of modifier genes genotyping into molecular diagnosis of Lynch syndrome is premature. *T. Frebourg[1], S. Houlle[1], F. Charbonnier[1], E. Houivet[2], S. Baert-Desurmont[1], M.-P. Buisine[3], J. Benichou[2].* 1) Dept Genetics, Inserm U614, Rouen Univ Hosp, Rouen, France; 2) Department of Biostatistics, Rouen University Hospital, France; 3) Laboratory of Molecular Biology, Lille University Hospital, France.

One of the first modifier genetic factors identified in Lynch syndrome corresponds to the IGF1 promoter CA repeat, and it had been shown, in 121 *MMR* mutation carriers, that a 17 CA repeat or less was significantly associated to a higher colorectal cancer (CRC) risk and an earlier age of tumour onset (Zecevic et al., J. Natl. Cancer Inst. 2006; 98: 139-43). Wijnen et al. (Gastroenterology 2009;136:131-7) reported, in 675 *MMR* mutations, a significant association between CRC risk and two SNPs, rs16892766 (8q23.3) and rs3802842 (11q23.1), initially detected by GWAS as risk factors for CRC in the general population. We analyzed the effect of these modifier genes in 750 French *MMR* mutation carriers derived from 292 families and including 330 CRC patients (mean age of CRC onset: 43 years). We also analyzed the effect of the Novel 1 SNP (18q21), which has recently been shown to be associated to CRC risk in the general population (Pittman et al., Genome Res. 2009;19:987-93). A significant difference was observed in the CRC-free survival time or the median age of onset of CRC between males and females (log-rank test P < 0.0001), between *MSH2* and *MSH6* mutation carriers (log-rank test P = 0.0315), between *MLH1* and *MSH6* mutation carriers (log-rank test P = 0.0049) but not between *MSH2* and *MSH6* mutation carriers (log-rank test P > 0.05), indicating that our series was representative of Lynch syndrome. In this series, the univariate Kaplan-Meier analysis showed that the CRC penetrance did not differ significantly for the three SNPs and the IGF1 CA repeats, when stratified by genotype and gender. The multivariate Cox analysis (taking into consideration the family effect) stratified for the gender, the mutated MMR gene and date of birth did not reveal that a specific genotype for these 4 genes was associated to a significant hazard ratio increase. We subsequently restricted the analysis to *MSH2* and *MSH2* carriers and did not neither observed any difference. Finally, we performed the statistical analyses only on affected subjects and these analyses did not reveal any significant difference. In conclusion, we did not reproduce the previously published results. We think that this discrepancy might be explain by artefactual associations generated by too small samples. We conclude that genotyping of these polymorphisms should not be integrated into the molecular diagnosis of Lynch syndrome to optimize the follow-up of *MMR* mutation carriers.

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Impact of family history on uptake of risk-reducing surgeries among BRCA mutation carriers. *K.R. Singh[1], J. Gross[2], B. Karlan[2], X. Yan[1], K. Yang[1], X. Guo[1], O. Gordon[1].* 1) Medical Genetics, Cedars-Sinai Medical Genetics Institute-Gilda Radner Program, Los Angeles, CA; 2) Women's Cancer Research Institue at the Samuel Oschin Comprehensive Cancer Center, Cedars-Sinai Medical Center, Los Angeles, CA.

Objective: Lifetime risk of breast and ovarian cancers among BRCA mutation carriers are markedly reduced with prophylactic surgeries, yet only a portion elect to undergo these procedures. This study investigates factors that might influence patients' decisions regarding risk reducing mastectomy (RRM) and risk reducing bilateral salpingo-oopherectomy (RRBSO). **Methods:** Subjects were women with no personal history of cancer and with deleterious mutations in the BRCA1/2 genes who underwent RRM or were participants in the high risk surveillance program at our institution. Medical records were reviewed for age, parity, family history, personal history of breast biopsies, cosmetic surgery, and uptake of prophylactic surgeries. **Results:** Of 134 BRCA carriers, 31(23.1%) elected RRM. 24 of 29(82.8%) with RRM had the procedure within 2 years of genetic testing (surgery dates for 2 were unknown). In the group with RRM, history of mother deceased from breast cancer was 35.5% vs. 8.7% in the no RRM group, p<0.0002. Two women (6.5%) in the RRM group had a sister affected with breast cancer without mother being affected vs. 5(4.9%) in the no RRM group. Uptake of RRBSO was 71.0% in the RRM group vs. 46.6% in the no RRM group, p<0.0025. Forty-six (95.8%) of 48 patients who had RRBSO in the no RRM group had history of mother with pelvic cancer. Parity was higher in the group with RRM than the group without (19% para 0 vs. 46%, p<0.0038). There were no significant differences in mean age at time of genetic testing, history of breast biopsy, or history of cosmetic surgery between the two groups. **Conclusions:** BRCA carriers who had lost their mother to breast cancer were most apt to choose RRM. Numbers of women with only a sister affected with breast cancer were small in both groups and did not suggest significant influence on decision-making. Women with RRM were more likely to have RRBSO than women without RRM. Women without RRM were less parous than those with RRM and may have opted against surgery until completion of childbearing. However, among those without RRM, uptake of RRBSO was highest in women whose mother had pelvic cancer. The impact of losing a mother to cancer may be of under recognized import in patient attitudes regarding prophylactic procedures. Additional studies are needed to assess whether directly addressing this life experience or lack thereof during genetic counseling influences patient behavior regarding surveillance versus prophylactic surgery.

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Mutation analysis of *TSC1* and *TSC2* genes in 2 randomized, double-blind, placebo-controlled studies of everolimus in the treatment of subependymal giant-cell astrocytomas (SEGAs) (EXIST-1) and angiomyolipomas (EXIST-2). *Z. Tsuchihashi[1], W. Liu[1], J. Sampson[2], D.N. Franz[3], J. Bissler[3], C. Kingswood[4], T. Sahmoud[1], D. Kwiatkowski[5].* 1) Novartis Pharmaceuticals Corporation, Florham Park, NJ; 2) Institute of Medical Genetics, Cardiff University, Cardiff, UK; 3) Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 4) Royal Sussex County Hospital, Brighton, UK; 5) Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

Objectives: Tuberous sclerosis complex (TSC), a genetic disease characterized by multisystemic hamartomas and disabling neurological disorders, is caused by mutations in *TSC1* or *TSC2* that result in constitutive activation of mTOR, a key regulator of cell growth and proliferation, metabolism, and angiogenesis. Lesions occur throughout the body, including the kidney (angiomyolipomas), lungs (lymphangioleiomyomatosis [LAM]), and brain (tubers and SEGAs). Angiomyolipomas can also occur in patients with sporadic LAM independent of TSC. In an open-label phase II trial, everolimus, an oral mTOR inhibitor, significantly reduced TSC-associated SEGA volume. The EXIST (**EX**amining everolimus **I**n a **S**tudy of **T**SC) trials are evaluating the efficacy and safety of everolimus in the treatment of SEGAs (EXIST-1) and angiomyolipomas (EXIST-2). **Methods:** EXIST-1 (NCT00789828) and EXIST-2 (NCT00790400) are randomized, prospective, double-blind, multicenter, phase III trials. In EXIST-1, patients of any age with SEGAs and evidence of serial growth are randomized 2:1 to receive everolimus (initial dose 4.5 mg/m^2/d with titration to trough concentration of 5-15 ng/mL) or placebo. In EXIST-2, patients ≥18 y with a definite diagnosis of TSC or sporadic LAM and ≥1 angiomyolipoma (≥3 cm) are randomized 2:1 to receive everolimus 10 mg/d or placebo. Patients will be treated until progression or unacceptable toxicity. Primary endpoint is SEGA response rate (RR, reduction in volume ≥50%) in EXIST-1 and angiomyolipoma RR in EXIST-2, determined by MRI per independent central radiology review. Secondary endpoints in EXIST-1 include change in frequency of epileptiform events, duration of and time to SEGA response, time to SEGA progression, skin lesion RR, biomarker analyses, and safety. EXIST-2 secondary endpoints include duration of and time to angiomyolipoma response, time to angiomyolipoma progression, skin lesion RR, biomarker analyses, and safety. Target enrollment is 99 patients per study. In both studies, blood DNA samples from all patients are analyzed for the mutation status of *TSC1* and *TSC2* genes. All of the exons and the adjacent intronic regions of these 2 genes are PCR-amplified and analyzed by Sanger dideoxy-sequencing method. Potential association between mutation status and disease phenotype and/or the patients' response to everolimus will be explored. Currently the mutation status of 21 patients has been determined and the updated result from this analysis will be presented.

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Gene Set Enrichment Analysis can detect specific biology to interpret and prioritize the results of a Coronary Artery Disease Genome Wide Association Study. *A.J. Stoddard[1], R.B. Lorier[1], A. Matter[1], E.A. Virlee[1], S.K. Pachowitz[1], J.P. Peete[1], D.L. Linzmeier[1], U. Broeckel[1,2,3,4].* 1) Human and Molecular Genetics Center, Medical College of Wisconsin, Milwaukee, WI; 2) Department of Pediatrics, Medical College of Wisconsin, Milwaukee, WI; 3) Department of Medicine, Medical College of Wisconsin, Milwaukee, WI; 4) Department of Physiology, Medical College of Wisconsin, Milwaukee, WI.

Background: Prioritizing and interpreting the results of Genome-wide association studies (GWAS) presents numerous challenges, especially for complex disease. Association analysis SNP-by-SNP provides little biological insight, with only rank order to assess priority. Many true associations will rank too low to merit attention. Even when single SNPs are strongly associated they seldom explain a large fraction of heritability for a complex trait. Gene Set Enrichment Analysis (GSEA),which allows associations to be tested as groups bringing biological context to bear, can be used to interpret GWAS results and is here applied to various inflammatory serum markers linked to Coronary Artery Disease (CAD).

Methods: A cohort of 607 Caucasian individuals with CAD was genotyped on Affymetrix 6.0 arrays. Covariate adjusted single SNP logistic regression based association values with plasma biomarker levels were calculated using the PLINK software. SNPs were mapped to genes taking the strongest associated marker per gene. GSEA v2.06 software (Broad) was used with 1229 supplied gene sets augmented with 32 cardiac expression and drug response lists generated from ArrayExpress and CMAP datasets to focus on cardiac context.

Results: For all 7 serum biomarkers considered SELE, ICAM1, IFNG, VCAM1, MPO, MPI and CRP, 3 or more gene sets from the cardiac expression library were in the top 50 most highly enriched gene sets by GSEA (for each biomarker the top 50 sets had an FDR of 0.2-0.3) showing a significant specific detection of cardiac biology (hypergeometric $p < 0.001$ or better in each case). Relevant biology was also revealed in non-targeted gene sets, e.g. the ETS pathway was the highest enriched pathway set for SELE associated genes ($p < 0.001$) with lower (between rank 2000-4000) ranking genes, RBL1, ETS1, CSF1 still contributing to the enrichment.

Conclusion: GSEA is shown to detect specific cardiac biology, combining gene expression with CAD association results, where individual genes would not be considered by association alone. Thus functional biological hypotheses are generated and can be prioritized for follow-up experiments.

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The rs10934857T>C of the GATA2 gene is a susceptibility variant for myocardial infarction. *M.M. Al-Rasheed[1,2,3], M. Al-Najai[1], M.M. Vigilla[1], S. Wakil[1], N. Dzimiri[1].* 1) Genetics Dept, King Faisal Specialist Hospital & Res. Ctr., Riyadh, KSA; 2) Clinical Pharmacy Dept., King Saud University, Riyadh, KSA; 3) School of Pharmacy & Biomolecular Sciences, University of Brighton, UK.

The predisposing effect of GATA2 to acquiring cardiovascular disease still remains to be elucidated. In this study, we first sequenced the GATA2 gene in 200 individuals to identify informative variants of interest. We then selected 3 familiar variants, rs2335052 (A>G), rs3803 (C>T), rs10934857 (T>C), and two novel SNPs herein denoted as ns2713579 (A>G) and ns1573949 (A>G) to test for possible association with myocardial infarction (MI) by the real-time PCR-based techniques. A total of 2633 MI cases versus 1230 controls, all of Saudi origin, were employed for the study. The results pointed to a significant causative association for the rs10934857 with MI [Odds ratio (95% Confidence Interval) = [1.12(1.01-1.24); p=0.031]. Further analysis pointed to the autosomal recessive mode of inherence [1.34(1.13-1.58); p= 0.002] as being responsible for this association. Interestingly, there was no definable link for this variant with coronary artery disease (CAD). Besides, no confounding effect of type 2 diabetes mellitus, hypertension, hyperlipidaemia, or any other variable was observed for this association. These results demonstrate that the GATA2 rs10934857 variant predisposes individual to acquiring MI, but not necessarily manifestation of CAD in our study population.

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Genetic Variation at the DPP4 Locus Influences ApoB Levels among South Asians and Reveals Heterogeneity in Europeans Caused by Adiposity. *S.D Bailey[1], C. Xie[2], A. Montpetit[3], S. Anand[2], J.C Engert[1,4] on behalf of the EpiDREAM investigators.* 1) Department of Human Genetics, McGill University, Montreal, Quebec, Canada; 2) Population Health Research Institute, McMaster University, Hamilton, Ontario, Canada; 3) McGill University and Genome Quebec Innovation Centre, Montreal, Quebec, Canada; 4) Department of Medicine, McGill University, Montreal, Quebec, Canada.

The majority of genome-wide association scans (GWAS) performed to date have focused primarily on individuals of European descent. Although some of the identified risk alleles are consistent across ethnicities, performing association scans in other ethnic groups could identify new genes. We used the ITMAT Broad CARe (IBC) array to test 31,739 common SNPs, from 2000 cardiovascular candidate genes, in 2625 South Asian individuals from the EpiDREAM study, individuals screened for the DREAM trial, for an association with levels of apolipoprotein B (apoB). We attempted to replicate seven SNPs, from previously unreported genes, in 1081 South Asian controls from the INTERHEART study. A SNP within the dipeptidyl peptidase IV (DPP4) gene was significantly associated with apoB levels in both the EpiDREAM and INTERHEART studies (β=-0.023, p=1.33x10-4 and β=-0.028, 7.28x10-3, combined n=3706, p=3.09x10-6). Interestingly, the SNP was not associated with apoB in 8973 EpiDREAM individuals of European descent (p=0.94). However, the South Asian samples were significantly leaner than the European samples (average BMI of 26.4±4.3 and 30.4±5.7, respectively). In the lowest European BMI tertile (average BMI=24.6±2.0) we observed a significant association between the SNP and apoB (β=-0.020, p=1.44x10-3). We restricted the analysis to only lean individuals (BMI < 25) and observed an increase in the effect of this SNP on ApoB (EpiDREAM Europeans n=1468, β=-0.029, p=4.38x10-4). This increase was also observed in lean South Asians from both the EpiDREAM (n=1028, β=-0.024, p=9.13x10-3) and INTERHEART (n=577, β=-0.045, p=1.78x10-3) studies. When we analyzed all lean individuals from the entire EpiDREAM study, which includes European, South Asian, Latin American, African American, Native North American and East Asian samples, we observe strong overall evidence of an association between the identified SNP in DPP4 and apoB (n=3207, p=6.98x10-6), which is supported further by the replication in lean South Asians from the INTERHEART sample (p=1.78x10-3). DPP4 is a target of anti-hyperglycemic medication used in the treatment of type-2 diabetes (T2D). DPP4 inactivates two insulinotropic hormones, the gastric inhibitory peptide and glucagon-like peptide 1, and its pharmacological inhibition has been shown to lower both fasting and postprandial glucose levels by prolonging their half-lives. These results increase our understanding of the relationship between glucose and lipid metabolism.

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Evaluation of Variants in the BCAT Gene as Metabolic Quantitative Trait Loci. *L. Elbadawi[1], C. Haynes[2], J. Johnson[2], J. Bain[3], M. Muehlbauer[3], R. Stevens[3], C. Newgard[3], K. Newby[1,4], S. Gregory[2], W. Kraus[1,2], E. Hauser[2], S. Shah[1,2].* 1) Department of Medicine, Duke University, Durham, NC; 2) Duke Center for Human Genetics, Durham, NC; 3) Sarah W. Stedman Nutrition and Metabolism Center, Durham, NC; 4) Duke Clinical Research Institute, Durham, NC.

Background. Metabolic biomarkers for coronary artery disease (CAD) are strongly heritable but the source of the genetic variation for these biomarkers remains to be identified. Derangements in branched-chain amino acid (BCAA) catabolism are heritable in families burdened with early onset CAD. Further, BCAA levels are higher in subjects with cardiovascular disease than in controls without CAD when controlling for confounders. BCAT1 encodes the cytosolic form of the enzyme branched-chain amino acid transaminase that catalyzes the reversible transamination of branched-chain alpha-keto acids to branched-chain L-amino acids essential for cell growth and plays a large role in the BCAA catabolic process. Thus, we tested whether BCAT1 is a metabolic quantitative trait locus (mQTL) modulating BCAA levels and serving as a CAD susceptibility gene. Objectives. Our primary hypothesis was that single nucleotide polymorphisms (SNPs) in BCAT1 would be associated with differential levels of peripheral blood BCAA in patients referred for evaluation of CAD. Secondarily, we hypothesized that these BCAT1 SNPs would be associated with CAD. Methods and Results: Patients undergoing cardiac catheterization for evaluation of CAD at Duke University Medical Center who were enrolled in the CATHGEN biorepository formed the study population. Of these, 2375 subjects had had mass spectrometry based metabolomic profiling of 69 metabolites performed, and were included in this analysis. Genotyping of SNPs RS2242400, RS10505955, RS2353486 in BCAT1 was performed using Taqman assays. Principal component analysis (PCA) was used for multidimensional data reduction, and linear and logistic regression examined the associations of metabolomic factors resulting from PCA with BCAT1 SNPS and BCAT1 SNPs with CAD, respectively. The three SNPs met Hardy-Weinberg equilibrium and had minor allele frequencies of: 0.11, 0.43, 0.36, respectively. PCA identified thirteen factors, including a factor composed of the branched chain amino acids, as previously observed. However, none of the BCAT1 SNPs were associated with levels of this BCAA factor. Further, BCAT1 SNPs themselves were not associated with the presence of CAD. Conclusions. Our data do not support the role of BCAT1 variants as a mQTL for CVD risk. Future studies should evaluate other genes in the BCAA catabolic process to understand the genetic architecture modulating BCAA levels, and predisposing to CAD.

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Identification of a novel candidate gene on chromosome 20p12 influencing variation in lipoprotein-associated phospholipase A2, a risk factor for cardiovascular disease. *P.B. Higgins, V.P. Diego, J.W. Kent Jr., M.A. Carless, T.D. Dyer, D.L. Rainwater, L.A. Almasy, A.G. Comuzzie, J.E. Curran, E.K. Moses, M.C. Mahaney, J. Blangero.* Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX.

Lipoprotein-associated phospholipase A2 (Lp-PLA2) is a novel biomarker of oxidative stress and inflammation, and is positively associated with risk of cardiovascular disease (CVD). Lp-PLA2 is transported in the bloodstream predominantly by low density lipoprotein (LDL) and partly by high density lipoprotein (HDL). The physiological dynamics of LDL and HDL concentrations in the circulation are intricately related to triglyceride levels. We hypothesized therefore that variation in Lp-PLA2 activity would be significantly influenced by the ambient lipid/lipoprotein milieu. To test this idea, we constructed discrete lipid/lipoprotein environments for LDL cholesterol (LDLC), HDLC, and serum triglycerides by scoring the upper tertile as 1, and 0 otherwise, and performed genotype-by-environment interaction (GEI) analyses of Lp-PLA2 activity in relation to the three lipid/lipoprotein environments. We focus on results from the genotype-by-triglyceride environment interaction (GTI) linkage analysis. From this GTI linkage analysis, we found strong evidence of a quantitative trait locus (QTL) on chromosome 20p12 (genome-wide maximum LOD score = 3.67), as well as evidence of a GTI effect at the QTL (p = 0.003). Within the 1-LOD interval about the chromosome 20p12 QTL, we typed 712 single nucleotide polymorphisms (SNPs). Because we desired to take a Bayesian model selection (BMS) approach toward detecting genetic association with Lp-PLA2 activity, we decided to delimit the model space by screening for SNPs that had a p-value less than 0.005 by a naïve measured genotype association test. This first screen resulted in a reduced set of six SNPs, thus giving a model space of 26 = 64 models of additive SNP effects. BMS identified the most supported model among the reduced set to be the model that contained two specific SNPs, namely rs11905991 and rs605589 (2 degrees of freedom measured genotype association test p = 4.3 × 10-6). The first SNP is in the vicinity of an open reading frame, and thus may be a novel functional gene. The second SNP is at the phospholipase Cβ1 (*PLCB1*) gene, which is known to protect cells against oxidative-stress-induced cell death and to promote mitogenesis. In conclusion, we have identified a novel candidate gene of relevance to the known functionality of Lp-PLA2.

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A two-stage matched case-control study on multiple hypertensive candidate genes in Han Chinese population. *T. Kuo[1], M. Kang[1], C. Chung[1], H. Ho[2], C. Ting[2], T. Lin[3], S. Sheu[3], W. Tsai[4], J. Chen[4], H. Leu[5], W. Yin[6], T. Chiu[7], Y. Chen[1], C. Chen[7], C. Fann[1], J. Wu[1], T. Lin[1], S. Lin[5], J. Chen[5], W. Pan[1].* 1) Biomedical Science, Academia Sinica, Taipei, Taiwan; 2) Taichung Veterans General Hospital, Taichung, Taiwan; 3) Kaohsiung Medical University Chung-Ho Memorial Hospital, Kaohsiung, Taiwan; 4) National Cheng Kung University Hospital, Tainan, Taiwan; 5) National Yang-Ming University School of Medicine and Taipei Veterans General Hospital, Taipei, Taiwan; 6) Cheng Hsin Rehabilitation Medical Center, Taipei, Taiwan; 7) Min Sheng General Hospital, Taoyuan, Taiwan.

We performed a two-stage association study of young-onset hypertension on genotype data of single nucleotide polymorphisms (SNPs) from 992 cases and 992 matched controls of Han Chinese in Taiwan. 239 SNPs of 37 hypertension candidate genes with functional importance were investigated. These 37 candidate genes were selected based on the website of genetic association database (GAD) and were classified into five main groups: (1) renin-angiotensin-aldosterone system (RAAS); (2) messenger-related; (3) channels of transporters; (4) energy related; and (5) others with functional importance related to hypertension. 239 SNPs across these genes were selected from those significantly reported in the previous publications and those given by the HaploView software based on their linkage disequilibrium (LD) structure. Single SNP analysis using conditional logistic regression was carried out for individual SNP in this matched case-control study. We identified two SNPs that were strongly associated with hypertension in both two stages. The first SNP (rs2301339) is located at guanine nucleotide binding protein beta 3 subunit (GNB3) and the other one (rs17254521) is located at insulin receptor (INSR). The former is perfectly linked in linkage disequilibrium (LD) with C825T(rs5443) which is associated with the occurrence of splice variant in GNB3 and has been found to be associated with hypertension in Caucasian, but less inconsistent in Asian populations. Despite significant evidence in both linked SNPs, their effect is opposite. This might be due to population heterogeneity and the younger subjects of hypertensive patients used in the present study.

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BRAP gene exerts a pro-atherogenic effect via prohibiting NF-κB nuclear translocation. *Y.C. Liao[1, 2, 5], H.F. Lin[3], Y.S. Wang[2], K.C. Chen[2], M.H. Chang[5], S.H.H. Juo[2, 3, 4].* 1) Graduate Institute of Medicine , Kaohsiung Medical University, Kaohsiung, Taiwan; 2) Graduate Institute of Medical Genetics, Kaohsiung Medical University, Kaohsiung, Taiwan; 3) Department of Neurology, Kaohsiung Medical University, Kaohsiung, Taiwan; 4) Department of Medical Research, Kaohsiung Medical University, Kaohsiung, Taiwan; 5) Section of Neurology, Taichung Veterans General Hospital, Taichung, Taiwan.

Background The BRCA-1 associated protein (BRAP) gene was recently identified as a susceptible gene to myocardial infarction (MI) in two Asian populations. The rare allele of functional SNP rs11066001 is associated with an enhanced BRAP expression and aggravated NF-κB activity. The present study sought to determine (1) the BRAP effect on stroke and carotid atherosclerosis, and (2) the mechanism beneath its pro-atherogenic effect. **Methods** Three phenotypes including stroke, carotid intimal-medial thickness (IMT), and plaque were used to evaluate the BRAP effect in a Chinese population. The study participants included 769 stroke patients and 1235 stroke- and MI-free volunteers who received carotid ultrasonogrpahy. SNP rs11066001 was genotyped by TagMan method. Human artery smooth muscle cells (HASMCs) were transfected with siRNA targeting at BRAP or scrambled siRNA before treated by lipopolysaccharide (LPS). MTT assay, transwell experiments and ELISA were used to measure the HASMC proliferation, migration and inflammation. **Results** Unlike MI, the SNP was not significant for stroke (p = 0.47). However, the rare homozygotes GG was associated with a 1.82-fold risk to have at least one carotid plaque (p = 0.026) among our healthy subjects. There was a trend of over-represented GG genotype in the subjects with higher plaque index compared to the AA+AG genotypes (GG frequencies in plaque index >= 4, index = 1-3, and index = 0 were 14.7%, 9.6%and 6.6% respectively, trend p = 0.030). There was no association between rs11066001 and IMT values. When cells were treated with LPS, there was an increase of BRAP expression level, HASMC proliferation, migration and secretion of inflammatory markers. Knocking down the BRAP expression led to attenuated proliferation and migration in HASMCs (p < 0.05). In addition, the MCP-1 and IL-8 levels were significantly lower in HASMCs transfected with BRAP siRNA. Knock-down the BRAP gene did not affect the NF-κB mRNA levels, but prohibit the NF-κB activation. In western blot, the nuclear-cytoplasm ratio of NF-κB gene increased when HASMCs were exposed to LPS. Such nuclear translocation was blocked after knocking down the BRAP gene. Immuno-fluorescence stains demonstrated identical findings. **Conclusions** We found BRAP gene confers risks for carotid atherosclerosis,coronary artery disease but not stroke. The pro-atherogenic effect of BRAP is mediated by influencing the NK-kB activity via nuclear translocation.

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The rs1333040 Genotypes on 9p21.3 Influence the CDKN2A/B Expression in Coronary Atherosclerotic Plaques. *N. Marziliano[1], F. Orsini[1], M.F. Notarangelo[2], D. Lina[2], S. Veronese[1], C. Berzuini[3], D. Ardissino[2], P. Merlini[1].* 1) Cardiovascular, Azienda Ospedaliera Niguarda Ca' Granda, Milano, MILANO, Italy; 2) Division of Cardiology, Azienda Ospedaliero-Universitaria di Parma, Parma, Italy; 3) Statistical Laboratory, Centre for Mathematical Sciences, Cambridge, United Kingdom.

Coronary artery disease (CAD) is significantly influenced by genetic background. Recent genome-wide association studies have shown that common genetic variants in tight linkage disequilibrium located in human chromosomal region 9p21.3 are associated with ischemic heart disease. However, the mechanisms by which these variants increase the risk of CAD is still unknown. In an elegant study of knock-out mice lacking the CAD locus interval in chromosome 4, Visel et al. (Nature 2010) found that this region is critically required for the normal cardiac expression of two neighbouring cyclin-dependent kinase inhibiting genes, Cdkn2a/b. We investigated the influence of the 9p21.3 variant rs1333040 on CDKN2A/B expression in 30 human coronary atherosclerotic plaques obtained by means of coronary atherectomy from patients with ischemic heart disease. The genotype distribution of rs1333040 was as follows: five atherosclerotic plaques had no risk allele (CC), 10 had one risk allele (CT) and 15 had two (TT). There was a significant (p=0.00013) trend of the expression levels of both genes with the number of copies of the risk allele (C), the expression in the CT plaques being in between that of CC and TT plaques, for both genes. Visel et al. suggested that the non-coding CAD risk interval affects vascular cell proliferation and senescence by modulating the expression of Cdkn2a and Cdkn2b. This view is complemented by our findings showing a link between the genotype of the rs1333040 locus and CDKN2A/B expression in diseased human tissues, which support the hypothesis that sequence variations in the CAD risk interval may act as distant regulatory sequences and be required for the correct vascular expression of CDKN2A/B. Further studies are needed to identify the target human cell in which the 9p21.3 risk allele most influences the reduction in CDKN2A/B gene expression.

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GENE-CENTRIC STUDY IDENTIFIES TWO NOVEL GENES, CLCN2 (A VOLTAGE-GATED CHLORIDE CHANNEL) AND KCNAB1 (A VOLTAGE-GATED POTASSIUM CHANNEL) ASSOCIATED WITH BLOOD PRESSURE IN TWO INDEPENDENT IRISH POPULATIONS. *N. McCarthy[1], C. Vangjeli[1], G. Cavalleri[1], K. Shianna[3], N. Delanty[1], E. O'Brien[2], B. Harvey[1], A. Stanton[1].* 1) Royal College of Surgeons in Ireland, Dublin, Ireland; 2) University College Dublin, Dublin, Ireland; 3) Duke Institute for Genome Sciences & Policy, North Carolina, USA.

Objective: Hypertension is highly heritable. Recent genome-wide association studies have identified 13 novel loci associated with BP. However these loci only explain a small proportion of total BP variation. It is recognized that identification of all genetic variants associated with BP will require complementary strategies. Here we report on a targeted candidate gene study with dense SNP coverage of multiple genes involved in electrolyte transport. Design and Method: Using the Illumina GoldenGate platform, we genotyped 1860 SNPs in 81 genes in 358 healthy bank employees who had undergone seated clinic BP measurements (screening population, SP). Only the 35 SNPs, in 21 genes, that were found to be associated with systolic, diastolic or pulse pressure (SBP, DBP or PP) within the SP (p£0.01), were genotyped (Illumina Veracode platform) in a second independent replication population (RP) - these 380 healthy bank employees had undergone repeated 24-hour ambulatory BP monitoring. Association analyses were performed using additive genetic models and adjusted for age and sex. Results: Quantile-quantile plots showed enrichment for significant P-values in both populations. Particularly strongly associated SNPs included a synonymous SNP in a voltage-gated chloride channel gene (*CLCN2*) and an intronic SNP in a voltage-gated potassium channel gene (*KCNAB1*). The *CLCN2* SNP was associated with increased clinic SBP(2.7mmHg, p0.02)in the SP and clinic, daytime and nighttime SBP(2.9mmHg, p0.003; 1.8mmHg, p0.009; 2.1mmHg, p0.001*) in the RP aswell as increased clinic DBP (2.1mmHg, p0.009) in the SP and increased daytime and nighttime DBP(2.0mmHg, p0.00003*; 2.0mmHg, p0.00003*)in the RP . The KCNAB1 SNP was associated with increased clinic SBP(5.5mmHg, p0.04) in the SP and clinic, daytime and nighttime SBP(7.0mmHg p0.0002*; 3.6mmHg, p0.006; 3.5mmHg, p0.005) in the RP aswell as clinic PP(5.9mmHg, p0.001) in the SP and clinic, daytime and nighttime PP(4.0mmHg, p0.003, 2.3mmHg p0.009, 2.5mmHg p0.001*) in the RP . * P-values in RP which exceed the Bonferroni correction for multiple testing. Conclusions: Variants in ion channels CLCN2 and KCNAB have not previously been implicated in the causation of hypertension. These findings indicate strong associations with clinically relevant BP changes; providing new insights into the pathophysiology of BP regulation and potentially pointing to novel drug targets for the treatment of hypertension.

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Common single nucleotide polymorphisms associated with QT interval and sudden cardiac death: the FinSCDgen study. *P.A. Noseworthy[1], V. Salomaa[2], A.S. Havulinna[2], A.M. Lahtinen[3], K. Porthan[4], A. Jula[2], P.J. Karhunen[5], M. Perola[6], K. Kontula[3], C. Newton-Cheh[1,7].* 1) Massachusetts General Hospital, Boston, MA 02114, USA; 2) National Institute for Health and Welfare, Helsinki, Finland; 3) Research Program for Molecular Medicine and Department of Medicine, University of Helsinki, Helsinki, Finland; 4) Department of Cardiology, University of Helsinki, Helsinki, Finland; 5) Department of Forensic Medicine, Tampere University Hospital, Tampere, Finland; 6) Institute of Molecular Medicine FIMM, University of Helsinki, and National Institute for Health and Welfare, Helsinki, Finland; 7) Broad Institute, Cambridge, MA 02114, USA.

Introduction: Sudden cardiac death (SCD) comprises 15% of all deaths in western countries and about 2/3 of all cardiac deaths. Although SCD is heritable, its genetic determinants are poorly characterized. We hypothesized that common genetic variants associated with QT interval, arrhythmias and other cardiac phenotypes, would also be associated with SCD.

Methods: We genotyped 36 common (MAF >1%) candidate SNPs in individuals in 4 cohort studies (FINRISK 1992, 1997, 2002 and Health 2000, total n=26,377), and 2 series of forensic autopsies from Helsinki and Tampere (n= 695). We constructed a QT genotype score using the allele copy number and the effect estimates for the SNPs associated with QT interval in prior studies. Adjudication of the cause of death revealed 717 SCDs. Regression models adjusted for age, sex and geographical area. Risk estimates were pooled using fixed effects meta-analysis using inverse variance weights.

Results: Among 5,769 Health2000 subjects in whom ECGs were available, the QT genotype score predicted the observed QT (p<1E-106); the observed QT interval was associated with SCD (p=0.01). The QT score was weakly associated with increased risk of SCD (p=0.04) in Health2000, but not on meta-analysis of all samples (p=0.10). Analyses of individual common variants revealed 5 SNPs associated with SCD at the p<0.05 level. The strongest associations included rs2200733 on chr4q25, previously related to atrial fibrillation risk, with a relative risk of 1.23 (95% CI 1.06 - 1.42, p= 0.006) per minor allele and rs41312391 in an intron of SCN5A, with RR of 1.25 (95% CI 1.10-1.43, p=0.001).

Conclusions: Our study identified five novel genetic variants associated with the risk of SCD. We confirmed the association between QT genotype score and observed QT interval, but failed to show a relationship between QT genotype score and SCD, possibly due to limited power. Larger studies are warranted to fully define the contribution of common genetic factors, alone or in aggregate, to SCD.

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Screening for copy number variation and sequencing candidate genes associated with conotruncal heart defects. *K. Osoegawa[1,2], D. Iovannisci[1], M. Ladner[1], K. Schultz[1], B. Lin[1], C. Parodi[1], S. White[1], S. Hawbecker[1], A. Borg[3], G. Shaw[4], E. Trachtenberg[1,2], E. Lammer[1].* 1) Research Inst, Childrens Hosp Oakland, Oakland, CA; 2) Center for Applied Genomics, Childrens Hosp Oakland, Oakland, CA; 3) Department of Oncology, Lund University, Sweden; 4) Pediatrics in the Division of Neonatal & Developmental Medicine, Stanford University School of Medicine.

Congenital heart defects are the most common anatomical grouping of human birth defects, yet little is known about their etiologies. Conotruncal defects are an important pathogenetic subset of congenital heart defects, comprising nearly 20 percent. We previously showed that array comparative genomic hybridization (array-CGH) analysis of DNA samples derived from cleft lip and palate subjects was an efficient and productive method for identifying candidate chromosomal loci, complementing traditional genetic mapping strategies. DNA samples from a population-based sample of ~400 California infants born during 1999-2004 with conotruncal defects were screened for chromosomal microdeletions/duplications as an approach to identify candidate genes for conotruncal defects. Previously identified deletion cases at 22q11.21 were confirmed using array-CGH to evaluate the compatibility of the technology with DNA samples from conotruncal defects. Of 220 cases analyzed, we identified one subject with a novel 3.9 Mb duplication at 8p23.1. Deletions overlapping this 8p23 region have been associated with congenital heart defects. Among males, we also identified four subjects who had 47,XYY or mosaic 47,XYY karyotypes. Each was diagnosed as tetralogy of Fallot. In addition to the genome wide screening, we began developing a strategy for identifying micro-deletions/duplications using multiplex ligation-dependent probe amplification (MLPA) and sequencing of 9 candidate genes that are expressed in secondary heart field cells or for which mutations have been reported in a few infants with conotruncal defects. For sequencing, DNA fragments containing exons from these genes have been amplified via PCR, pooled, and sequenced using the Roche 454 GS FLX system. The current technology allows us to obtain ~1 million sequences per run with an average read length of 450 bases. We will compare DNA sequences obtained from children born with conotruncal defects with reference sequences to identify nucleotide changes associated with conotruncal defects.

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Germline GJA1 (Connexin 43), GJA5 (Connexin 40) and GJC1 (Connexin 45) mutations in atrioventricular nodal reentrant tachycardia (AVNRT). *H. Ak Celik[1], C. Hasdemir[2], H.H. Aydin[1]*. 1) Department of Medical Biochemistry, Ege University School of Medicine, Izmir, Turkey; 2) Department of Cardiology, Ege University School of Medicine, Izmir, Turkey.

Connexins are a group of structurally-related transmembrane proteins that assemble to form gap junctions. Multiple connexin types are expressed in heart muscle including Connexin 40 (GJA5), Connexin 43 (GJA1) and Connexin 45 (GJC1). Although, differences in connexin expressions are well documented in acquired adult hearth diseases, their mutations have only rarely been identified. The study population consisted of 4 patients (4 female, age range 37-53 y/o). All patients presented with palpitations. All patients underwent electrophysiologic study and radiofrequency catheter ablation. Transthoracic echocardiography was performed for left/right ventricular function and atrial sizes. All patients had slow/fast type AVNRT. One patient had also nonsustained right atrial tachycardia. All patients had structurally normal heart. We sequenced GJA1, GJA5 and GJC1 genes from genomic DNA isolated from peripheral lymphocytes. Silent and heterozygous missense GJA1 (Connexin 43) mutations were identified in all patients. GJA5 (Connexin 40) mutations were indentified in 3 patients and GJC1 (Connexin 45) mutations were determined only in 2 patients. We hypothesize that atrioventricular nodal reentrant tachycardia has a genetic basis and the genes encoding heart muscle connexins may play role in pathogenesis of AVNRT.

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Gene and protein activation of mitogen activated protein kinases in post-infarcted patients. *R. Akbarzadeh Najar[1], S.M.H. Ghaderian[1], A.S. Tabatabaei Panah[2]*. 1) Department of Medical Genetics, Faculty of Medicine, Shahid Beheshti University of Medical Sciences and Health Services, Tehran, Iran; 2) Department of Biology, Basic Sciences Faculty, Islamic Azad University, East Tehran Branch (Ghiamdasht), Tehran, Iran.

Purpose Remodeling in the failing heart involves several cell signal transduction pathways which are regulated in the heart in direct response to changes in mechanical loading conditions. Activation of mitogen-activated protein kinases (MAPKs) signaling cascade are important pathophysiologic regulators during the development of heart failure (HF). In present study, we designed to monitor the activity of these MAPKs in Iranian patients with acute myocardial infarction (AMI) comparing with controls. Methods The degree of activation (Phosphorylation) of p38 kinase, p44/42 extracellular regulated kinase, and c-Jun N-terminal kinase (JNK1/2) and their corresponding activity levels were analyzed in peripheral blood mononuclear cells (PBMCs) of 258 patients with AMI and 250 normal subjects. The expression of p38α mRNA was determined by real time RT-PCR. These analysis were carried out immediately and 12 hours after AMI. The investigation conforms to the principles outlined in the 1964 Declaration of Helsinki. A P value less than 0.05 was considered statistically significant. Results Activity of p38 and JNK1/2 MAPKs were significantly increased in patients with AMI than controls immediately after infarction which was reduced during 12 hours after AMI. There was statistical significant increase in the ratio of phosphorylated p38:total p38 ($P<0.05$) and also a significant increase in the level of phosphorylation of ATF-2 ($P<0.05$) in the patients than healthy individual. However, there were no statistically differences in activation and activity of p44/42 in the patients and controls. A comparison of the patients with control subjects demonstrated that in AMI, p38α mRNA expression was 3.1-fold and 2.2-fold higher than control samples respectively. Conclusion Results of this study indicate that these MAPKs signaling pathway might be activated by AMI which signal transduction involves kinase phosphorylation and play important roles in their activity. Activation of MAPKs mainly p38 and c-Jun N-terminal kinase are associated with different forms of cardiac pathology such as AMI and their signal transduction involves kinase phosphorylation, which play significant roles in gene expression and protein function. Elevated activity of p38 and JNK1/2 MAPKs suggests that they may potentially play significant roles in HF. These observations are particularly intriguing in light of the fact that these regulating factors are important in pathophysiology of AMI and HF.

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Genetic Determinants of Plasma Apolipoprotein A2 Levels are Associated with Atherosclerotic Burden and Progression in Stable Cardiac Patients. *H. Allayee[1], W.H. Tang[2], J. Hartiala[1], Y. Patel[1], S. Hazen[2,3]*. 1) Dept Preventive Med, Univ Southern California, Los Angeles, CA; 2) Department of Cardiovascular Medicine, Cleveland Clinic, Cleveland, OH; 3) Department of Cell Biology, Cleveland Clinic, Cleveland, OH.

Background: Apolipoprotein A2 (ApoA2), the second most abundant protein on high-density lipoprotein (HDL) particles, has been associated with triglyceride metabolism and insulin resistance in animal models, but its role in humans is unclear. We investigated the genetic relationship of HDL cholesterol-adjusted ApoA2 levels with atherosclerotic disease burden and progression in humans. **Methods:** We carried out genome-wide association study (GWAS) for ApoA2 levels adjusted for HDL with 1 million SNPs in 2367 subjects undergoing elective coronary angiography. SNPs that demonstrated genome-wide significant association ($p<10^{-8}$) with ApoA2 levels were also tested for association with future need for revascularization after three years of follow up. **Results:** In our cohort (age 63±11 years, 66% male), a higher ApoA2 levels, expressed as a ratio to HDL, were associated with increased risk of prevalent cardiovascular disease (odds ratio [OR] 1.54; 95%CI 1.42-1.66, $p<0.001$) and need for future revascularization (HR 1.18; 95%CI 1.13-1.24, $p<0.001$). Five loci located on chromosomes 1, 2, 3, 4, and 10 exhibited significant p-values for adjusted ApoA2 levels ranging from 10^{-9} to 10^{-13}. The SNP on chromosome 1 is located upstream of the ApoA2 structural gene and lowers ApoA2 levels by 1 mg/dl per minor allele copy. Of these five loci, subjects carrying the minor allele for SNPs on chromosome 2p12 (6% frequency) and chromosome 4p16.3 (7% frequency) also demonstrated a 2-fold increased risk in need for future revascularization (both HR 2.0, 95%CI 1.6-2.4, $p<0.0001$), consistent with their effects on raising plasma ApoA2 levels. **Conclusions:** In stable cardiac patients, ApoA2, when analyzed in relation to HDL, provides independent association with CVD and risk for future revascularization. The results from our GWAS also support this notion since SNPs that lead to higher ApoA2 levels are also associated with increased risk for future revascularization.

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The Effect of Survivorship Bias on Cross-Sectional Case-Control Genetic Studies of Highly Lethal Diseases. *C.D. Anderson[1,2,3], M.A. Nalls[4], A. Biffi[1,2,3], N.S. Rost[1,2,3], S.M. Greenberg[2], A.B. Singleton[4], J.F. Meschia[5], J. Rosand[1,2,3]*. 1) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA; 2) Hemorrhagic Stroke Research Group, Department of Neurology, Massachusetts General Hospital, Boston, MA; 3) Program in Medical and Population Genetics, Broad Institute, Cambridge, MA; 4) Laboratory of Neurogenetics, Intramural Research Program, National Institute on Aging, Bethesda, MD; 5) Department of Neurology, Mayo Clinic, Jacksonville, FL.

Background: Survivorship bias is the phenomenon by which individuals are excluded from analysis of a trait because of mortality related to the expression of that trait. In genetic association studies, variants increasing risk for disease onset as well as risk of disease-related mortality (lethality) could be difficult to detect in cross-sectional case-control designs, possibly leading to underestimation of a variant's effect on disease risk.

Methods and Results: We modeled cohorts for three diseases of high lethality (intracerebral hemorrhage, ischemic stroke, and myocardial infarction) using existing longitudinal data. Based on these models, we simulated case-control genetic association studies for genetic risk factors of varying effect sizes, lethality, and minor allele frequencies (MAF). For each disease, erosion of detected effect size was larger for case-control studies of individuals of advanced age (age > 75 years) and/or variants with very high event-associated lethality (Genetic-conferred Relative Risk for event-related death > 2.0). We found that survivorship bias results in no more than 20% effect size erosion for cohorts with mean age < 75 years, even for variants that double lethality risk. Ascertainment of at least 40% of lethal cases in the population further reduces this phenomenon, resulting in < 20% effect size erosion even for cohorts of advanced age with extremely lethal phenotypes. Furthermore, we found that increasing effect size erosion was accompanied by depletion of MAF in the case population, yielding a "signature" of the presence of survivorship bias. Once again, ascertainment of 40% of lethal cases mitigated this MAF depletion.

Conclusion: Our results demonstrate that failure to enroll lethal cases can distort the measured effect sizes for genetic variants affecting both disease incidence and lethality. However, this bias results in less than 20% erosion in observed effect sizes for cohort ages < 75 years, and is further minimized by enrollment of a small portion (40%) of lethal cases in the population. Our simulation provides formulae to allow estimation of effect size erosion given a variant's odds-ratio (OR) of disease, OR of lethality, and MAF. These formulae will add precision to power calculation and replication efforts for case-control genetic studies. Our approach requires validation using prospective data.

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Genomewide studies of Interleukin-6 levels: meta-analysis from the CHARGE Consortium. *J.C. Bis[1], M. Barbalic[2], A. Dehghan[3,16], J. Baumert[4], M.E. Rudock[5], J. Lahti[6], H. Snieder[7], H. Wallaschofski[8], Y. Jamshidi[9], C. Lu[10], R. Tracy[11], C.M. Ballantyne[12], J. Witteman[3,16], W. Koenig[13], Y. Liu[5], J.G. Eriksson[14], B.Z. Alizadeh[7], A. Teumer[15], I.M. Nolte[7], E.J. Benjamin[17], CHARGE Consortium Inflammation Working Group.* 1) Department of Medicine and Cardiovascular Health Research Unit, University of Washington, Seattle, WA, USA; 2) Human Genetics Center, University of Texas Health Science Center at Houston, Houston, TX, USA; 3) Department of Epidemiology, Erasmus Medical Center, Rotterdam, The Netherlands; 4) Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Epidemiology, Neuherberg, Germany; 5) Department of Epidemiology & Prevention, Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem, NC, USA; 6) Institute of Behavioural Sciences, University of Helsinki, Helsinki, Finland; 7) Unit of Genetic Epidemiology & Bioinformatics, Department of Epidemiology, University Medical Center Groningen, University of Groningen, the Netherlands; 8) Institute of Clinical Chemistry and Laboratory Medicine, University of Greifswald, Germany; 9) Division of Clinical Developmental Sciences, St George's University of London, London, UK; 10) Department of Biostatistics, Boston University School of Public Health, Boston, MA, USA; 11) Departments of Pathology and Biochemistry, University of Vermont College of Medicine, Burlington, VT, USA; 12) Department of Medicine, Baylor College of Medicine and Center for Cardiovascular Prevention, Methodist DeBakey Heart and Vascular Center, Houston, TX, USA; 13) Department of Internal Medicine II-Cardiology, University of Ulm Medical Center, Ulm, Germany; 14) National Public Health Institute, Helsinki, Finland and Department of General Practice and Primary Health Care, University of Helsinki, Helsinki, Finland; 15) Interfaculty Institute for Genetics and Functional Genomics, University of Greifswald, Germany; 16) Member of the Netherlands Genomic Initiative (NGI); 17) National Heart, Lung, and Blood Institute's and Boston University's Framingham Heart Study, Framingham, MA, USA.

Background: To identify genetic variants associated with circulating Interleukin-6 (IL-6), a cytokine marker of inflammation and a risk factor for cardiovascular events, we conducted a meta-analysis of genome-wide association data in subjects of European ancestry from ten large studies in the setting of the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium. **Methods:** Participants were from ten community-based studies: the Atherosclerosis Risk in Communities study, the Cardiovascular Health Study, the Framingham Heart Study, Health ABC, the Helsinki Birth Cohort Study, MONICA/KORA, the Netherlands Study of Depression and Anxiety, the Rotterdam Study, the Study of Health in Pomerania, and TwinsUK. Each study used whole genome genotype information to impute to HapMap's CEU panel and employed regression models to relate ~2.5 million single nucleotide polymorphisms (SNPs) to log-transformed IL-6 levels. We then combined study-specific findings in a fixed-effects meta-analysis including up to 20,084 individuals. **Results:** We identified 70 SNPs at two genomic loci whose p-values for association with IL-6 levels surpassed our genome-wide significance threshold corresponding to one expected false positive ($p < 4.0\times10^{-7}$). On chromosome 1, we identified associations with 68 SNPs within and around the gene encoding the IL-6 receptor ($p = 5.3\times10^{-40}$), a part of a protein complex through which IL-6 exerts its actions. Additionally, we identified an association for 2 SNPs on chromosome 6 near the Notch homolog 4 gene ($p = 6.2\times10^{-8}$), whose product is part of an evolutionarily conserved intercellular signaling pathway. **Conclusions:** Our community-based genome-wide association meta-analysis identified two regions that were associated with circulating IL-6 levels. One, in the cytokine's receptor, confirms previous studies. The other, in a region of chromosome 6, may highlight other inflammation-related pathways of importance.

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Replication study of plasma lipoprotein levels-associated SNPs identified in recent GWAS. *E.K. Bryant[1], A.S. Dressen[1], J.E. Hokanson[2], R.F. Hamman[2], C.M. Kammerer[1], M.I. Kamboh[1], F.Y. Demirci[1].* 1) Human Genetics, GSPH, Univ Pittsburgh, Pittsburgh, PA; 2) Epidemiology, Colorado School of Public Health, Univ Colorado Denver, Aurora, CO.

Genome-wide association studies (GWAS) have identified a number of genes/SNPs associated with plasma total cholesterol (T-C), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), and triglyceride (TG) levels. Most of the previously established lipid genes also confirmed by these GWAS are being comprehensively evaluated by our group using deep sequencing based-SNP discovery strategy. The purpose of this study was to test the replication of GWAS signals from other genes that were not targeted by our sequencing effort in two epidemiological samples, U.S. Non-Hispanic Whites (NHWs, *n*=623) and Hispanics (*n*=410). For each SNP, the association analysis was performed with all lipoproteins regardless of previously reported specific associations. To date nine SNPs have been genotyped and the screening of additional SNPs is underway. Six SNPs showed significant associations with one or more lipoprotein traits among NHWs and the strongest associations ($P<0.01$ after adjusting for gender, age, BMI, smoking) were: *GRIN3A*/rs1323432 with T-C ($P=0.002$) and LDL-C ($P=0.009$), *NUTF2*/rs2271293 with HDL-C ($P=0.009$), and *DOCK7*/rs10889353 ($P=5\text{x}10^{-5}$) & *GALNT2*/rs2144300 ($P=0.006$) with TG. Among Hispanics, the associations were either weaker ($P=0.004$ for rs10889353 with TG) or non-significant but with a similar trend (rs1323432, rs2271293, and rs2144300), probably partly due to the smaller sample size. Ongoing efforts towards defining the spectrum of lipoprotein traits-associated genetic loci/variants and their consistent replication by independent groups across various ethnic populations will help to improve our understanding of genetic regulation of these traits and will likely guide new therapeutic interventions.

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A Genome-wide Copy Number Variation association study of Young-Onset Hypertension in Han Chinese Population of Taiwan. *K.M. Chiang[1,3], H.C. Yang[2], Y.J. Liang[2], W.H. Pan[1].* 1) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 2) Institute of Statistical Sciences, Academia Sinica, Taipei, Taiwan; 3) Graduate Institute of Life Sciences, National Defense Medical Center, Taipei, Taiwan.

Hypertension is a common and complex disorder. Although many large scale genome-wide association studies of SNPs have been performed, only a few studies have successfully identified the loci that are related to the hypertension, not to mention the scanty Asian studies. Besides single nucleotide polymorphisms (SNPs), copy number variation (CNV) is now being known as an important role in genetic susceptibility to common disease. Young-onset hypertension (YOH) may be a more feasible target disorder to investigate than the late-onset one due to its stronger genetic component. To address this issue, we performed a genome-wide study of association between CNVs and Young onset hypertension. Using the Illumina HumanHap550 BeadChips which contains nearly 4,300 SNPs in approximately 500 reported copy number variant regions of the genome, we typed 400 YOH cases and 400 age and gender matched controls. CNV regions were identified by PennCNV which implements a hidden Markov model (HMM) that integrates multiple sources of information to infer CNV calls for individual genotyped samples. The CNV calls which contain more than 10 SNPs and larger than 50kb were used to analyze. Two different analyses, regional association test and the association tests with sliding-window procedure, were used to identify potential CNVs. We identified some CNVs on the chromosome 1p36.3, 4p16.3, 9q34.3, 11q11, 14q32.33, 16p13.3, 16q24.3, 19p13.3 and 19q13.31, seemed to be associated with YOH. Changes in the copy number level of dosage-sensitive gene may alter the gene dosage. All of the expression levels of the genes which were spanned by these CNVs are currently being tested with the 400 YOH case-control pairs.

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Clinical Diagnosis of Marfan Syndrome using an electronic guideline. *J. Dean, A. Ross, C. Brown.* Medical Genetics, University of Aberdeen, Aberdeen, United Kingdom.

The International Nosology for the diagnosis of Marfan Syndrome published in 1996 has become the gold standard for making the diagnosis. The Scottish Marfan Clinical Guideline facilitates use of this nosology in the clinic. In recent years, the discovery of Marfan like disorders such as the Loeys-Dietz syndrome has added weight to the need for a revision of the nosology to take account of these disorders and of increasing knowledge about the clinical spectrum of the fibrillinopathies. The development of targeted treatments such as ARBs currently under trial has also increased the need for accurate diagnosis. A clinical Marfan database has been devised which implements the Scottish Marfan Guideline electronically. Using this database, we have compared the outcome of clinical assessment of the Aberdeen Marfan clinic patients using the International nosology with the results of molecular testing of fibrillin-1, TGFBR1 and TGFBR2. There is a close but not complete correlation between clinical diagnosis and molecular diagnosis. The discrepancy is attributable to several factors including the well known reduced effectiveness of the nosology in children, the difficulty of confirming the clinical features in relatives when family history is needed as a diagnostic criterion, the non-specificity of dural ectasia in connective tissue disorders, and the existence of patients with mild disease and fibrillin mutation. The information presented may help to inform the diagnostic process and the revision of the international nosology.

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Identification of novel MYBPC3 gene mutations associated with hypertrophic cardiomyopathy. *J-H. Ding[1], W-J. Yin[2], S. Greene[2], N. Akilla[2], B-Z. Yang[1], P.A. Grayburn[3].* 1) Institute of Metabolic Disease, Baylor Research Institute, Dallas, TX; 2) Institute of Biomedical Studies, Baylor University, Waco, TX; 3) Institute of Cardiovascular Diseases, Baylor University Medical Center, Dallas, TX.

Hypertrophic cardiomyopathy (HCM), an autosomal dominant disorder, represents the most common inherited cardiovascular disorder with a prevalence of 1/500 in the general population. HCM manifests a wide range of clinical features and is the primary cause of sudden cardiac death in young adults. Sarcomeric gene mutations are a well known cause of HCM in both adults and children. More than 450 different pathogenic mutations in at least 17 genes have been identified with large allelic and genetic heterogeneity. The two most frequently mutated genes are the MYBPC3 gene (OMIM #600958) and the MYH7 gene (OMIM #160760). Here we report two novel mutations in two unrelated patients with HCM. Case 1 is a 46-year old Middle Eastern man with severe asymmetric septal hypertrophy (septal thickness 3.3 cm) on echocardiography but no resting outflow tract gradient. He has no family history of sudden death or heart failure. To investigate the molecular defect, genomic DNA was extracted from blood and all exons and flanking intronic regions of MYH7 and MYBPC3 genes were amplified. The PCR products were purified and sequenced. Sequence analysis revealed that the patient is a homozygous for an A-to-G nucleotide change at position 935 in exon 13, which leads to p.K312R mutation in the MYBPC3 gene. This novel mutation was verified by a PCR/restriction test, but was not detected in the normal control subjects. Case 2 is a 53-year old Asian man who underwent implantation of a cardiac defibrillator after being resuscitated from sudden cardiac death. His echocardiogram showed mild asymmetric septal hypertrophy with no resting outflow tract gradient. His DNA sequencing revealed a single base-pair deletion at position 453 in exon 5 (c.453delT), resulting in a frameshift at code 151 (p.Asp151GlufsX8) in MYBPC3. Subsequent subcloning and sequencing also confirmed that the patient was a heterozygous for this novel deletion. Our study suggests that despite the numerous reports of mutations in the MYBPC3 gene, there exist novel mutations in HCM patients of non-European ethnic origins. Further studies will improve our understanding of genetic causes in HCM patients.

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Genes for Aging-Dependent Atherosclerosis. *J.R. Dungan[1], A.S. Allen[2], S.H. Shah[3,4], W.E. Kraus[4], E.R. Hauser[3].* 1) Sch Nursing, Duke Univ, Durham, NC; 2) Biostatistics & Bioinformatics, Duke Univ, Durham, NC; 3) Center for Human Genetics, Duke Univ, Durham, NC; 4) Dept of Medicine (Cardiology), Duke University, Durham, NC.

Atherosclerosis is a disease of aging; prevalence and mortality increase with age. The aging process contributes to known cardiovascular effects leading to CAD. Biomarker risk factors for CAD also show age-dependent distributions. We hypothesized that some candidate genes for CAD may also be implicated in aging-dependent atherosclerosis. Our primary aim was to examine statistical interactions between age and CAD candidate genes to generate formal evidence of genetic effects mediated by age. We performed a secondary analysis of 598 SNPs for 498 previously genotyped CAD candidate genes in 1,885 subjects in the Duke Catheterization Genetics (CATHGEN) case-control study. Participants were recruited sequentially through the cardiac catheterization laboratories at Duke University Hospital (Durham, NC, USA). SNPs were genotyped using either TaqMan or Illumina BeadArray. We evaluated age-by-genotype interactions in a gene association model of CAD severity, as quantified by the CAD index, a numerical summery of coronary angiographic data that incorporates extent and anatomical distribution of coronary disease. We fitted a weighted regression model with CAD index as the dependent variable and known CAD risk factors, age at Duke catheterization, genotype (dominant model) and an age*genotype interaction term, with sample weights as implemented in PROC SURVEYREG (SAS 9.2, Cary, NC) to control for the CATHGEN case-control sampling scheme. We defined age as age at cardiac catheterization as this was subject's age at the time the CAD index variable was determined on cath. In addition, we calculated moving average plots of minor allele frequencies (MAF) across age, incorporating both sampling and kernel weights, in order to visualize the unique distributions of MAF across age. We report eight SNPs corresponding to eight different genes that had significant age*genotype interactions. After Bonferroni correction for 598 tests, three SNPs remained highly significant: RS12528807 (PLA2G7 gene); RS12610791 (SPINT2 gene); and, RS1800206 (PPARA gene). Variants in these genes have previously been implicated in cardiovascular, metabolic, and aging phenotypes. Eight SNPs represent candidate genes for aging-specific CAD progression. Finding genes for cardiovascular aging are important to improve care of older people with heart disease. Characterizing the influence of age interactions with gene associations is also critical to refining gene associations with CAD.

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Identifying mitral valve prolapse mutations on chromosomes 11 and 13 using next-generation sequencing. *R. Durst[1,2], M. Leyne[1], RA. Levine[2], SA. Slagenhaupt[1] on behalf of the Leducq Mitral Consortium.* 1) CHGR, MGH, Boston, MA; 2) Cardiology Division, Massachusetts General Hospital, Boston, MA.

Mitral valve prolapse (MVP) is a common cardiac disorder that exhibits a strong hereditary component. Twenty percent of MVP patients will develop severe complications, including congestive heart failure, endocarditis, atrial arrhythmias, embolic events and even sudden death. Surprisingly, very little is known about the developmental etiology of MVP. To date, only mutations in FLNA have been shown to cause non-syndromic MVP. Previously, we identified two MVP loci using genetic linkage analysis in large families: MMVP2 on chromosome 11p15.4 and MMVP3 on chromosome 13q31.3-32.1. We were recently awarded an R102 NHLBI Resequencing and Genotyping Service grant that allowed us to capture and sequence these loci. All sequencing was performed at the Venter Institute. A DNA library of the two loci was prepared using SureSelect technology. After excluding repeat elements we targeted ~50% of each locus. Four individuals from each family, who share only the disease allele, were selected for sequencing. Sequencing was performed on the Solexa sequencer. 97% of the targeted area was sequenced and the average coverage was 310X. Single nucleotide polymorphisms (SNPs) were identified and characterized as potential mutations if they were 1) shared by all four individuals in the family and 2) not present in dbSNP or other public databases. After analyzing familial sharing, there were 155 potential mutations on chromosome 11 and 308 on chromosome 13. These potential mutations were prioritized and evaluated in the following order: 1) coding sequence changes, 2) promotor or splice site changes, and 3) changes in evolutionary conserved regions. Using these criteria, we identified several potential missense mutations in the MMVP2 locus. There are no coding changes in the MMVP3 locus, but there are 25 changes in highly conserved regions adjacent to three genes, GPC6, GPC5 and HS6ST3. We are currently evaluating these mutations for potential function and initiating sequencing in a large cohort of familial and sporadic MVP patients in order to identify additional mutations.

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GALNT2, a novel GWAS locus associated with HDL-C, does not alter HDL catabolism. *A.C. Edmondson[1], A. Raghavan[1], J.S. Millar[1], S.A. Khetarpal[1], S. Kathiresan[2], D.J. Rader[1].* 1) Institute of Translational Medicine and Therapeutics, University of Pennsylvania, Philadelphia, PA; 2) Broad Institute and Massachusetts General Hospital, Boston, MA.

Genome wide association studies (GWAS) for quantitative traits, such as blood lipid concentrations, have successfully identified many genes previously implicated in rare Mendelian disorders of lipoprotein metabolism and have also mapped many novel loci. One such novel locus for plasma high density lipoprotein cholesterol (HDL-C) is on chromosome 1 and contains the GALNT2 gene with the most significant SNP located in the first GALNT2 intron. GALNT2 is involved in the first step of O-linked glycosylation of proteins and was never previously linked to HDL metabolism. Hepatic overexpression of GALNT2 decrease HDL-C and knock-down of endogenous mGALNT2 increases HDL-C. However, the mechanism of HDL-C modulation by GALNT2 is unknown. To elucidate this mechanism, mice were injected with human HDL radiolabeled on the protein and cholesteryl ester moieties and kinetic parameters were studied in WT mice and mice overexpressing GALNT2. The mice overexpressing GALNT2 had an approximate 25% decrease in HDL total cholesterol (P = 0.002), reflecting decreased free cholesterol (P=0.004) and decreased cholesterol ester (P=0.002), but an unaltered cholesterol ester/free cholesterol ratio (P=0.603) suggesting normal cholesterol esterification. GALNT2 overexpressing mice did not exhibit significantly altered ApoA-I protein concentration (P=0.10), ApoA-I production rate (P=0.12), and did not have a change in fractional catabolic rate of protein (P=0.68) or cholesteryl ester (P=0.235). GALNT2 overexpressing mice did have a decreased HDL-C/ApoA-I ratio (P=0.0006). There were no major alterations in tissue uptake of HDL cholesterol or protein in GALNT2 overexpressing mice. These results suggest that GALNT2 may affect HDL-C production, rather than catabolism.

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Heritability and Linkage of Left Ventricular Diastolic Filling Velocity in the Jackson Heart Study. *L. Ekunwe[1], E. Fox[2], S.G. Buxbaum[1].* 1) Jackson Heart Study, Jackson State University, Jackson, MS; 2) University of Mississippi Medical Center, Jackson, MS.

Background: Left ventricular diastolic filling velocity ratio is frequently used to define diastolic function and is predictive of cardiovascular morbidity and mortality. In other words, it can be thought of as an endophenotype for Coronary Heart Disease (CHD). Other studies have shown a genetic link between diastolic function and its relationship to coronary heart disease. This study's aim is to determine if there is a heritable component to diastolic function with evidence of linkage. The prevalence of diastolic dysfunction is high in this cohort and therefore it is of interest to determine whether there are genetic factors underlying this trait in this African American population sample.

Methods: Three measures of diastolic function were measured: Mitral valve A and E wave velocity, and the ratio of valve A to valve E. These were adjusted for age, sex, body mass Index (BMI) and waist circumference (WC) using SAS PROC GLM. Heritability (h^2) of the residuals was determined using ASSOC in S.A.G.E. Subsequently, multipoint model-free linkage analysis was performed in SIBPAL in S.A.G.E.

Results: Heritability of mitral valve E (E), mitral valve A (A), and ratio of E/A (E/A) were 18, 21%, and 16%, respectively. Suggestive evidence for linkage of both E and A wave velocities were found at the same locus on chromosome 1 at or near microsatellite marker D1S3721 (LOD 1.83 at 72.6 cM and 2.45 at 76 cM, respectively). Strong evidence for linkage was found for E/A (LOD 3.60), however, this was at a different locus on chromosome 1, at 274 cM near D1S1609. Strong evidence of linkage for E/A was also found on chromosome 3p26 (LOD 3.62)and 18p21.2 (LOD 4.08). A LOD of 5.2 was found on 3q26.1 for E wave.

Conclusion: Each of these endophenotypes for CHD were moderately heritable. Evidence for linkage was found on chromosome 18 and at two loci on chromosomes 1 and 3.

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A novel missense mutation M185V in the TAZ gene associated with atypical Barth syndrome. *Y. Fan[1], R. Chang[2, 3], M. Fox[4], B.A. Westerfield[1], J. Steller[3], A.S. Batra[3], R.Y. Wang[2,3], K. Dipple[4], N. Gallant[4], L.S. Pena[1], H. Wang[1], E.R. McCabe[4], V.E. Kimonis[3].* 1) John Welsh Cardiovascular Diagnostic Laboratory, Section of Cardiology, Department of Pediatrics, Texas Children's Hospital, Baylor College of Medicine, Houston, TX; 2) Division of Metabolic Disorders, Children's Hospital of Orange County, Orange County, CA; 3) Children's Hospital of Orange County and Department of Pediatrics, University of California Irvine, CA; 4) Department of Pediatrics, University of California Los Angeles, CA.

Barth syndrome is an X-linked recessive disorder characterized by dilated cardiomyopathy, neutropenia, 3-methylglutaconic aciduria, abnormal mitochondria, variably expressed skeletal myopathy, and short stature. The disorder is caused by mutations in the tafazzin (TAZ/G4.5) gene located on Xq28, as first described by Bione et. al. in 1996. Alternative splicing of the gene yields several different proteins, the functions of which remain largely uncharacterized. Sequencing of the TAZ gene is currently the most reliable diagnostic approach for Barth syndrome. We describe an Irish/German family in which the 4-month-old proband presented with respiratory distress, neutropenia and dilated cardiomyopathy with reduced ejection fraction of 10% by echocardiogram. 3-methylglutaconic aciduria was not detected on three urine organic acid analyses in the proband. Family history indicated that his maternal uncle died of endocardial fibroelastosis and dilated cardiomyopathy at the age of 26 months. A novel missense mutation 553A>G (M185V) in exon 7 of the TAZ gene was identified in the proband through bidirectional sequencing. Family studies revealed that the proband's mother, maternal aunt and grandmother carry the same missense mutation. Bioinformatic analysis predicted that this sequence alteration is deleterious by PolyPhen-2 algorithm. This missense mutation was not present in 115 X chromosomes from 81 ethnically-matched control subjects (47 males and 34 females). The identification of TAZ gene mutations is important for the diagnosis and genetic counseling in this family with atypical Barth syndrome that is not associated with 3-methylglutaconic aciduria.

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***ATP1B1*, *RGS5* and *SELE* polymorphisms and association with hypertension in African Americans.** *M.U. Faruque[1], G. Chen[2], A. Doumatey[2], H. Huang[2], J. Zhou[2], G.M. Dunston[1], C.N. Rotimi[2], A.A. Adeyemo[2].* 1) National Human Genome Center, Howard University College of Medicine, Washington, DC; 2) Center for Research on Genomics and Global Health, National Institutes of Health, Bethesda, MD.

Background and Purpose: Hypertension is a global public-health burden because of its high incidence and concomitant risks of cardiovascular and kidney diseases, leading to significant mortality and morbidity worldwide. It is known to disproportionately affect populations of African ancestry compared to their European counterparts. In addition to other factors, genetics is thought to play a role in the disease-associated disparity. While increasing number of hypertension associated genetic variants are being reported, few have been replicated in independent studies. Association of chromosome 1q with blood pressure (BP) related phenotypes have been reported in independent linkage studies. Animal studies have showed evidence of linkage of BP-related QTLs in mouse and rat to human 1q syntenic chromosomal loci. Several genes in this hypertension linkage region, including *ATP1B1*, *RGS5* and *SELE*, have been reported to be associated with hypertension. We examined the genetic role of these three genes in hypertension development in African Americans. Materials and Methods: We examined 87 tag single nucleotide polymorphisms (tSNPs) from the *ATP1B1*, *RGS5* and *SELE* genes in a well-characterized cohort of 968 African Americans from Washington D.C. metropolitan area. Single SNP and haplotype association testing was done under an additive genetic model and adjusting for age, gender, body mass index and ancestry-by-genotype (principal components). Results: A total of 12 SNPs were found associated with hypertension and associated traits at a replication p-value < 0.05. The strongest signal for hypertension was for rs2815272 in the *RGS5* gene ($p = 9.3X10^{-3}$). For systolic blood pressure (SBP), rs3917420 in the *SELE* gene ($p = 9.0X10^{-4}$) and rs4657251 in the *RGS5* gene ($p = 9.7X10^{-3}$) were the top hits. Individual variants in these genes had phenotypic effects ranging from -2.2 to 7.2 mm Hg for SBP and -2.0 to 2.8 mm Hg for diastolic blood pressure (DBP). One *SELE* gene haplotype, (CCAGC: rs4656701-rs4363475-rs12038818-rs4786-rs5368) showed statistically significant association with SBP after correction for multiple testing ($p < 0.01$). Conclusions: These findings further confirm the genetic role of *ATP1B1*, *RGS5* and *SELE* in hypertension. Given their physiological role in blood pressure regulation, further studies are warranted to examine the functional significance of this genetic finding.

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Generalization of GWAS-based genetic effects for blood pressure: The Strong Heart Family Study. *N. Franceschini[1], V.S. Voruganti[2], S. Laston[2], K. Haack[2], E.T. Lee[3], L.G. Best[4], J.W. MacCluer[2], J.G. Umans[5], T.D. Dyer[2], S.A. Cole[2], K.E. North[1,6].* 1) Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, NC; 2) Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX; 3) University of Oklahoma Health Sciences Center, Oklahoma City, OK; 4) Missouri Breaks Industries Research Inc, Timber Lake, SD; 5) Medstar Research Institute, Washington, DC; 6) Carolina Center for Genome Sciences, University of North Carolina, Chapel Hill, NC.

Hypertension is one of the leading causes of morbidity and mortality in the US and disproportionately prevalent among minority populations. Whereas recent genome wide association (GWA) studies have identified several loci associated with blood pressure (BP) traits, the impact of these variants in populations of different ancestral backgrounds is largely unknown. The purpose of the current study was to identify genetic loci influencing BP traits in a biomedically understudied minority population, American Indians. We selected 43 recently identified GWA single nucleotide polymorphisms (SNPs) in 23 loci for genotyping in 3807 American Indian participants of the Strong Heart Family Study (three centers: Arizona, Dakotas and Oklahoma). We applied standard quality control to the genotyped data (call rate>90%). One SNP failed (rs16998073 in the *FGF5* gene) and the remaining 42 were considered further. Center-specific BP residuals were obtained from linear regression models adjusted for age, sex, age^2 and age-by-sex interaction. We then performed an inverse normalization of the center-specific residuals which were used as phenotypes. The residuals were regressed onto SNP dosage using variance component models to account for family relatedness and population history. Summary estimates across centers were combined using a weighted average of point estimates meta-analyses (fixed effects). Interestingly, notable differences in allele frequencies across centers were observed. Six SNPs in five loci were associated with systolic BP (alpha= 0.05): rs9815354 in *ULK4* (p=0.03), rs10491334 in *CAMK4* (p=0.02), rs11191548 near *NT5C2* (p=0.009), rs381815 and rs11024074 in *PLEKHA7* (p=0.02 both) and rs2681492 in *ATP2B1* (p=0.02). In addition, several of the SNPs had effects that were in the correct direction although with p values between 0.05 and 0.20, due to the inherent small effects sizes of these variants and the limited sample size. These findings suggest some genetic susceptibility similarities to BP related traits across European and American Indian populations. Future work in this population should fine map these regions as trans-racial mapping may narrow the implicated regions and help identify the underlying functional alleles.

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Replication of genetic association studies in calcific aortic valve stenosis. *N. Gaudreault[1], V. Ducharme[1], M. Lamontagne[1], P. Mathieu[1], P. Pibarot[1], Y. Bossé[1,2].* 1) Centre de recherche Institut universitaire de cardiologie et pneumologie de Québec, Laval University, Quebec, Canada; 2) Laval University Hospital Research Center (CRCHUL), Quebec, Canada.

Calcific aortic valve stenosis (AVS) is a life-threatening disease of the aortic valve with currently no medical treatment available. The incidence of AVS increases with age and is thus a growing public health problem. Previous genetic studies suggest an important genetic component. However, only a handful of studies have attempted to unravel the genetic architecture of AVS. The goal of this study is to validate genes previously associated with AVS. Seven genes were assessed including APOB, APOE, CTGF, IL10, PTH, TGFB1, and VDR. For each gene, we selected SNPs previously associated with AVS and complement with a maximally informative set of common SNPs (tagSNPs) using the European-derived (CEU) HapMap dataset. SNPs were genotyped using the Illumina® BeadXpress platform in 467 patients that underwent surgical aortic valve replacement. These patients were compared to 3294 controls taken from the Illumina® iControlDB genotyped on the HumanHap550 genotyping BeadChip. SNPs not genotyped in controls were imputed using the MACH program and a reference set consisting of the known phased haplotypes of 60 unrelated individuals in the HapMap CEU. Significant p-value thresholds were applied for each gene using the Bonferroni correction. A missense mutation in the APOB gene was significantly associated with AVS (rs1042031, E4181K, p = 1.4E-5). A second SNP located 5.6 kb upstream of the APOB stop codon was also associated with the disease (rs6725189, p = 2.0E-5). Six SNPs surrounding the IL10 locus were strongly associated with AVS (0.006 > p > 8.4E-11). The most compelling association for IL10 was found with a promoter polymorphism (rs1800872) well-known to regulate the production of the encoded anti-inflammatory cytokine. The frequency of the low-producing allele was greater in cases compared to controls (30% vs. 20%, p = 8.4E-11). SNPs in PTH, TGFB1, and VDR had nominal p-values < 0.05, but did not resist Bonferroni correction. This study suggests that individuals carrying specific polymorphisms in the IL10 and APOB genes are at higher risk of developing AVS.

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Tumor necrosis factor-α: investigation of gene polymorphism and regulation of TACE-TNF-α system in patients with acute myocardial infarction. *S.M.H. Ghaderian[1], R. Akbarzadeh Najar[1], A.S. Tabatabaei Panah[2].* 1) Department of Medical Genetics, Faculty of Medicine, Shahid Beheshti University of Medical Sciences and Health Services, Tehran, Iran; 2) Department of Biology, Basic Sciences Faculty, Islamic Azad University, East Tehran Branch (Ghiamdasht), Tehran, Iran.

Purpose Tumor necrosis factor-α (TNF-α) plays an important role in the pathophysiological process of acute myocardial infarction (AMI). A polymorphism within TNF-α gene promoter and contribution of TNF-α converting enzyme (TACE) have been reported to be associated with TNF-α production which may increase susceptibility to heart failure such as AMI. However, the relationship between this polymorphism and susceptibility to AMI and the mechanism of TACE-TNF-α system regulation has poorly been studied. Methods Genomic DNA and peripheral blood mononuclear cells (PBMCs) of 996 patients with AMI and 510 control subjects were extracted within first 24 hours after the onset of AMI. The investigation conforms to the principles outlined in the 1964 Declaration of Helsinki. The -308 G/A TNF-α polymorphism was detected. The mRNA transcription and protein expression levels of TNF-α and TACE were analyzed by real time RT-PCR and flow cytometry respectively as well as plasma TNF-α by ELISA. Intracellular TACE and TNF-α levels were measured using median fluorescence intensity (MFI). A P value less than 0.05 was considered statistically significant. Results The 'A' allele frequency of TNF-α was significantly more frequent in the patients than controls (19.2% vs. 11.5% respectively, P<0.001). Differences in circulating levels of TNF-α were significantly higher in the patients comparing with controls (17.59±0.01 via 11.78±0.02 respectively, P<0.001). The TNF-α and TACE mRNA and protein levels were higher in the patients than controls [(TNF-α/GAPDH, 2.71±0.03 via 1.73±0.02, P<0.001; TACE/GAPDH, 3.21±0.04 via 2.78±0.11, P<0.001) (TNF-α MFI levels: 3.51±0.06 vs. 2.93±0.02, P<0.001; TACE MFI levels: 3.84±0.06 vs. 3.46±0.01, P<0.001) respectively]. There were significant positive correlation between these mRNA and protein expression levels (r=0.66, P<0.001, r=0.78, P<0.001 respectively). Conclusion These data suggest that genetic polymorphism in TNF-α might be helpful for determining susceptibility to AMI in Iranian patients. The TACE-TNF-α system in circulating leucocytes is stimulated which demonstrate that in patients with AMI, TACE expression in PBMC increases with TNF-α expression and processing of TNF-α in PBMC might be regulated by TACE at transcriptional, translational, and post-translational levels in AMI. In this regard, the TACE, an important regulator of TNF-α maturation, could be used as a potential target for inhibition of a cellular source of TNF-α in AMI.

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Genome-wide meta-analyses of SNP by environmental factor interactions on echocardiographic traits: a CHARGE-EchoGen study. *N.L. Glazer[1], J.F. Felix[5], M. Dörr[3], M-H. Chen[8,18], R. Schmidt[12], A. Schillert[15], J.I. Rotter[2], A.A. Hicks[9], P.P. Pramstaller[9,10,11], A. Pfeufer[6], S. Kääb[7], E. Bisping[14], B. Pieske[14], T. Lumley[16], D. Arnett[4], J.C.M. Witteman[5], S.B. Felix[3], H. Schmidt[13], P. Wild[17], R.S. Vasan[8, 19], CHARGE-EchoGen Consortium.* 1) Cardiovascular Health Research Unit and Department of Medicine, University of Washington, Seattle, WA, USA; 2) Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA; 3) Institute for Community Medicine SHIP/ Clinical-Epidemiological Research Greifswald, Germany; 4) University of Alabama at Birmingham Department of Epidemiology, Birmingham, AL, USA; 5) Department of Epidemiology, Erasmus MC, Rotterdam, the Netherlands; Member of the Netherlands Consortium on Healthy Aging; 6) Institute of Human Genetics, TU Munich and HMGU Munich, Germany; 7) Med. Klinik, Klinikum der Universität München - Grosshadern, Munich, Germany; 8) The National Heart Lung and Blood Institute's Framingham Heart Study, Framingham, MA, USA; 9) Institute of Genetic Medicine, European Academy Bozen/Bolzano (EURAC), Bolzano, Italy (Affiliated Institute of the University of Lübeck, Lübeck, Germany); 10) Department of Neurology, General Central Hospital, Bolzano, Italy; 11) Department of Neurology, University of Lübeck, Lübeck, Germany; 12) Department of Neurology, Medical University Graz, Austria; 13) Institute of Molecular Biology and Biochemistry, Medical University Graz, Austria; 14) Department of Pneumology and Cardiology, University Medicine Göttingen, Germany; 15) Institut für Medizinische Biometrie und Statistik, Universität zu Lübeck, Universitätsklinikum Schleswig-Holstein, Campus Lübeck, Lübeck, Germany; 16) Department of Biostatistics, University of Washington, Seattle, WA, USA; 17) Department of Medicine 2, University Medical Center Mainz, Germany; 18) Department of Neurology, Boston University School of Medicine, Boston, MA, USA; 19) Department of Medicine, Preventive Medicine and Cardiology Sections, Boston University School of Medicine, Boston, MA, USA.

Many common, complex traits are believed to be a result of the combined effect of genes, environmental factors, and their interactions. Echocardiographic measures of left ventricular (LV) structure and function are heritable phenotypes of cardiovascular disease. In a previous genome-wide association (GWA) study of echocardiographic traits, we identified 5 genetic loci harboring common single nucleotide polymorphism (SNP) variants that were associated with variation in LV diastolic dimensions and aortic root size. These variants' main effects explained a small proportion of trait variance. We conducted a genome-wide study of gene-environment interaction in population-based cohort studies participating in the EchoGen and Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortia. Our aim was to identify genetic loci that may interact with environmental factors (EF) to effect echocardiographic traits. Within each of 6 community-based cohorts (N=17,000 individuals of European ancestry), we conducted SNP x EF interaction regressions, using a 1-df test for each of the approximately 2.5 million SNPs across the genome (imputed to the HapMap CEU panel). The echocardiographic outcomes were LV mass, internal dimensions, wall thickness, fractional shortening, aortic root and left atrial size; the environmental factors investigated were age, sex, height and weight. Using a pre-specified P value threshold of 5x10-7, we performed an inverse variance-weighted fixed-effects meta-analysis of GWA data from each cohort. In total, 51 genetic loci met the P-value threshold, 10 of which had a minor allele frequency (MAF) >5%. We observed significant interactions on aortic root size with 1 locus each for age, height and weight, 1 locus with weight on LV mass, 1 locus with height on LV wall thickness, 1 locus with sex on LV internal dimensions, 1 each interacting with sex and weight on fractional shortening, and 1 each interacting with sex and weight on left atrial size. We identified 10 genetic loci harboring common variants that may interact with EFs to influence cardiac structure and function. These findings are novel and are different from loci currently known to have main effects on these traits. Replication studies are needed to confirm; if replicated, these findings warrant further investigation into their functional significance and possible relationship to clinical cardiovascular disease.

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Identification of Novel Locus for Familial Thoracic Aortic Aneurysms and Dissections. *D. Guo[1], C. Minn[1], V. Tran-Fadulu[1], E. Regalado[1], J. Coney[1], J. Cao[1], M. Wang[1], R. Yu[2], S. Shete[2.], D. Milewicz[1].* 1) Univ Texas/Houston Med Sch, Houston, TX; 2) Univ Texas/MD Anderson Cancer Center, Houston, TX.

Thoracic aortic aneurysms typically enlarge over time and present as acute aortic dissections (termed TAAD), lead to sudden death. Approximately 20% of patients with TAAD have an affected first-degree relative, suggesting a significant genetic basis to the disease. A large family (TAA254) was identified with an autosomal dominant inheritance pattern of TAAD, with decreased penetrance in women. The aortic disease in TAA254 is relatively stable, with a low risk of dissection compared to previously reported TAAD loci and gene mutations. . Of the nine affected family members diagnosed with ascending aortic aneurysms (ranging from 4.2 to 6.6 cm, with average aortic diameter of 4.8 ± 0.7 cm), only one individual presented with an acute dissection, with an aortic diameter of 6.6 cm. To identify the defected gene for TAAD in this family, SNP-based whole genome linkage analysis was performed, followed with microsatellite fine-mapping. A novel TAAD locus has been identified for the defective gene in this family on chromosome 12, termed TAAD5 locus, with a maximum parametric LOD score of 2.7 and a maximum non-parametric LOD of 3.6 (exact NPL P-value =0.01). Interestingly, four women in the family over 40 years of age (average age of 55.3 ± 20.2 years old) and carrying the affected disease haplotype did not have TAAD, confirming decreased penetrance of this disease in women. Among nine males who were older than 30 years of age (average age of diagnosis is 52.6 ± 13.4 years of age) with the affected haplotype, only one of them had a type A dissection. Our data support the hypothesis that the defective gene causing TAAD in this family is associated with ascending aneurysms having a low risk of dissection. This is in contrast to other TAAD genes, such as TGFBR2, in which there is a high risk of dissection with minimal dilatation. Therefore, this family provides further evidence that understanding the underlying genetic defects causing TAAD can impact the management of families with familial TAAD.

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The role of *CAV3*, coding for caveolin-3, in long QT syndrome, Brugada syndrome and sudden adult death syndrome. *P. Hedley[1], J. Kanters[2], E. Behr[3], V. Corfield[4], W. McKenna[5], M. Christiansen[1].* 1) Statens Serum Inst, Copenhagen, Denmark; 2) University of Copenhagen, Copenhagen, Denmark; 3) St George's Hospital, London, United Kingdom; 4) University of Stellenbosch, Cape Town, South Africa; 5) University College London, London, United Kingdom.

Background: Mutations in CAV3, coding for the major constituent of caveolae and a scaffolding protein caveolin-3, have been associated with skeletal muscle disease, cardiomyopathy and most recently, and with a low prevalence, with long QT syndrome and sudden adult death syndrome. The pathogeneic mechanism of these caveolinopathies vary from decreased to increased expression of caveolin-3 to more subtle changes in the interaction between caveolin-3 and signalling molecules and ion channels. We examined the prevalence of CV3 mutations in a large cohort of index patients with long QT syndrome (n = 248), Brugada syndrome (n = 14)and sudden adult death syndrome (n = 34). **Materials and Methods**: *CAV3* was screened for mutations using direct DNA sequencing of exons and exon-close intronic regions of the gene. **Results**: One missense mutation, T78M, was found in one long QT patient. This patient was also carrier of the I400N mutation in KCNH2 and is thus a compound heterozygous carrier of CAV3 and KCNH2 mutations. Family studies revealed that the two mutations were separated in family memebrs and one carrier of the T78M mutation had a normal ECG but a history of syncopes. **Conclusion**: CAV3 mutations are a very rare cause of long QT syndrome and does not seem to play a major role in neither Brugada syndrome nor sudden adult death syndrome.

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Genome-wide association study reveals RYR1 as a genetic risk factor for electrocardiogram left ventricular hypertrophy. *K. Hong[1], D. Shin[2,3], S. Lee[2,4], M. Go[5], J. Lim[1], Y. Jang[2,4], B. Oh[1].* 1) Biomedical Engineering, Kyung Hee Univ, Seoul, Korea; 2) Cardiovascular Genome Center, College of Medicine, Yonsei University, Seoul, Korea; 3) 3Yonsei University Research Institute of Science for Aging, College of Medicine, Yonsei University, Seoul, Korea; 4) Severans Medical Research Institute, College of Medicine, Yonsei University, Seoul, Korea; 5) Center for Genome Science, National Institute of Health, Seoul, Korea.

Left ventricular hypertrophy (LVH) is one of the predictors of future cardiovascular events. Recently, genome-wide association studies (GWASs) have led us to new understanding of complex pathologies and LVH is one of the traits. To identify the genetic risk factor for LVH, we performed a GWAS and a replication study for the LVH in this study. The LVH were diagnosed by electrocardiogram and the genome-wide association study was conducted for community-based cohorts [KARE (control, n = 3653 and case, n = 398)] by using Affymetrix SNP array 5.0. The replication subjects came from a hospital of Yonsei University (control, n = 623 and case, n = 402) and their genotypes were determined by TaqMan™ assay. We examined replication analysis in two ways: hospital case-hospital control used in the replication set 1, and in set 2 was hospital case-cohort control. The initial electrocardiogram-LVH GWAS revealed 13 SNPs in four suggestive association loci (p-value < 1×10^{-5}), including *RYR1* locus (rs2960321, P = 1.9×10^{-6}), 8p24.23 (rs7825068, P = 4.7×10^{-6}), *DYNC2H1* locus (rs11225822, P = 2.6×10^{-6}), and *DNAJC7* locus (rs4239268, P = 8.8×10^{-6}). While tendency of association was found in the replication set 1, the replication set 2 revealed significant association to three SNPs of *RYR1* locus (rs2960321, P = 0.002). Meta-analysis indicates the p-value (rs2960321, meta-analysis odds ratio = 1.39, CI 1.24 - 1.57, P = 2.9×10^{-8}) became stronger than the original study. Ryanodine receptors (RyR) are intracellular Ca^{2+}-permeable channels that provide the sarcoplasmic reticulum Ca^{2+} release required for skeletal and cardiac muscle contractions. RyR1 underlies skeletal muscle contraction, and RyR2 fulfills this role in cardiac muscle. Malignant hyperthermia, central core disease, and catecholaminergic polymorphic ventricular tachycardia have been genetically linked to mutations in either RyR1 or RyR2. To confirm whether the *RYR1* and *RYR2* gene have epistatic interaction for the development of LVH, we investigated the epistatic interaction using PLINK program between the *RYR1* and *RYR2* gene, and the results suggested the epistatic interaction between rs2960321 of *RYR1* gene and rs559344 of *RYR2* gene (p = 0.042). We identified a novel candidate gene for left ventricular hypertrophy. The epistatic interaction indicated that biological interaction between two *RYR* genes would be importance for normal cardiac-muscle function.

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Comprehensive resequencing of the *CD36* gene in subjects with extremely low or high plasma HDL-C levels. *S.C. Hughes[1], F.Y. Demirci[1], A.S Dressen[1], C.H. Bunker[2], J.E. Hokanson[3], R.F. Hamman[3], C.M. Kammerer[1], M.I. Kamboh[1].* 1) Human Genetics, GSPH, Univ Pittsburgh, Pittsburgh, PA; 2) Epidemiology, GSPH, Univ Pittsburgh, Pittsburgh, PA; 3) Epidemiology, Colorado School of Public Health, Univ Colorado Denver, Aurora, CO.

Coronary heart disease (CHD) is a primary public health concern and among the leading causes of death every year in the United States. Plasma lipid levels are among the major risk factors that influence CHD risk and high levels of plasma high density lipoprotein cholesterol (HDL-C) have been shown to be protective against CHD. *CD36* belongs to the class B scavenger receptor family of cell surface proteins and binds many ligands including thrombospondin and lipoproteins. The aim of this study was to comprehensively evaluate the relation between common and rare variation in *CD36* gene (located at 7q11.2) and plasma HDL-C levels by resequencing ~30 kb-long genomic fragment in individuals with extremely low or high HDL-C levels from two epidemiological samples, U.S. Non-Hispanic Whites (NHWs) and African Blacks. The analysis of sequencing data from individuals with HDL-C levels in the upper 5th percentile (47 NHWs and 48 African Blacks) and the lower 5th percentile (48 NHWs and 47 African Blacks) identified a total of 343 variants, of which 69 were shared by both populations. Of 131 variants observed in NHWs, ~4% were located in exons, ~11% were indels, and ~34% had ≥ 5% minor allele frequency (MAF). Of 281 variants observed in Blacks, ~5% were located in exons, ~15% were indels, and ~37% had ≥ 5% MAF. When considering only exonic variants, 2 out of 5 exonic variants (~40%) in NHWs lead to amino acid changes while 9 out of 13 (69%) in Blacks lead to amino acid changes. No striking differences were observed between the cumulative distribution of rare variants among high and low HDL-C groups in either ethnic group, but some common alleles did exhibit different frequencies between the two HDL-C groups. No striking differences were observed between the allele frequencies of common indels or common variants that result in amino acid changes. Genotyping of all identified rare variants and common tag SNPs in the entire sample sets (623 NHWs and 788 Blacks) is underway and will help to unravel the extent to which the *CD36* genetic variation influences the regulation of plasma HDL-C levels and other lipoproteins.

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Using Electronic Medical Records for Genome-wide Association Studies and Admixture Mapping to Identify SNPs Associated with ECG Traits in African Americans. *J. Jeff[1], A. Ramirez[2], J. Denny[2,3], A. Kho[6], M. Ritchie[1], M. Hayes[7], L. Armstrong[7], M. Basford[4], W. Wolf[8], J. Pacheco[8], R. Chisholm[8], D. Roden[2,4,5], D. Crawford[1].* 1) Human Gen, Vanderbilt Univ, Nashville, TN; 2) Department of Medicine, Division of Clinical Pharmacology, Vanderbilt Univ, Nashville, TN; 3) Department of Biomedical Informatics, Vanderbilt Univ, Nashville, TN; 4) Office of Personalized Medicine,Vanderbilt Univ, Nashville, TN; 5) Department of Pharmacology,Vanderbilt Univ, Nashville, TN; 6) Division of General Internal Medicine, Northwestern University, Chicago, IL; 7) Division of Endocrinology, Metabolism, and Molecular Medicine,Northwestern University, Chicago, IL; 8) Center for Genetic Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL.

Several genome wide association studies (GWAS) have identified loci that contribute to electrocardiographic (ECG) parameters, and most have been performed in European-American populations collected from population-based cohorts or surveys. As part of the electronic Medical Records and Genomics (eMERGE) network, we conducted a GWAS in 455 African Americans from the Vanderbilt Genome-Electronic Records Project and Northwestern University NUgene Project. African American individuals that had a normal ECG without evidence of cardiac disease before or within one month following the ECG, without concurrent use of medications that interfere with QRS duration, and who did not have abnormal electrolyte values at the time of the ECG were included. Over 930K SNPs from the Illumina 1M BeadChip were tested for an association with PR interval, QRS duration, QTc interval, and heart rate using linear regression assuming an additive genetic model. Tests of association were performed unadjusted and adjusted for age, sex, PR/QT drug usage and principle components. None of the four traits were associated with a tested SNP at p<5.0x10-8. Chromosome 3 GWAS-identified SNPs that influence ECG traits (SCN5A-SCN10A) in European Americans were not associated in this African American sample (p>0.10); however, the effect sizes trended in the same direction. Interestingly, the minor allele frequencies (MAF) for the GWAS-identified SNPs in European Americans (such as SCN10A nonsynonymous rs6795970; MAF= 43%) are much higher than in African Americans (0.8%). Given these frequency differences, we hypothesized SCN5A-SCNA10A, if associated with ECG traits in African Americans, will have more European ancestry along this genomic region compared to the rest of the genome. We therefore performed an admixture scan for ~800K independent SNPs (r2 <0.80) across the genome using ANCESTRYMAP. For the QRS duration trait, the genome-wide LOD scores ranged from 0.65 to 9.63, and 19 of the 22 autosomes had LOD scores >2.0. Chromosome 3 had the highest genome-wide LOD score (9.63) indicating that chromosome 3 has the most QRS-associated SNPs that differ in allele frequency between the two ancestral populations compared to the entire genome. These results suggest that for ECG traits, European-identified GWAS associations may be generalizeable to other populations such as African Americans.

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Functional Analysis of Newly Identified Promoter Polymorphisms of Endothelial Lipase (*LIPG*) in Human Endothelial Cells. *S. Khetarpal[1], A. Edmondson[1], A. Raghavan[1], H. Neeli[2], S. Kathiresan[3], L. Cupples[4], S. Demissie[4], A. Manning[4], S. DerOhannessian[1], M. Wolfe[1], D. Rader[1].* 1) Dept Med, Univ Pennsylvania, Philadelphia, PA; 2) Dept Hospital Med, Temple Univ, Philadelphia, PA; 3) Broad Instit of Harvard and MIT, Cambridge, MA; 4) Boston University and Framingham Heart Study.

Increased plasma levels of HDL cholesterol (HDL-C) are associated with decreased risk of developing cardiovascular disease. Endothelial lipase (*LIPG*) has been associated with HDL-C in GWAS and loss-of-function variants are associated with increased HDL-C in humans. We have also demonstrated association of plasma EL concentrations with metabolic syndrome, coronary atherosclerosis, and inflammation in humans. We further sought to understand how regulatory variation in *LIPG* affects expression of EL and HDL-C levels in humans. We thus performed medical resequencing of the *LIPG* promoter in human subjects from extremes of the HDL-C phenotypic distribution. We identified an excess of rare promoter variants unique to subjects with elevated HDL compared to rare variants in subjects with decreased HDL. We also identified a common promoter haplotype. We hypothesized that promoter variants may alter *LIPG* expression and thus alter HDL-C metabolism. To test this hypothesis, we generated a firefly luciferase reporter construct driven by the *LIPG* promoter. Regulatory variants identified through resequencing were introduced into the luciferase reporter construct via site-directed mutagenesis. We tested these variant constructs in luciferase reporter assays in both HEK293 cells and human umbilical vein endothelial cells (HUVECs), the latter of which endogenously express *LIPG* and thus allow us to assess how promoter variants may affect *LIPG* expression in vivo. Many of the variants identified specifically in high HDL-C subjects were found to significantly decrease gene expression in vitro, consistent with reduced *LIPG* expression leading to elevated HDL-C. Genetic association analysis of the common promoter haplotype in the Framingham Heart Study showed that the common *LIPG* promoter haplotype is significantly associated with decreased HDL-C. The luciferase reporter construct containing the promoter haplotype exhibited increased expression in vitro, as predicted. Subjects with the common *LIPG* promoter haplotype were also found to have significantly increased plasma levels of EL protein, as determined by ELISA. Our results suggest that EL is a physiologically significant modulator of HDL-C metabolism in humans, with regulatory variation of LIPG playing an important role in modulating HDL-C concentration.

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Association of the Adiponectin Gene Variations with Risk for Ischemic Stroke in a Korean Population. *Y. Kim[1], O. Bang[2], M. Cha[2], Y. Park[1].* 1) Med Genome Res Ctr, KRIBB, Daejeon, Korea; 2) Dept. of Medical Research, KIOM, Daejeon, Korea.

Stroke is the second-leading cause of death and a major cause of morbidity and mortality worldwide. The evidence of variations in adiponectin (AdipoQ) gene that are associated with ischemic stroke has not been consistent, and it is unclear whether the same loci contribute to associations in the Korean population. Using a Korean population, we tested ischemic stroke- associated AdipoQ markers. In a preliminary genome-wide association study using 320 Affymetrix 250k NSP chips, AdipoQ was found to be associated with ischemic stroke in Koreans. To study AdipoQ, further 673 ischemic stroke patients and 267 unrelated individuals without a history of stroke or transient ischemic attack were examined in a case-control study. Six polymorphisms (rs182052G>A, rs16861205G>A, rs822391T>C, rs822396A>G, rs12495941G>T and rs3774261A>G) that had a minor allele frequency over 1% were strongly associated with stroke ($P < 0.05$). The haplotypes in ht 1 (AGGCGG and AAGTAG) were also significantly associated with susceptibility to stroke ($P < 0.05$). Our findings show that polymorphisms in AdipoQ are associated with a risk for ischemic stroke in the Korean population. This study lends further support to the putative role of AdipoQ in stroke. <<<<.

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Transcriptome and exome sequencing for gene discovery in dilated cardiomyopathy. *D. Li, J. Gonzalez-Quintana, N. Norton, J. Siegfried, R.E. Hershberger.* Dept Medicine/Cardiology, University of Miami, Mlami, FL.

Dilated cardiomyopathy (DCM), a primary myocardial disease, is a common cause of heart failure and heart transplantation. Genetic causation of most DCM cases remains unknown even though mutations in numerous genes have been identified. Sanger sequencing of candidate genes, whether for linkage analysis and positional cloning or sequencing of hypothesis-based candidate genes, is challenging, costly and labor intensive. To circumvent these limitations and to accelerate DCM gene discovery, we utilized next generation sequencing (NGS) technology to sequence the transcriptomes of failing left ventricular (LV) myocardium from patients in two multi-generational DCM families. mRNA was isolated from explanted DCM hearts and from nonfailing LV myocardium. The cDNA libraries were sequenced (paired-end 2x76 bp) with Illumina GA II. More than 40 million cDNA sequence reads were produced from each tissue sample, which gave adequate coverage for abundant and rare transcripts (as few as 1 copy/per cell). Genetic variants were identified by comparing the transcriptome sequences with NCBI human reference sequences. Novel variants were identified by filtering out SNPs in publicly accessible databases and those appearing in normal LV myocardium. Conservation analysis (PhastCons and Genomic Evolutionary Rate Profiling methods) and protein functional prediction by PolyPhen were performed for all novel variants. This analysis resulted in a list of 79 genes with novel rare nonsynonymous variants. To narrow the candiate gene list, exome sequencing using NGS with filtering from an additional affected subject was conducted that decreased the shared variant list of candidates to 7 genes. Desmoplakin (DSP), phosphoglycerate mutase 2, and latent transforming growth factor beta binding protein 1 were considered as leading candidate genes due to their relevance to cardiac pathophysiology. A novel nonsense mutation in DSP segregated with multiple affected members in the family and was absent from 370 normal control DNAs. A similar genetic analysis for 6 remaining candidate variants is ongoing. Our study demonstrates that transcriptome and exome sequencing is a promising approach to discover DCM genes.

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Phenotypic Spectrum and Genotype-Phenotype Correlations in Loeys-Dietz syndrome. *H. Morisaki[1], H. Ogino[2], Y. Tsutsumi[3], K. Akutsu[1], A. Ono[1], A. Kono[4], M. Higashi[4], T. Kosho[5], S. Mizuno[6], T. Morisaki[1,7].* 1) Dept Bioscience & Genetics, Natl Cerebr & Cardiovasc Ctr Res Inst, Suita, Osaka, Japan; 2) Dept Cardiovascular Surgery, Natl Cerebr & Cardiovasc Ctr, Suita, Osaka, Japan; 3) Dept Cardiovascular Medicine, Natl Cerebr & Cardiovasc Ctr, Suita, Osaka, Japan; 4) Dept Radiology, Natl Cerebr & Cardiovasc Ctr, Suita, Osaka, Japan; 5) Dept Medical Genetics, Shinshu Univ Sch Medicine, Matsumoto, Nagano, Japan; 6) Dept Pediatrics, Aichi Pref Colony Central Hosp, Kasugai, Aichi, Japan; 7) Dept Molecular Pathophysiology, Osaka Univ Grad Sch Pharm Sci, Suita, Osaka, Japan.

Loeys-Dietz syndrome (LDS) is a systemic connective tissue disorder characterized by vascular and skeletal manifestations caused by mutations in *TGFBR1* or *TGFBR2*. Although characteristic craniofacial and arterial manifestations are helpful for diagnosis of the disorder, many features overlap between LDS and Marfan syndrome (MFS), and diagnostic genetic testing is needed for most patients. It is also generally accepted that there is no genotype-phenotype correlation in LDS. In this study, we analyzed the clinical details of 30 Japanese LDS patients with *TGFBR1* (14 patients in 11 families) and *TGFBR2* (16 patients in 14 families) mutations, and compared them with those with MFS to clarify LDS-characteristic features. We also report several phenotypic differences between *TGFBR1* and *TGFBR2* mutation carriers. When compared with patients genetically diagnosed as MFS, LDS patients were physically less dolichostenomelic and more had ocular hypertelorism. Annuloaortic ectasia (AAE) was observed in most of the LDS patients, although it was absent in 11% of those who had already experienced TAAD. Ectopia lentis, a characteristic feature of MFS, was not observed, while congenital retinal abnormalities were observed in 20% of our LDS patients. In radiological findings, the incidence of arterial tortuosity was significantly different in the vertebral arteries as compared with MFS. Dural ectasia was observed in both LDS and MFS, however, the ectatic pattern was different between the groups. Significant differences between patients with *TGFBR1* and those with *TGFBR2* mutations were observed in regard to age at diagnosis, cleft/uvula abnormalities, skeletal involvement, lung involvement, and fulfillment of Ghent diagnostic criteria for MFS. Although hypertelorism and aortic involvement were observed at equally high frequency in both groups, LDS patients with *TGFBR2* mutations tended to have more severe skeletal involvement and be diagnosed at a younger age, often initially as MFS, while those with *TGFBR1* mutations had a greater chance to be diagnosed only when aortic symptoms occurred and diagnosed as familial TAAD. Based on our findings, we conclude that patients suspected of LDS should be carefully examined for LDS-specific features based on both physical characteristics and vascular imaging findings, and should also be tested for *TGFBR1*/*TGFBR2* mutations even if skeletal features are not characteristic.

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Identification of a locus for recessive Dilated-Cardiomyopathy on chromosome 2 by linkage analysis in a Bedouin familiy. *E. Muhammad[1], A. Levitas[2], V. Chalifa Caspi[3], E. Manor[4], J. C. Beck[5], V. C. Sheffield[5], R. Parvari[1,3].* 1) Dept Gen & Virology, Ben Gurion Univ, Beer Sheva, Israel; 2) Division of Pediatrics, Soroka Medical Center, Beer Sheva 84105 and Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva 84105, Israel; 3) National Institute of Biotechnology Negev, Ben Gurion University of the Negev, Beer Sheva 84105, Israel; 4) Institute of Genetics, Soroka Medical Center, Beer Sheva 84105 and Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva 84101, Israel; 5) Dept. of Pediatrics - Division of Medical Genetics; Howard Hughes Medical Institute, University of Iowa, Iowa City, IA 52242, USA.

Cardiomyopaties are the most common disorders resulting in heart failure. Dilated cardiomyopathy (DCM), a disorder characterized by cardiac dilatation and reduced systolic function, is the most frequent cause. However, recessive neonatal isolated dilated cardiomyopathy has scarcely been associated with a mutation. We have identified patients with acute DCM in a consanguineous Bedouin family presenting an autosomal recessive pattern of inheritance. Linkage analysis was carried out on five patients, their parents and four healthy sibling using SNPs array and microsatellite markers. One region on chromosome 2[2q35-2q36.3] in an interval of 10cM and 9.5Mb was consistent with linkage. The lod scores for linkage to this region were 3.5 and 4.5 for two point and multiple points, respectively. Two candidate genes encoding structural myocite proteins were sequenced and negated. One of them was the Desmin gene, in which mutations were previously reported to cause a dominant pattern of DCM.

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Effects of Rare, Putatively Damaging Variants in Essential Hypertension in the Population-based CLUE and the Family-based FBPP Samples. *K.H. Nguyen[1], V. Pihur[1], G.B. Ehret[1], S.K. Ganesh[3], A.B. Weder[3], J. Coresh[2], W.H.L. Kao[2], A. Chakravarti[1].* 1) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins Univ, Baltimore, MD; 2) Dept of Epidemiology, Johns Hopkins Univ, Baltimore, MD; 3) Dept. of Internal Medicine, Univ of Michigan, Ann Arbor, MI.

Essential hypertension (EH), or high blood pressure (BP) with no identifiable secondary cause, is a major risk factor for cardiovascular disease and its complications. While the genetic causes of EH are largely unknown, Guyton's hypothesis contends that genes that regulate salt re-absorption in the kidneys are a primary cause, buttressed by genes identified from Mendelian hypo- and hypertension syndromes. We conducted a pilot medical resequencing project of 11 syndromic hypo-/hypertensive genes in 560 individuals at the extremes of systolic BP. We further selected 11 non-synonymous variants, all predicted to be damaging by both the genetic algorithms in the software SIFT (Sorting Intolerant From Tolerant) and PolyPhen (Polymorphism Phenotyping), as our putative functional candidates and genotyped these in ~7,000 unrelated samples from the population-based CLUE cohort and ~6,500 individuals from the family-based Family Blood Pressure Program (FBPP) using Sequenom technology. The top SNP, rs2681472 in *ATP2B1*, from published genome-wide association studies were also genotyped in the same samples. The genotype data were analyzed for association with four BP traits (SBP, DBP, PP, MAP) using the computer software MERLIN. Due to the rarity of these coding variants (MAF < 5% except one at 8% and one at 12%), only 6 of the 11 variants were present in both cohorts. Two coding variants in the α and γ subunits of the epithelial sodium channel (ENaC) protein complex reached statistical significance in FBPP. rs5742912 (Trp493Arg) in SCNN1G was associated with a decrease of 3 mmHg (residual corrected for age, age2, gender, BMI) in SBP (p= 0.013) and 2 mmHg (residual) in PP (p=0.009), and Thr259Asp in *SCNN1A* was associated with a increase of 2 mmHg (residual) in PP (p=0.002). Both mutations are located in the extracellular loop of the ENaC subunits that is suggested to be the channels' site of action. *SCNN1G* and *SCNN1A* mutations are known in Pseudohypoaldosteronism type 1 and Liddle syndrome, respectively.

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MBL and MIF gene polymorphisms in Turkish children with cardiomyopathy: no association with MBL (codon 54) genotypes, but an association between MIF (-173) polymorphism and CMP. *S. Oguzkan Balci[1], N. Col Araz[2], T. Sever[1], O. Baspinar[3], A. Balat[4], S. Pehlivan[1].* 1) University of Gaziantep, Faculty of Medicine, Department of Medical Biology and Genetics, Gaziantep, Turkey; 2) University of Gaziantep, Faculty of Medicine, Department of Pediatrics; 3) University of Gaziantep, Faculty of Medicine, Department of Pediatric Cardiology; 4) University of Gaziantep, Faculty of Medicine, Department of Pediatric Nephrology.

Objectives of study: Cardiomyopathy (CMP) is defined as structural and/or functional disorders of the hearth muscle, and it is an important cause of chronic congestive cardiac failure in children. Disturbances of the cellular and humoral immune system are frequently observed in CMPs, and myocardial inflammation is one of the commonest mechanisms in cardiomyopathy. Mannose binding lectin (MBL) is a key molecule in innate immunity with the capacity to bind to microorganisms and kill them by initiating the lectin pathway of complement activation. Macrophage migration inhibitory factor (MIF) is a constitutive element of the host antimicrobial defences and stress response that promotes proinflammatory function of the innate and acquired immune system. It plays an important role in the pathogenesis of acute and chronic infammatory or autoimmune disorders. The aim of present study was to investigate any possible association between polymorphisms of MBL and MIF genes and CMP in a group of Turkish children. **Methods:** The study was approved by the local ethical committee and informed consents were obtained from the patients. Fourteen-children with CMP and 21 age- and sex-matched healthy controls were analyzed for codon 54 A/B polymorphism in MBL gene and -173 G/C polymorphism in MIF gene by using PCR-RFLP methods. **Results:** The distribution of AA, AB, and BB genotypes for MBL codon 54 were 57.1 %, 28.6 % and 14.3 % in CMP compared with 76.2 %, 23.8 % and 0 % in the controls (p=0.261). The allele frequency of A/B in MBL was 71.4 %, 28.6 % in CMP compared with 88 %, 12 % in the controls. (p=0.078). The distribution of GG, GC, and CC genotypes for MIF (-173) were 50 %, 28.6 %, and 21.4 % in CMP compared with 52.4 %, 47.6 % and 0 % in the controls (p=0.157). CC genotype was higher than GG, GC and GG+GC genotypes in patient group (p=0.049, p=0.022, p=0.026, respectively). The allele frequency of G/C in MBL was 72 %, 28 % in CMP compared with 76.2 %, 23.8 % in the controls (p=0.280). The observed genotype counts were not deviated significantly from those expected according to the Hardy-Weinberg Equilibrium for MBL and MIF gene polymorphisms (P>0.05). **Conclusion:** This study is the first to investigate the MBL and MIF gene polymorphisms in Turkish children with CMP. We conclude that CC genotype of MIF (-173) polymorphism may be a risk factor for CMP patients. However, further studies with larger samples are needed to address the exact role of this polymorphism in CMP.

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SNPs on Chromosome 5p are Associated with Coronary Artery Disease in the Japanese Population. *K. Ozaki[1], A. Aoki[1], H. Sato[2], A. Takahashi[3], Y. Sakata[2], Y. Onouchi[1], T. Tsunoda[4], M. Kubo[5], S. Ikegawa[6], N. Kamatani[3], M. Hori[2], Y. Nakamura[7], T. Tanaka[1].* 1) Laboratory for Cardiovascular Disease, Center for Genomic Medicine, RIKEN, Yokohama, Japan; 2) Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, Suita, Japan; 3) Laboratory for Statistical Analysis, Center for Genomic Medicine, RIKEN, Yokohama, Japan; 4) Laboratory for Medical Informatics, Center for Genomic Medicine, RIKEN, Yokohama, Japan; 5) Laboratory for Genotyping Development, Center for Genomic Medicine, RIKEN, Yokohama, Japan; 6) Laboratory for Bone and Joint Disease, Center for Genomic Medicine, RIKEN, Tokyo, Japan; 7) Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan.

Coronary artery disease (CAD) including myocardial infarction (MI), leading cause of death in many countries, results from complex interactions multiple genetic and environmental factors, indicating the importance of clarifying genetic risk factors in their pathogenesis. By means of a genome wide association study in a Japanese population using 250k single nucleotide polymorphism (SNP) markers, we identified a novel susceptible locus for MI on chromosome 5p. A marker SNP showed significant association for MI in several independent Japanese cohorts (combined $P < 10^{-10}$, OR = 1.25; comparison of allele frequency; approximately 5,300 cases and 7,600 controls). Association study using tagging SNPs based on Linkage disequilibrium (LD) revealed that additional two SNPs in modest LD with the marker SNP were also associated with MI. Furthermore, we found that the genotype frequency of these SNPs in individuals with unstable angina pectoris, another severe CAD based coronary atherosclerosis, were nearly identical with those of MI and were significantly associated with the disease. These findings indicate that the SNPs on chromosome 5p are novel genetic risk factor for CAD.

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Chromosomal locus 9p21.3: a marker of the angiographic severity of the coronary artery disease. *N. Rivera[1], R. Roncarati[1,2], C. Viviani-Anselmi[1], F. De Micco[3], A. Mezzelani[2], G. Condorelli[4], A. Puca[1,2], F. Airoldi[1], G. Condorelli[1,2], C. Briguori[3].* 1) Multimedica Research Hospital, Milan, Italy; 2) Institute of Biomedical Technologies, CNR, Milan Italy; 3) Clinica Mediterranea, Naples, Italy; 4) University Federico II, Naples, Italy.

Background— Several Genome-wide Association Studies (GWAS) revealed a highly significant link between variations of chromosome 9p21.3 and the risk of coronary artery disease (CAD). Our aims were (1) to assess cardiovascular risk factors along with the function of twelve 9p21.3 genetic variants in Italian Caucasians with CAD and (2) to identify the relationship between genetic susceptibility and coronary vessel anatomic phenotypes and lesion severity. Methods and Findings— We performed a cross-sectional study in an Italian Caucasian group of 1696 subjects with CAD involving one-, two-, or three-vessel CAD. Twelve SNPs from GWAS of CAD were assessed for links between genetic variants at this locus and significant disease in major epicardial coronary vessels. Six single nucleotide polymorphisms (SNPs) (i.e., rs10757274, rs4977574, rs2383207, rs10738610, rs10757278, rs1333049) were strongly associated with both the diameter stenosis and the number of coronary vessels affected. More specifically, in each of the six SNPs, we noticed an increase of the risk-allele frequency as the number of diseased coronary vessels increased. We also observed that (1) the percentage of risk-allele carriers increased dramatically, particularly in subjects diagnosed with significant lesions in the left anterior descending and circumflex arteries, and (2) a strong genetic link between these polymorphisms and the number of diseased coronary vessels Conclusions. The present study showed that six genetic variants at locus 9p21.3 are associated with the angiographic severity and extensions of CAD. Keywords: CAD; severity of coronary vessel; stenosis, cardiovascular genetics; single nucleotide polymorphism; 9p21.

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Progressive systemic and stenotic vasculopathy: Candidate genes identified by comparative exome sequencing. *R. Rupps[1,2], C.D. van Karnebeek[3], A.P. Fejes[4], M. Morimoto[1,2], C. Shuen[1,2], T. Markello[5], A. Delaney[4], S. Jones[4], M. Marra[4], C. Boerkoel[1,2].* 1) Dept Medical Genetics, University of British Columbia, Vancouver BC, Canada; 2) Child & Family Research Institute, Children's & Womens th Ctr-BC, Vancouver, BC, Canada; 3) Div Biochemical Diseases, Dept Pediatrics, BC Children's Hospital, Vancouver, BC, Canada; 4) BC Cancer Agency, Genome Sciences Centre, Vancouver BC, Canada; 5) Undiagnosed Diseases Program, National Institutes of Health, Bethesda, MD, USA.

Background: Fibromuscular dysplasia (FMD) is a group of non-atherosclerotic, non-inflammatory diseases of the musculature of arterial walls, leading to stenosis of small and medium-sized arteries. It most commonly affects renal and carotid vessels, but more distal sites may also be involved. A genetic etiology of this systemic disorder is suggested by concordance among monozygotic twins and a 10% familial recurrence rate, but has not yet been identified. **Aim:** To define a cause of FMD, we applied comparative whole exome sequencing in an extreme syndromic form of this vascular disease involving all major arteries. **Case:** This 15 year old boy with an unremarkable family history has a diffuse ischemic vasculopathy involving the non-ostial portion of his medium-sized vessels. Stenosis of the carotid arteries, mesenteric and renal arteries has caused recurrent cerebral infarctions, severe abdominal pain, progressive hypertension and revascularization resembling Moya Moya disease. His other features include an ASD, branch pulmonary artery stenosis, intestinal non-rotation, anal stenosis, and osteopenia with isolated recurrent fractures. Extensive genetic and metabolic investigations yielded normal results. Histopathology of internal carotid artery showed: mild to moderate intimal thickening, calcification and focal fragmentation of the internal elastic lamina. **Methods:** Exonic DNA from the patient and both parents was selected using the Agilent SureSelect kit and sequenced using the Illumina NexGen sequencing platform. Using an autosomal recessive model of disease, rare and novel non-synonymous homozygous and compound heterozygous mutations were identified by comparison of the unique variant positions to dbSNP and 1046 libraries. **Results & Conclusions:** We identified several genes with bi-allelic mutations; one functioning in the RAS pathway is a likely candidate since it modulates formation of functional tight junctions. Biological investigations currently underway will determine if any of these mutations are causative of disease. Understanding the disease in this patient will improve our understanding of arterial biology and provide insight into FMD-like conditions.

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KCNH2 (HERG) gene polymorphisms associated with increased genetic risk condition of non-valvular atrial fibrillation in Koreans. *D. Shin[1,3], A. Park[1], H. Hwang[2], N. Son[1], K. Lee[1], E. Shin[4], J. Lee[4], B. Joung[2], H. Pak[2], M. Lee[2], S. Kim[2], Y. Jang[1,2].* 1) Cardiovascular Genome Ctr, Yonsei Col Med, Seoul, Korea; 2) Division of Cardiology, Yonsei University Health System; 3) Yonsei University Research Institute of Science for Aging; 4) DNA Link Inc, Seoul, Korea.

Atrial fibrillation (AF) is the most frequently and seriously encountered cardiac rhythm disorder in humans and is characterized by chaotic electrical activity of the atrial. Recent evidence indicates the phenotypes of non-valvular AF are more likely to occur in patient with a genetic susceptibility. KCNH2 gene encodes for the HERG protein, a α-subunit of the cardiac IKr channel, which contributes to the repolarization of the cardiac action potential. In this study, we sought to determine the association of genetic polymorphisms of KCNH2 gene with non-familial non-valvular AF. We investigated hospital-based case-control study for a total of 754 Korean subjects, comprising of 354 cases with AF and 400 controls without AF. Genetic screening was carried out by direct DNA sequencing and SNaPShot assay. We examined 9 tagging single nucleotide polymorphisms (tSNPs) in the KCNH2 gene. We did not observe the significant differences in the allele and genotype distributions between patients with AF and control subjects. However, logistic regression analysis represented that tSNP, rs740952 (T25399C; I489I), in exon 6 confers the risk of non-valvular AF in Korean males. The significant association was observed under a dominant model for TT genotype with a decreasing risk of AF (OR, 0.687; 95% CI, 0.481-0.980; P=0.038). In addition, rs740952 was significantly associated with left atrial volume (P=0.013), left atrial dimension (P=0.006) in female group. Haplotype analysis of KCNH2 polymorphisms did not show a significantly different distribution of their haplotype condition between the patients with AF and the controls. However, left atrial dimension increased significantly with the haplotype CGTG in apparent recessive model in males (CGTG/other haplotypes, P= 0.006). We provide evidence that KCNH2 rs740952 SNP may contribute to protect Korean individuals to the risk of non-valvular AF, and may play a role in cardiac remodeling after the development of AF. Further studies together with other IKr channel genes are required to determine the association between AF susceptibility and potassium channel gene functional variants.

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Family-based evaluation of polygenic variance explained by genes associated with HDL, LDL, and triglyceride levels. *B.H. Shirts[1], S.J. Hasstedt[2], P.N. Hopkins[3], S.C. Hunt[3].* 1) Pathology, University of Utah, Salt Lake City, UT; 2) Human Genetics, University of Utah, Salt Lake City, UT; 3) Cardiovascular Genetics, Medicine, University of Utah, Salt Lake City, UT.

Over 30 genes have been identified that influence HDL, LDL, and/or triglyceride levels, which are known risk factors for cardiovascular disease. Only a small portion of the total variance in the serum levels of these markers can be explained by these markers. However, case-control studies are unable to estimate the proportion of heritability attributable to associated genetic polymorphisms. We used a sample of over 2200 individuals in 96 pedigrees to evaluate the proportion of heritability explained by 15 SNPs previously associated with HDL, LDL, and/or triglyceride levels while adjusting for age, sex, and BMI using jPAP software for maximum likelihood analysis.

Heritability for HDL, LDL, and triglycerides were 0.54, 0.41, and 0.26 respectively. Of the 15 loci investigated, 6 contributed to the heritable component of HDL level (LIPC, LPL, APOC-APOE, LIPG, APOB, TRIB1), although less than 5% of heritability could be attributed to these loci in a combined model. Eight loci contributed to heritability of LDL level (APOC-APOE, CELSR, APOB, HMGCR, TRIB1, FADS, CETP, LIPC); together these account for less than 10% of the heritability. Five loci contributed to heritability of triglyceride levels (APOA1, TRIB1, GCKR, MLXIPL, APOC-APOE), which together account for approximately 10% of the heritability. Heritability estimates for most loci were larger than estimates of total variance for most loci examined, which is consistent with heritability being only a portion of total variance; however, previously identified associations were not replicated at all loci.

Analysis of family-based samples allows estimation of proportion of polygenic attributable specific genetic loci. Our estimates suggest that known loci do explain a significant amount of variance in HDL, LDL, and triglyceride levels, but that there are additional sources of familial variance yet to be identified.

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Titin mutations in arrhythmogenic right ventricular cardiomyopathy. *M. Taylor[1], S. Graw[2], C. Barnes[1], D. Slavov[1], F. Brun[3], B. Pinamonti[3], E. Salcedo[1], W. Sauer[1], S. Pyxaras[3], G. Sinagra[3], H. Granzier[4], L. Mestroni[1].* 1) Adult Med Gen Prog and Division of Cardiology, Univ Colorado Denver, Aurora, CO; 2) Department of Psychiatry, Univ Colorado Denver, Aurora, CO; 3) Department of Cardiology, Hospital and University of Trieste, Trieste, Italy; 4) Department of Physiology, University of Arizona, Tucson, AZ.

Purpose: Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a rare inherited myocardial disease characterized by fibrofatty replacement of the myocardium and a predisposition to cardiac arrhythmias and sudden death. ARVC is familial in up to 50% of cases and the current disease model implicates desmosome dysfunction as the principal pathogenic defect. We evaluated the cardiomyopathy gene titin (TTN) as an ARVC candidate as it is close to the ARVD4 locus and the titin protein connects to the transitional junction at intercalated disks. **Methods:** Patients from 38 ARVC families underwent TTN DNA resequencing provided by the University of Washington, Department of Genome Sciences. Exons and peri-exonic regions of titin isoform N2A (NM_133378) along with additional exons unique to the principal cardiac isoform N2B (NM_003319) were amplified from genomic DNA using PCR. This covered 312 exons (311 expressed as titin protein) and the complete 3' untranslated region. Four of the ARVC families were sufficiently large to test for cosegregation of TTN mutations with the ARVC phenotype and are reported here. **Results:** Four, unique TTN variants were detected in the four families (Thr2896Ile, Ile16949Thr, Pro17706Leu, Ala19309Ser). The Thr2896Ile variant showed compelling genetic evidence that is pathogenic: present in 9 confirmed/obligate ARVC subjects including 2 fifth-degree relatives, absent in over 350 cardiomyopathy and 176 control chromosomes, and was scored 'intolerant' by SIFT and PolyPhen predictive algorithms. Ile16949Thr and Ala19309 were absent in controls and segregated with 'affected' status in smaller ARVC pedigrees. Pro17706Leu, although 'intolerant' and unique, is likely a rare benign polymorphism since it was inherited from the unaffected parent. **Conclusions:** Titin mutations may lead to the ARVC phenotype; disruption of normal spring-recoil properties of the titin protein may account for the phenotype manifestations in the thinner-walled stress-susceptible right ventricle.

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CHIP-based sequence analysis of 34 cardiomyopathy genes reveals new genes involved in HCM and DCM and multiple pathogenic mutations in single patients. *A. van den Wijngaard[1], I. Krapels[1], W. Van Dijk[1], A. Stassen[1], P. Lindsey[1,2], Y. Arens[1], P. Helderman[1], C. Marcelis[3], S. Heymans[2,4], P. Volders[2,4], R. Jongbloed[1,2], H. Smeets[1,2].* 1) Clinical Genetics, Maastricht University Medical Centre, Maastricht, Netherlands; 2) Research School CARIM, Univ Maastricht, Maastricht, The Netherlands; 3) Dept Clinic Genet, UMC St. Radboud, Nijmegen, The Netherlands; 4) Dept Cardiol, Maastricht UMC, Maastricht, The Netherlands.

Purpose: Inherited cardiomyopathy is a cardiac disease with an estimated prevalence of 1:500 for hypertrophic cardiomyopathy (HCM) and 1: 2500 for dilated cardiomyopathy (DCM). Both cardiomyopathies show large genetic and clinical heterogeneity and require high-throughput and affordable mutation detection technologies to efficiently integrate molecular screening into clinical practice. Current diagnostic screening of a limited number of genes solves about 60-70% of the familial cases. However, double pathogenic mutations seem to be present in 5-10% of the familial cases. **Methods:** To create a fast genetic screening method for inherited cardiomyopathy, we designed a DNA resequencing array (CardioCHIP 150K34) covering 34 genes in duplicate. We included genes involved in DCM, HCM, non compaction cardiomyopathy, Limb Girdle Muscular Dystrophy and candidate genes based on their presence or protein-protein interaction in the sarcomere. All exons, flanking introns (38bp), the 5'UTR and 3'UTR regions, covering heart- and muscle-specific RNA-isoforms are included. Genomic regions were amplified in 152 LR-PCR's covering 395 exons. **Results:** Recently 250 patients were resequenced for all 34 genes. The mutation detection accuracy is >99%. About 98% of the novel exonic variants were confirmed by conventional sequence analysis. In addition to the mutations detected in the 13 routinely tested genes, we also identified mutations in the additional genes and candidate genes. In several patients up to 4 pathogenic mutations were identified, involved in different pathogenic processes. **Conclusion:** Our data indicate that parallel analysis of multiple genes is a prerequisite for genetic testing in HCM and DCM to rapidly identify the underlying genetic defect(s).

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Gene Expression Analyses in Marathon Runners. *D. Vance[1], M. Stoutenberg[2], G. Chen[1], R. Myerberg[1], D. Tekin[1], L. Nathanson[1], K. Jacobs[2], J. Clark[2], A. Perry[2], P.J. Goldschmidt-Clermont[1], D. Seo[1], E. Rampersand[1].* 1) University of Miami Miller School of Medicine, Miami, FL; 2) University of Miami, Coral Gables, FL.

Individual differences in the prevalence of hypertension and other cardiovascular related traits have been observed in individuals who train for marathons, suggesting underlying genetic variations in fitness levels. Gene expression analyses can be used to identify genes whose expression levels are correlated with quantifiable measures of fitness, such as VO2max, an established metric of cardio-respiratory fitness. To explore the relationship between genetics and fitness, we studied the global gene expression in the leukocytes of sedentary individuals collected before and after a 17-week half marathon training program. Twenty men (29.3 ±1.0yr) were enrolled in the marathon training program. An additional 22 men (27.8 ± 1.4 yr) who performed aerobic activity less that 2 hours per·week-1 served as sedimentary controls. Fasting blood samples, VO2max and measures of body fat composition were collected at baseline and at the completion of the training program for all participants. Leukocyte samples were collected and stored using the Leukolock system (Ambion). To examine the participants' gene expression profiles, the extracted samples were run on the Affymetrix GeneChip Human Gene ST array, which covers 29000 different transcripts. We will use Significance Analysis of Microarrays (SAM) to determine genes that are differentially expressed before and after exercise training. We will also use other methods such as multivariate sparse ANOVA modeling to find genes whose expression corresponds to different levels of VO2max.

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Association of Variants in Candidate Genes Influencing Autonomic Nervous System Functionality with Blood Pressure Level. *C. Vangjeli[1], N. McCarthy[1], G. Cavalleri[1], K. Shianna[2], N. Delanty[1], E. O'Brien[3], A.V. Stanton[1].* 1) Molecular & Cellular Therapeutics, Royal College of Surgeons in Ireland, Dublin 9, Ireland; 2) Duke Institute for Genome Sciences and Policy, 450 Research Drive- B wing, Box 91009 Durham, NC 27708; 3) The Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin 4, Ireland.

Genomewide association studies have identified 13 novel loci associated with blood pressure (BP). However only a small proportion of total BP variation is explained by these findings - identification of all BP associated SNPs will require other strategies. Here we report on a candidate gene study with dense SNP coverage of multiple genes involved in the autonomic nervous system (ANS), namely neurotransmitter receptors, metabolisers, and transporters. Using the Illumina GoldenGate platform, we genotyped 2364 SNPs in 168 genes in 358 healthy bank employees who had undergone seated clinic BP measurements (screening population, SP). Only the 58 SNPs in 33 genes, found to be associated with systolic, diastolic or pulse pressure (SBP, DBP or PP) within the SP (p£0.01), were genotyped (Illumina Veracode platform) in a second independent replication population (RP) - these 380 healthy bank employees had undergone repeated 24-hour ambulatory BP monitoring. Association analyses were performed using additive genetic models. Quantile-quantile plots showed enrichment for significant P-values in both populations. Differences in BP (mean,p-value, age and sex adjusted) per copy of minor allele for the top associations signals are tabulated below.

	Clin-icSBP	DaySBP	NightSBP	Clin-icDBP	DayDBP	NightDBP
SLC17A8	4.0*	1.9	-0.3	2.6*	1.9*	0.02
GABRR1	0.9	-0.9	-1.3*	0.7	-0.1	-0.5

Significant results indicated by *. SLC17A8 encodes a sodium-dependent inorganic phosphate cotransporter and GABRR1 encodes the α subunit of the ionotrophic gamma-aminobutyric acid receptors. These genes have not previously been implicated in the causation of hypertension though the influence of the ANS on BP makes the observed associations biologically very plausible.

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A comprehensive genetic study on left atrium size in Caribbean Hispanics identifies candidate genes in 17p10. *L. Wang[1], M.R. Di Tullio[2], A. Beecham[1], S. Slifer[1], T. Rundek[3], S. Homma[2], S.H. Blanton[1], R.L. Sacco[3].* 1) John T. Macdonald Foundation Department of Human Genetics, John P. Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL; 2) Department of Medicine, Columbia University, New York, NY; 3) Department of Neurology and Epidemiology, University of Miami Miller School of Medicine, Miami, FL.

Background: Left atrial enlargement is associated with cardiovascular disease and stroke. Genetic factors contributing to the left atrium (LA) dimension are poorly understood. We sought to map susceptibility genes for LA size in a large Dominican family dataset and an independent population-based sample from the Northern Manhattan Study (NOMAS). Methods and Results: 100 Dominican families comprising 1350 individuals were studied to estimate heritability and map quantitative trait loci for LA size using variance components analysis. LA dimension was measured by transthoracic echocardiography. A polygenic covariate screening was used to identify significant covariates. LA size had a moderate estimate of heritability (h2=0.42), after adjusting for significant covariates. Linkage analysis revealed suggestive evidence on chromosome 10p19 (D10S1423, MLOD=2.00) and 17p10 (D17S974, MLOD=2.05). Ordered subset analysis found significantly enhanced ($p<0.05$ for increase of LOD score) evidence for linkage at 17p10 (MLOD=2.9) in families with lower LDL level. 2233 single nucleotide polymophisms (SNPs) were used to perform a peak-wide association mapping across 17p10 in 825 NOMAS individuals. Evidence for association were found in NTN1, MYH10, COX10, and MYOCD genes (p=0.00005 to 0.005). Conclusions: Using non-biased genome-wide linkage followed by peak-wide association analysis, we identified several possible susceptibility genes affecting LA size. Among them, MYOCD has been shown to serve as a key transducer of hypertrophic signals in cardiomyocytes. Our data support that polymorphisms in MYOCD modify LA size.

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The Combination of Genetics and Corus CAD Gene-Expression Score for the Detection of Obstructive Coronary Artery Disease. *J.A. Wingrove[1], H. Tao[1], M.R. Elashoff[1], J. Blanchard[1], P. Beineke[1], S.E. Daniels[1], S. Rosenberg[1], W.E. Kraus[2], R.S. Schwartz[3], S. Voros[4], E.J. Topol[5], The PREDICT Investigators.* 1) CardioDx, Palo Alto, CA; 2) Duke University, Durham, NC; 3) Minneapolis Heart Institute and Foundation, Minneapolis, MN; 4) Piedmont Heart Institute, Atlanta, GA; 5) Scripps Translational Science Institute, La Jolla, CA.

Background: Coronary artery disease (CAD) etiology and progression is driven by a complex combination of genetic and environmental factors. Peripheral blood gene expression is sensitive to the presence of CAD, but does not directly address the role of other tissues, such as the endothelium and vascular wall, that may be captured by genetic information. We have initiated a study to determine if genetics can complement gene expression and report the impact of 9p21 genotype on the performance of a clinically validated, gene-expression based algorithm designed to detect significant coronary artery disease. **Methods**: DNA and RNA were isolated from 975 Caucasian subjects (369 cases) undergoing coronary angiography and participating in PREDICT, a multi-center trial designed to compare peripheral blood gene expression with the extent of CAD. Cases had > 50% stenosis in >1 major coronary artery; controls had < 25% luminal stenosis in any major coronary artery by quantitative coronary angiography. The gene-expression based test, comprised of expression levels of 23 genes as well as age and gender risk functions, was performed on the 975 subjects. TaqMan was used for genotyping rs10757278, a SNP associated with likelihood of developing CAD that is located at the 9p21 locus. **Results**: Receiver-operator characteristics (ROC) analysis and Net-Reclassification Index (NRI) were used to assess algorithm performance. The baseline AUC of the algorithm was 0.70 ± 0.02; inclusion of 9p21 genotype in the algorithm increased the AUC by 1% to 0.71. Stratification by gender showed a larger increase in AUC in women (0.65 to 0.68) than men (0.66 to 0.67). NRI of the baseline algorithm score compared to the Diamond-Forrester clinical risk score (age, gender, chest pain type) was 20%; addition of 9p21 genotype increased this to 24.6%. **Conclusions**: Combining 9p21 genotype with an existing gene-expression based algorithm for CAD detection resulted in a modest increase in algorithm performance, suggesting that the combination of DNA sequence variants and gene-expression profiling may improve CAD detection. An additional set of previously identified CAD-associated SNPs have also been genotyped and assessed for enhancement of algorithm performance.

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Prolonged corrected QT interval in a patient with Paramyotonia Congenital/Hyperkalemic Periodic Paralysis overlap phenotype. *T. Greer[1], B. Powell[1], J. Caton[2], A. Dubin[3].* 1) Med Gen/Metabolism, Children's Hosp Central CA, Madera, CA; 2) Cardiology, Children's Hosp Central CA, Madera, CA; 3) Pediatric Heart Center, Lucile Packard Children's Hosp at Stanford, Palo Alto, CA.

Human skeletal muscle channelopathies are a group of disorders characterized by abnormal ion channel function resulting in muscle fiber overactivity, leading to generalized myotonia, muscle stiffness, cramping, or pain in affected individuals. Mutations occur in genes that encode for potassium (KCNJ2), calcium (CACNA1S), or sodium channels (SCN4A) cause ineffective activation and inactivation. Paramyotonia Congenita (PC) and Hyperkalemic Periodic Paralysis (hyperPP) are just two of six channelopathies that have been well described. Both have autosomal dominant mode of inheritance, with variable expression, even within families. Over 30 disease-causing mutations have been identified in the SCN4A gene that encodes the skeletal muscle voltage-gated sodium channel Nav1.4 in patients with PC and hyperPP. HyperPP is characterized by weakness that occurs with rest after exercise, fasting, cold exposure, and oral potassium loads. Patients with PC generally have attacks of stiffness induced and exacerbated by continued exercise or exposure to cold. There is an overlap phenotype with episodes of paralysis and myotonia that occur independently. Cardiac conduction abnormalities are not generally seen in these phenotypes. Periodic paralysis due to mutations in the KCNJ2 gene (Andersen-Tawil syndrome) can be associated with fatal ventricular dysrhythmias due to prolonged QTc intervals. Also, mutations in the sodium channel gene SCN5A, known to be exclusively expressed in heart tissue, are the cause of a rare cardiac dysrhythmia disorder (Long QT-3 variant syndrome). Péréon, Lande, et al. were the first to demonstrate expression of SCN4A in human heart and reported a family with a SCN4A mutation (R1448C) with the HyperPP/PC overlap phenotype and prolonged QTc intervals. We will describe a second patient with a SCN4A mutation, T704M (n2111C>T), who had the hyperPP/PC overlap muscle phenotype and asymptomatic prolonged QTc. We will describe this unique clinical presentation and the electrophysiological findings. We will also review the evolving understanding of gated sodium channel function in cardiac muscle physiology.

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In-depth metabolic characterization of genetic loci underlying serum-lipids. *A.K. Petersen[1], S.Y. Shin[2], W. Römisch-Margl[3], G. Zhai[4], K. Small[4], R. Wang-Sattler[1], E. Grundberg[2,4], J.S. Ried[1], A. Peters[1], B. Kato[4], A. Döring[1], H.E. Wichmann[1,5,6], P. Deloukas[2], M. Hrabé de Angelis[7,8], H.W. Mewes[3,9], T. Illig[1], T.D. Spector[4], J. Adamski[7,8], K. Suhre[3,10], N. Soranzo[2,4], C. Gieger[1].* 1) Institute of Epidemiology, Helmholtz Zentrum Muenchen, Neuherberg, Germany; 2) Wellcome Trust Sanger Institute, Genome Campus, Hinxton, CB10 1HH, United Kingdom; 3) Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum Muenchen, Neuherberg, Germany; 4) Department of Twin Research & Genetic Epidemiology, King's College London, London SE1 7EH, United Kingdom; 5) Institute of Medical Informatics, Biometry and Epidemiology, Ludwig-Maximilians-Universität Munich, Germany; 6) Klinikum Grosshadern, Munich, Germany; 7) Institute of Experimental Genetics, Genome Analysis Center, Helmholtz Zentrum Muenchen, Neuherberg, Germany; 8) Institute of Experimental Genetics, Life and Food Science Center Weihenstephan, Technische Universität Muenchen, Munich, Germany; 9) Department of Genome-oriented Bioinformatics, Life and Food Science Weihenstephan, Technische Universität Muenchen, Munich, Germany; 10) Faculty of Biology, Ludwig-Maximilians-Universität, Munich, Germany.

Emerging technologies based on mass spectrometry and nuclear magnetic resonance enable the monitoring of hundreds of small metabolites from tissues or body fluids. Because metabolites change rapidly in response to physiologic perturbations, such metabolite concentrations provide a direct readout of the physiological state in the human body, allowing the discovery of novel proximal biomarkers of disease phenotypes. Furthermore, profiling of metabolites in relevant biological pathways can help elucidate the contribution of genetic variants underlying inherited variation in established risk factors. Among the major risk factors for coronary artery disease and myocardial infarction are serum lipids, including total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG). Using a high-throughput metabolomics platform we quantify metabolites covering a biologically relevant panel of amino acids, sugars, acylcarnitines, and phospholipids. Together with cholesterol, phosphatidylcholines and sphingomyelines species are the major building blocks of the cell membrane. They are biochemically closely linked to TGs by the intermediate of diacyl-glyceride in the Kennedy pathway and by the shared pool of fatty acids that make up their respective side chains. To dissect the effect of published genetic variants influencing serum lipid levels, we tested 151 metabolites in 1,797 participants from the KORA population (Germany) for association and replicated the results in 1,176 participants of the TwinsUK cohort. By analysing lipid concentrations in conjunction with genetic data and metabolite traits (and their ratios), we aim to identify cases where a genetic locus is associated with both a lipid and a metabolite concentration, which would provide new functional information about the underlying biological processes. We report here the initial results of this effort and discuss methodological approaches to metabolite analyses in the context of large population studies. Among others, we identify associations of variants in the *APOA* gene cluster with different ratios of phosphatidylcholines and sphingomyelines ($P = 2.5 \times 10^{-15}$). The here reported co-associations of the *APOA* gene cluster with TG and HDL together with phosphatidylcholines and sphingomyelines now allow us to dissect the effect of this gene cluster on the biochemistry of Apolipoproteins.

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FREQUENCY OF VARIANTS OF LONG QT DISEASE GENES IN PATIENTS WITH DRUG-INDUCED LONG QT SYNDROME. *T. Zhang, F. Del-carpio, S. Yao, M. Ahearn, R. Myerburg, N. Bishopic.* University of MIami Miller school of Medicine, Miami, FL.

Background: The long QT syndrome (LQTS) is a hereditary disorder of myocardial repolarization, resulting from mutations in cardiac ion channel genes, and associated with increased risk of arrhythmia and sudden death. Similar arrhythmias and QT prolongation can be induced by certain drugs in a fraction of patients (drug-induced LQTS, diLQTS), and it has been hypothesized that susceptibility to diLQTS may be genetically based. We performed a prospective study of the prevalence of mutations and polymorphisms in genes associated with congenital LQTS among otherwise unselected patients with drug-induced QT lengthening, with or without torsade de pointes (TdP). Method and results: 45 patients with pharmacologically-induced QT prolongation (490-601 msec) and/or TdP were recruited (mean age 55, range 27-95; M:F 1:0.7). Drugs most frequently associated with QT changes were levofloxacin, azithromycin, amiodarone, and haloperidol. Individual exons and intron/exon boundaries of 5 LQTS-associated genes (KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2) were screened for genetic variants. At present complete results of mutational analysis in 28 patients are available. In those, we identified 10 non-synonymous polymorphisms (2 in KCNQ1, 3 in KCNH2, 4 in SCN5A and 1 in KCNE1), 10 synonymous polymorphisms (3 in KCNQ1, 5 in KCNH2 and 2 in SCN5A), and 5 intronic polymorphisms. No variants were detected in KCNE2. Of these 25 variants, 11 have not previously been reported. Conclusion: In this single hospital study of diLQTS, we identified a high frequency of variants in genes associated with congenital LQTs, including 11 novel variants. Locally relevant and ethnically matched control samples have been developed and functional analysis in the novel variants is underway.

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Genetic modifiers predisposing to congenital heart disease in a sensitized population. *H. Li[1], S. Cherry[1], C. Maslen[2], T. Wiltshire[3], R. Reeves[1].* 1) Department of Physiology and Institute for Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA; 2) Department of Medicine, Division of Endocrinology, Oregon Health & Science University, Portland, Oregon, USA; 3) Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC, USA.

Congenital heart disease (CHD), the most frequent birth defect in human beings, has long been associated with complex genetic syndromes. Trisomy for human chromosome 21 (Hsa21) is the most frequent risk factor for CHD. The frequency of CHD in individuals with Down syndrome (DS) is about 50 times higher than in the general population. However, 50% of people with DS have a normal heart, thus, trisomy 21 is not sufficient to cause CHD. Additional genetic components may contribute to the perturbations resulting from gene dosage effects in trisomy. We have identified polymorphisms in the *CRELD1* gene that are associated with CHD in individuals with DS. To establish a biological basis for this interaction, we have initiated studies of CHD in mouse models of Down syndrome. The *Creld1* null allele was introduced into Ts65Dn mice, which are trisomic for orthologs of about half of the genes on Hsa21. We provide direct evidence that reduced *Creld1* expression exacerbates the occurrence of septal defects in *Ts65Dn, Creld1+/-* trisomic mice. To focus the search for trisomic genes that interact with *Creld1*, we introduced the *Creld1* null allele into Ts1Cje mice. Ts1Cje mice are trisomic for approximately 80% of the trisomic genes in Ts65Dn. Reducing *Creld1* expression in Ts1Cje by crossing in the null allele did not affect the frequency of heart defects in this model, thus it appears that one or more of the 23 conserved genes that is trisomic in Ts65Dn but disomic in Ts1Cje is necessary for the Creld1 interaction affecting heart development. We have undertaken a genome scan in mice to find more disomic CHD modifiers by comparing *Ts65Dn, Creld1+/-* mice with or without septal defects. These results will be compared with an analogous approach in a case/ control study of people who have Down syndrome with or without CHD.

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A fine-mapping study for left ventricular mass on chromosome 12p11 identifies potential candidate genes. *A. Beecham[1], D. Della-Morte[2], M.R. Di Tullio[3], L. Wang[1], M.S. McClendon[2], S. Slifer[1], T. Rundek[2], R.L. Sacco[1,2], S.H. Blanton[1].* 1) Department of Human Genetics, Hussman Institute for Human Genomics, Miller School of Medicine, University of Miami, Miami, FL, USA; 2) Department of Neurology and Epidemiology, Miller School of Medicine, University of Miami, Miami, FL, USA; 3) Department of Medicine, Columbia University, New York, NY, USA.

Left ventricular mass (LVM) is an important risk factor for stroke and cardiovascular disease. Previously we found evidence for linkage on chromosome 12p11 (LOD = 3.11) in 1360 individuals from 100 Dominican families, with a significant increase (LOD = 4.45) in a subset of families with high average waist circumference (WC) using ordered subset analysis (OSA). In the present study, we use fine-mapping to confirm and further study the genetic effect on LVM. Association analysis was done in the 1 LOD critical region of the linkage peak in an independent sample of 895 primarily Hispanic individuals (65%). Genotype data were available on 7085 SNPs in the region from 23 to 53 MB. An additive genetic model was used, and adjustment was made for age, sex, BMI, SBP, diabetes, and population substructure (PCA1 and PCA2 from Eigenstrat). In order to follow-up on the OSA, analysis was subset by high and low WC. High WC was defined as ≥ 40 inches in men and 35 inches in women. There were 488 individuals with high and 396 individuals with low WC. Genotype data were available on 4334 SNPs in the 1 LOD critical region of the OSA peak from 23 to 41 MB. To test the difference in genetic effects between the high and low subsets, a Z-score was computed for the difference in β coefficients and significance assessed. The most significant association was in rs10743465, located downstream of the SOX5 gene (p=9.68E-06), which remained significant after multiple testing correction by SimpleM. 17 additional SNPs had p < 0.001, 15 of which were located in or near genes: SOX5(5), SLC38A1(3), BICD1(2), RACGAP1(1), C12orf68(1), PKP2(1), and BHLHE41(2). The most significant difference in genetic effect between those with high and low WC occurred at rs10877703 (p=6.41E-05) in an intron of SLC2A13. 11 additional SNPs had p < 0.001, all located in or near genes: SLC2A13(1), TMTC1(3), ITPR2 (1), CCDC91(2), DDX11(3), and PKP2(1). Evidence for a genetic effect on LVM has been shown on chromosome 12p using both linkage and fine-mapping. SOX5 may play a role in the regulation of LVM and is a good candidate as it has been found associated with other cardiac variables, such as PR interval in a recent genome-wide association study. An interaction of SLC2A13 and other genes with abdominal obesity may contribute to phenotypic variation of LVM. Validation studies are needed to confirm associations, particularly in other populations.

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C4BPB/C4BPA Locus as a New Susceptibility Locus for Venous Thrombosis: Results from Genome-Wide Association and Gene Expression Analyses Followed by Case-Control Studies. *A. Buil[1], D.A. Tregouet[2], J.C. Souto[3], N. Saut[4], M. Germain[2], M. Rotival[2], L. Tiret[2], F. Cambien[2], M. Lathrop[5], T. Zeller[6], M.C. Alessi[4], S. Rodriguez de Cordoba[7], T. Munzel[6], P. Wild[6], J. Fontcuberta[3], F. Gagnon[8], J. Emmerich[9], L. Almasy[10], S. Blankenberg[6], J.M. Soria[1], P.E. Morange[4].* 1) Unit of Genomics of Complex Diseases, Research Institut Hospital de la Santa Creu i Sant Pau, Barcelona, Spain; 2) INSERM UMRS 937; Université Pierre et Marie Curie, Paris, France; 3) Haematolgy Department. Hospital de la Santa Creu i Sant Pau, Barcelona, Spain; 4) INSERM, UMR_S 626, F-13385, Marseille, France; 5) Centre National de Génotypage, Evry, France; 6) Medizinische Klinik und Poliklinik, Johannes-Gutenberg Universität Mainz, Germany; 7) Departamento de Inmunología, Centro de Investigaciones Biológicas, CSIC. Madrid; 8) Dalla Lana School of Public Health, University of Toronto, Ontario, Canada; 9) INSERM U765, médecine vasculaire - HTA, hôpital européen Georges-Pompidou, Paris, France; 10) Population Genetic Department. Southwest Foundation for Biomedical Research. San Antonio (TX), USA.

The C4b-binding protein (C4BP) has been hypothesized to be involved in the susceptibility to venous thrombosis (VT) through its binding with protein S (PS), a key element of the coagulation/fibrinolysis cascade. To identify genetic factors that may influence the plasma levels of the three C4BP existing isoforms, Alpha7Beta1, Alpha6Beta1, Alpha7Beta0, we conducted a genome-wide association study by analyzing 283,437 single nucleotide polymorphisms (SNPs) in the GAIT study composed of 387 individuals. Three SNPs at the C4BPB/C4BPA locus were found genome-wide significantly associated with Alpha7Beta0 levels. One of these SNPs was further found to explain ~11% of the variability of mRNA C4BPA expression in the Gutenberg Heart Study composed of 1,490 individuals, with no effect on C4BPB mRNA expression. The allele associated with increased Alpha7-Beta0 plasma levels and increased C4BPA expression was further found associated with increased risk of VT (OR = 1.24 [1.03 - 1.53]) in two independent case-control studies (MARTHA and FARIVE) gathering 1706 cases and 1379 controls. This SNP was not associated to free PS nor total PS. In conclusion, we observed strong evidence that the C4BPB/C4BPA locus is a new susceptibility locus for VT through a PS independent mechanism that remains to be elucidated.

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Study of 9p21 genomic region and *USF1* gene associated with predisposition to cardiovascular diseases in Azorean healthy population. *M. Correia[1], N. Bruffaerts[1], A. Balagué[1], T. Pereirinha[1], C. C. Branco[1,2], L. Mota-Vieira[1,2].* 1) Molecular Genetics and Pathology Unit, Hospital of Divino Espírito Santo of Ponta Delgada, EPE, Azores, Portugal; 2) Instituto Gulbenkian de Ciência, Oeiras, Portugal.

Cardiovascular diseases (CVD) are a major source of morbidity and mortality worldwide, including the Azores archipelago where the CVD has a higher mortality rate when compared to mainland Portugal. In order to investigate this question, we characterized 11 SNPs in two regions associated with risk of CVD: 4 on *USF1* gene (rs10908821, rs2516839, rs1556259, rs2774279) and 7 on 9p21 region (rs10116277, rs10757274, rs4977574, rs2833206, rs2383207, rs107557278, rs1333049), covering 9.0 and 44.1 kbp, respectively. Genotyping was performed by real time PCR using TaqMan Assays in a sample of 170 healthy blood donors. The average gene diversity was higher for markers in 9p21 (0.491) than for *USF1* (0.327). Although Azoreans' allele frequencies for 10 SNPs were in agreement with those reported for other European populations, the rs10116277 in 9p21 showed significantly different values (χ^2, p<0,05). All markers were in HWE, excluding two SNPs - rs10116277 and rs2833206 - in 9p21 region, which presented the highest heterogosity (0.776 and 0.759, respectively) and did not show homozygous variant (TT) neither homozygous normal (AA), respectively. Among 18 haplotypes, the most frequent (0.317) was the haplotype risk "**TGGGGGC**" for CAD, MI, stroke and atherosclerosis. Regarding the *USF1* gene 8 haplotypes were estimated, being the "C**T**AA" (0.291) and the "C**T**A**G**" (0.354) the two most frequent. The last one seems to be the haplotype with the highest CVD risk in females, corroborating previous literature data. In order to improve the knowledge of CVD in Azoreans, we are carrying out the joint analysis of all variants of the 9p21 genomic region and the *USF1* gene. We also intend to extend the present study to other candidate gene or regions, which has been recently associated with CVD. Collectively, the results will constitute a valuable resource for future case-control studies on CVD in the Azorean population. (lmotavieira@hdes.pt, Funded by the Azores Government, M 1.2.1./I/002/2008).

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Generalizability of GWAS-identified SNPs associated with lipid traits in the Population Architecture using Genomics and Epidemiology (PAGE) study. *D.C. Crawford[1, 2], L. Dumitrescu[1], S.A. Pendergrass[1], K. Taylor[4], C. Carty[6], M. Quibrera[5], N. Franceschini[4], S.A. Cole[7], F. Schumacher[8], M. Fornage[9, 10], C.B. Eaton[11], S. Buyske[12], L.A. Hindorff[13], T.A. Manolio[13], J. MacCluer[7], K. Brown-Gentry[1], P. Buzkova[14], C.S. Carlson[6], J. Haessler[6], G. Anderson[6], K. Johnson[15], S. Laston[7], B. Cochran[9], E.T. Lee[16], L.G. Best[17], R.B. Devereux[18], B. Howard[19], C. Kooperberg[6], K.E. North[3,4].* 1) Center for Human Genetic Research, Vanderbilt University, Nashville, TN; 2) Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN; 3) Carolina Center for Genome Sciences, University of North Carolina, Chapel Hill, NC; 4) Department of Epidemiology, University of North Carolina, Chapel Hill, NC; 5) School of Public Health, University of North Carolina, Chapel Hill, NC; 6) Public Health Sciences, Fred Hutchison Cancer Research Center, Seattle, WA; 7) Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX; 8) Keck School of Medicine, University of Southern California, Los Angeles, CA; 9) Institute of Molecular Medicine, University of Texas Health Sciences Center, Houston, TX; 10) Division of Epidemiology School of Public Health, University of Texas Health Sciences Center, Houston, TX; 11) Alpert Medical School of Brown University School of Medicine, Providence, RI; 12) Department of Statistics & Biostatistics, Rutgers University, Piscataway, NJ; 13) Office of Population Genomics, National Human Genome Research Institute, Bethesda, MD; 14) Department of Biostatistics, University of Washington, Seattle, WA; 15) Department of Preventive Medicine, University of Tennessee, Memphis, TN; 16) University of Oklahoma Health Sciences Center, Oklahoma City, OK; 17) Missouri Breaks Industries Research Inc, Timber Lake, SD; 18) Department of Medicine, Weill Cornell Medical College, New York, NY; 19) Medstar Research Institute, Washington, DC.

Genome-wide (GWAS) and candidate gene association studies have identified ~40 genes/genomic regions assoicated with high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), and triglycerides (TG). The majority of these studies have been performed in populations of European-descent, and it is unclear if these associations will generalize to other populations. The Population Architecture using Genomics and Epidemiology (PAGE) network was established in 2008 to characterize GWAS-identified variants in diverse population-based studies including Atherosclerosis Risk in Communities, Cardiovascular Health Study, Coronary Artery Risk Development in Young Adults, Multiethnic Cohort, National Health and Nutrition Examination Surveys, Strong Heart Family Study, and Women's Health Inititative. We targeted GWAS-identified SNPs in at least two PAGE studies and performed tests of association assuming an additive genetic model adjusted for age and sex followed by a fixed effects meta-analysis for fasting HDL-C, LDL-C, and ln(TG) levels in ~20,000, ~9,000, and ~2,500 self-identified European American (EA), African American (AA), and Mexican American (MA) adults regardless of lipid lowering medication use. With these sample sizes, we had 80% power at $p<0.05$ to detect effect sizes (β) as low as 0.46, 0.69, and 1.30 for HDL-C; 0.86, 1.28, and 2.43 for LDL-C; and 0.02, 0.03, 0.05 for ln(TG) for EA, AA, and MA, respectively. As expected, most SNPs tested were significant at $p<0.05$ for EA (21/23, 18/19, 10/11) and less so for AA (8/23, 9/19, 5/11) and MA (6/16, 6/12, 7/8) for HDL-C, LDL-C, and ln(TG), respectively. Little overlap was identified across the populations for the three traits: 8/23, 8/19, and 4/11 were significant in both EA and AA while 4/16, 3/12, and 3/8 were significant in all three groups for HDL-C, LDL-C, and ln(TG), respectively. All overlapping associations were significant in the same direction. SNPs that were significant across all three groups included *LPL* rs2197089, *LPL* rs328, *LPL* rs6586891, and *APOA5* rs3135506 for HDL-C; *PCSK9* rs11206510, *PCSK9* rs11591147, and *LDLR* rs6511720 for LDL-C; and *GCKR* rs1260326, *MLXIPL* rs17145738, and *ANGPTL3* rs1748195 for ln(TG). Results from this PAGE study suggest that GWAS-identified variants from studies of European-descent may not necessarily generalize to other populations possibly due to differences in effect sizes, allele frequencies, and linkage disequilibrium.

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Impact of Coagulation Factors on Myocardial Infarction-A hospital based study. *S. Dayakar[1,2], S. Komandur[3,4], P. K. Parvathi Thavanati[5], S. Naik[6], V. Bhupesh Kumar[1], B. Shyamala Sasekeran[2], M. D. Sadhnani[1].* 1) Department of Clinical Biochemistry, Institute of Genetics and Hospital for Genetic Diseases,Osmania University,Begumpet,Hyderabad-500016,India,Email:dayaphdgenetics@gmail.com; 2) Apollo Hospitals Educational & Research Foundation, Apollo Health city, Jubilee hills, Hyderabad-500033, India; 3) Department of Molecular Medicine, Apollo Health city, Jubilee hills, Hyderabad-500033, India; 4) Department of Cytogenetics, Vijaya Diagnostic Centre, Himayatnagar, Hyderabad -500029, India; 5) Instituto de Genetica,Dpto.de Biologia Molecular Y Genomica,Centro Universitario de Ciencias de la Salud,Universidad de Guadalajara, Guadalajara, Jalisco, Mexico; 6) Heart Institute, Apollo Health city, Jubilee hills, Hyderabad-500033, India.

Advances in understanding the pathophysiology of Myocardial Infarction (MI) have led to a marked increase in the development of biomarkers for diagnosis, risk stratification, therapeutic decision-making, and assessment of clinical outcomes. It has been shown that,the incidence of MI was related to be high in the presence of various polymorphisms among the genes encoding for various factors, including haemostatic under the influence of environmental predisposition i.e., life styles factors like smoking, alcohol consumption, dietary habits etc. However, their contribution in the development of myocardial infarction (MI) remains controversial.

In the present study, we assessed the sequence variations in three coagulation factors, glycoprotein IIb/IIIa (PI^A), factor V Leiden (FVL) and Prothrombin (FII), in 102 patients with MI and 112 controls(who had not documented history of either stable or unstable angina or myocardial infarction). $PI^{A1/A1}$ genotype was observed in 58.7%, $PI^{A1/A2}$ in 20.61% and $PI^{A2/A2}$ in 20.61% among the patients group. While in control group, 83.92% had $PI^{A1/A1}$ genotype,12.5%$PI^{A1/A2}$ and 3.57% carried $PI^{A2/A2}$, which indicated that PI^{A2} was 2.4 times higher in patient group. Only one patient carried FV Leiden (G1619A) while another patient showed FII mutation (G20210A), both in heterozygous state. Owing to a small number with mutation, statistical analysis was carried out for risk factors (smoking status, hypertension, hypercholesteremia and diabetes mellitus) that differed significantly (P value 0.001, <0.0001, 0.0024 and <0.0001 respectively) between the patient and the control groups.

It can be concluded that, an understanding of gene-environment interactions along with inherited markers can ultimately be used to manage complex diseases through a better utilization of diagnosis,application of pharmacogenetics and genetic counseling.

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Genome-wide linkage and follow-up association analyses of carotid plaque in Caribbean Hispanics. *C. Dong[1], A. Beecham[2], D. Cabral[1], S.H. Blanton[2], R.L. Sacco[1,2,3], T. Rundek[1].* 1) Department of Neurology, University of Miami, Miami, FL; 2) John T. McDonald Department of Human Genetics, John P Hussman Institute for Human Genomics, University of Miami, Miami, FL; 3) Department of Epidemiology, University of Miami, Miami, FL.

Atherosclerosis, a complex subclinical cardiovascular disorder with a substantial genetic component, is the pathology underlying most ischemic strokes and heart attacks. Dissecting the genetic underpinnings of atherosclerosis, therefore, is of great value in assessing an individual's future risk for stroke and cardiovascular disease. This study sought to identify genetic loci influencing carotid plaque in two independent samples. B-mode ultrasound was performed to determine the presence and area of carotid plaque. Power transformation on total plaque area was first conducted to reduce the skewness and kurtosis in both linkage and follow-up association analyses. Variance components analysis was performed to test for linkage using 383 autosomal microsatellite markers in 1,308 subjects from 100 Dominican families. Generalized linear models were used to investigate the association between plaque traits and 18,917 single nucleotide polymorphisms (SNPs) under 1-LOD down regions of linkage peaks in an independent community-based dataset (N=928, 41% Dominicans) from the Northern Manhattan Study (NOMAS). Principal component approach was employed to control for population stratification in the follow-up association analysis. After adjustment for age, hypertension, diabetes mellitus, cigarette pack-years, body mass index, and waist-to-hip ratio, significant heritability was detected for plaque presence ($h^2=0.50\pm0.14$, $p<0.0001$) and plaque area ($h^2=0.17\pm0.04$, $p<0.0001$). Quantitative and dichotomous trait linkage analyses obtained similar results and identified four regions with multipoint LOD scores ≥2.00 on 7q36, 11p15, 14q32 and 15q23. In the follow-up association analysis of four linkage peaks, several SNPs in or near *SOX6, FSD2, AP3S2* and *EFTUD1* were associated with carotid plaque traits with a nominal p £0.0005 in the NOMAS dataset and with a p £0.01 in a Dominican subset. Our findings indicate that carotid plaque has considerable heritability and may be influenced by loci on chromosomes 11p15, 14q32 and 15q23 in Caribbean Hispanics. The *SOX6* gene within the Bone Morphogenic Protein pathway may be of particular interest and could be a candidate for carotid plaque. Further studies in larger populations are needed to validate the observed association, as well as in depth investigation of the genes in these regions to provide fundamental insight to the understanding of atherosclerotic disease.

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***LPA* common variation is associated with Lp(a) levels and myocardial infarction in non-Hispanic blacks in the Third National Health and Nutrition Examination Survey.** *L. Dumitrescu[1,2], K. Glenn[1], K. Brown-Gentry[1], C. Shephard[3], M. Wong[3], M.J. Rieder[3], J.D. Smith[3], D.A. Nickerson[3], D.C. Crawford[1,2].* 1) Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 2) Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN; 3) Department of Genome Sciences, University of Washington, Seattle, WA.

Lipoprotein(a) [Lp(a)] levels are an emerging risk factor for cardiovascular disease (CVD) and have been shown to be highly heritable. While much of the genetics impacting Lp(a) levels has been attributed to the kringle IV-2 (KIV-2) copy number variant in *LPA*, the gene that encodes the apo(a) component of Lp(a), the remaining variance has yet to be explained. Currently, the role of other cis-acting factors in *LPA* to the contribution of Lp(a) levels and its relevance to CVD is not completely understood. Furthermore, the extent to which these genetic variants can help to explain the significant differences in levels and risk across diverse populations is sorely understudied. To identify genetic variants associated with Lp(a) levels and possibly myocardial infarction (MI), 20 tagSNPs in *LPA* were genotyped in 7,159 individuals from the Third National Health and Nutrition Examination Survey (NHANES III). NHANES III is a diverse population-based survey with samples linked to hundreds of quantitative traits, including serum Lp(a). Using linear regression, 19 SNPs were tested for an association with transformed Lp(a) levels adjusting for age and sex. Tests of association between *LPA* variants and possible/probable myocardial infarction (MI) were also performed on a subset of participants that underwent electrocardiogram examination. Across the three different NHANES subpopulations (non-Hispanic whites, non-Hispanic blacks, and Mexican Americans), 15 of the 19 SNPs tested were strongly associated with Lp(a) levels in at least one subpopulation at $p<0.0001$. The strongest association (rs1652507, $p=5.4x10^{-34}$, $\beta=-0.54$) was found in Mexican Americans. This association was also observed in non-Hispanic blacks ($p=1.1x10^{-10}$, $\beta=-0.45$) but not in non-Hispanic whites ($p=0.57$, $\beta=0.21$). The additive effects of these associated alleles explained up to 12% of the variance in observed for Lp(a) levels. Additionally, five SNPs were associated with MI risk in non-Hispanic blacks at $p<0.05$. In contrast to previous reports in non-Hispanic whites, alleles associated with increased Lp(a) levels were also associated with decreased risk of MI in non-Hispanic blacks. These results demonstrate that genetic variation within *LPA*, outside and independent of the KIV-2 region, is associated with Lp(a) levels in the general population and may be associated with MI risk in non-Hispanic blacks in a paradoxical manner compared to non-Hispanic whites.

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The transcriptome of human epicardial, mediastinal and subcutaneous adipose tissues in men with coronary artery disease. *S. Guauque-Olarte[1], N. Gaudreault[1], D. Fournier[1], P. Mauriège[1,2], P. Mathieu[1], Y. Bossé[1,3].* 1) Centre de recherche Institut universitaire de cardiologie et de pneumologie de Québec, Laval University, Québec, Canada; 2) Division of kinesiology, Department of social and preventive medicine, Faculty of medicine, Laval University, Québec, Canada; 3) Laval University Hospital Research Center (CRCHUL), Québec, Canada.

The biological functions of epicardial adipose tissue (EAT) remain largely elusive. However, the proximity of EAT to the coronary arteries suggests a role in the pathogenesis of coronary artery disease (CAD). Objectives of this study were to identify genes that are up- or down-regulated among three adipose tissues (AT), namely EAT, mediastinal and subcutaneous and to study their possible relationships with the development of cardiovascular diseases. Samples were collected from patients undergoing coronary artery bypass grafting surgeries. Gene expression was evaluated in all three AT depots of six men using the Illumina® HumanWG-6 v3.0 expression BeadChips. The Significant Analysis of Microarrays (SAM) method was utilized to identify genes differentially expressed among the fat depots. The false discovery rate and the fold change thresholds were set at 10% and 2.0, respectively. The Ingenuity Pathway Analysis system was used to identify the principal biofunctions among differentially expressed genes. The expression of four genes of interest was verified by quantitative PCR (qPCR) in six technical and 25 biological replicates. Twenty-two and 72 genes were up-regulated in EAT compared to mediastinal and subcutaneous AT, respectively. Ninety-four were down-regulated in EAT compared to subcutaneous adipose depot. However, none were significantly down-regulated in EAT compared to mediastinal fat. The expression of the adenosine A1 receptor (ADORA1), involved in myocardial ischemia, was significantly up-regulated in EAT. Levels of the prostaglandin D2 synthase (PTGDS) gene, recently associated with the progression of atherosclerosis, were significantly different in the three pairwise comparisons (epicardial > mediastinal > subcutaneous). The results of ADORA1 and PTGDS were confirmed by qPCR in biological replicates. Overall, the transcriptional profiles of EAT and mediastinal AT were similar compared to the subcutaneous compartment. Despite this similarity, two genes involved in cardiovascular diseases, ADORA1 and PTGDS, were up-regulated in EAT. These results provide great insights about the biology of the EAT and its potential implication in CAD.

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Stem Cell Regulatory Gene Polymorphisms Associated with the Promotion of Coronary Artery Disease in Tobacco Smokers. *K.L. Hamilton-Nelson[1], I. Konidari[1], P. Goldschmidt-Clermont[2], M.A. Pericak-Vance[1], D. Seo[2], R. Levitt[3], G.W. Beecham[1].* 1) John P Hussman Institute for Human Genomics, Univ Miami, Miller School of Medicine, Miami, FL; 2) Department of Medicine, Division of Cardiology, Univ Miami, Miller School of Medicine, Miami, FL; 3) Department of Anesthesiology, Univ Miami, Miller School of Medicine, Miami, FL.

BACKGROUND While it is known that CAD is heritable and significantly impacted by tobacco smoke, the mechanisms remain elusive. Recent evidence suggests that biologic variability in stem cell mediated vascular repair may play an important role in coronary artery disease (CAD). We hypothesize that functional polymorphisms in stem cell regulatory genes underlie the promotion of CVD by tobacco smoke in susceptible individuals. To investigate this further, we analyzed a set of 43 candidate genes known to be involved in the regulation of stem senescence and that could affect stem cell mediated vascular repair. METHODS An existing dataset of 1050 cardiac catheterization patients, used in the first genome-wide association study of anatomic coronary artery disease served as the dataset for these analyses. Cardiac angiograms were used to assign a CAD burden index based on size and location of coronary lesions. Smoking status was self-reported. Genotype data were generated using the Affymetrix SNP array 6.0 platform for 900,000 SNPs. Data integrity was ensured with extensive quality controls including sample filters (gender consistencies, efficiency thresholds, etc), SNP filters (Hardy-Weinberg equilibrium, genotyping efficiency, MAF, etc). EIGENSTRAT methods were used to correct for population substructure. Linear regression implemented with PLINK software was used to conduct candidate gene association analyses. Analyses were adjusted for cholesterol, blood pressure, age, sex, and population substructure. Since the stem cell reservoir is known to decrease with age and smoking status, interaction terms between SNP and age, and SNP and smoking status were included in the model. RESULTS Preliminary analyses indicate significant associations between CAD and SNPs in stem cell regulatory genes WNT7A, WNT5A, and MEF2A among smokers ($p<0.006$). More comprehensive analyses are being conducted to further define the association between these candidate genes and smoking status in these subjects. CONCLUSION These data indicate for the first time that WNT7A, WNT5A and MEF2A may be implicated in the promotion of CAD by tobacco smoke. Confirmation in our larger dataset is underway as well as analyses in different populations with differing racial representation. These data provide new insights into how tobacco smoke may promote CAD by altering stem cell mediated vascular repair.

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Evidence for a novel locus influencing longitudinal adult body weight change from the CHARGE Consortium. *C.E. Jaquish[1,5], C.C. White[2], K. North[3], K. Monda[3], M.H.J Lee[4], A.D. Johnson[5], M. Zillikens[6], T.B. Harris[7], A.V. Smith[8], V. Gundnason[8,9], C.M. van Duijn[6,10], A.C.J.W. Janssens[6], A. Hofman[6], A.G. Uitterlinden[6], M. Feitosa[11], I. Borecki[11], L.A. Atwood[4], J. Dupuis[2], L.A. Cupples[2], C.S. Fox[5].* 1) NHLBI/NIH, Bethesda, MD; 2) Department of Biostatistics, Boston University School of Public Health, Boston, MA; 3) University of North Carolina at Chapel Hill, School of Public Health, Chapel Hill, NC; 4) Department of Neurology, Boston University School of Medicine, Boston, MA; 5) Division of Intramural Research, National Heart, Lung and Blood Institute (NHLBI),NHLBI's Framingham Heart Study; 6) Department of Internal Medicine, Erasmus Medical Center, Rotterdam, The Netherlands; 7) Laboratory of Epidemiology, Demography, and Biometry, Intramural Research Program, National Institute on Aging, Bethesda, MD; 8) 8Icelandic Heart Association, Kopavogur Iceland; 9 University of Iceland, Reykjavik, Iceland; 9) University of Iceland, Reykjavik, Iceland; 10) 10Department of Epidemiology and Biostatistics, Erasmus University Medical Center,Rotterdam, The Netherlands; 11) Division of Statistical Genomics, Department of Genetics, Washington University, St. Louis, MO.

Weight gain is adversely associated with many cardiovascular risk factors such as blood pressure, lipids and glycemia. Few studies have looked at the underlying genetic contribution to change in body weight over the adult life span. We conducted a genome wide association study (GWAS), meta-analysi,s and expression SNP (eSNP) analysis of weight change between 20 and 65 years of age in 17,730 individuals of European descent from 6 cohorts participating in the CHARGE consortium. Longitudinal weight change was modeled using a mixed model with random effects, adjusting for age, age squared and sex among individuals with at least two weight measures at study examinations. GWAS and meta analysis revealed that no loci achieved genome - wide significance. However, several loci showed suggestive evidence of association (p<9x10-5). To further characterize these associations, we utilized eSNPs previously reported in the literature to be associated with altered expression in adipose tissue . After correcting for the number of eSNPs tested (5,182), we observed a suggestive signal at the PAX8 locus (p=7.69x10-5) . PAX genes encode a family of transcription factors that are required for the formation of several tissues in the mammalian embryo. In the thyroid gland, PAX8 is essential in thyroid development and function. PAX8 has been shown to activate expression of several thyroid-specific genes and has been associated with congenital hypothyroidism. Thus, through its action in the thyroid gland PAX8 provides a novel candidate gene and plausible biological pathway for its association with weight change. Further studies are required to replicate and validate these findings.

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Rare coding mutations and risk for early-onset myocardial infarction: an exome sequencing study of 1,300 cases and controls. *S. Kathiresan for the NHLBI's Exome Sequencing Program - Early-onset Myocardial Infarction Project.* Program in Med & Pop Genetics, Broad Institute, Cambridge, MA.

Myocardial infarction (MI), the leading cause of death in the United States, is a heritable phenotype and the role for inheritance is greatest when MI occurs early in life. Genome-wide association studies of common variants have identified at least 13 loci associated with early-onset MI; however, the modest proportion of overall heritability explained by common variants suggests that low frequency (0.5% to 5% frequency) or rare (<0.5% frequency) variants may contribute to risk for early-onset MI. To test the hypothesis that rare coding mutations contribute to early MI risk, the U.S. National Heart, Lung, and Blood Institute's Exome Sequencing Program Early-onset MI Project is conducting a large-scale exome sequencing experiment. We are exome sequencing 650 cases with early onset-MI (men £50 and women £60; 450 of whom are of European ancestry and 200 of whom are of African American ancestry) and 650 controls free of MI. To enrich for protective alleles, we have specifically selected controls from population-based cohort studies with a high predicted risk for MI (based on traditional risk factors) but who are free of incident MI on follow-up. We are using solution-based hybrid selection to selectively capture and enrich the exome and next-generation sequencers (Illumina GAII) to sequence the exome. We have targeted 32.7 megabases at 188,260 exons from 18,560 genes. In the first 108 exomes sequenced, for each individual, we have generated ~6 billion bases of sequence, allowing for each targeted base to be read, on average, 177 times. For each individual, approximately 86% of all bases were covered with at least 20x depth. We are comparing several statistical methods recently developed to analyze the contribution of rare variants to complex traits including combined multivariate and collapsing methods (Li and Leal), weighted sum tests (Madsen and Browning), variable threshold tests (Price, Kryukov and Sunyaev), and c-alpha (Neale, Roeder, and Daly)]. The tests incorporate computational predictions of functional effects of rare variants. We have also implemented a network-based test that searches for sub-networks of genes with a significant difference in rare variant burden between cases and controls. We will present initial analyses for 1300 exomes at ASHG 2010.

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Effect of rs662799 (APO A5/A4/C3/A1) on HDL-C concentration is modified by physical activity. *H. Kitajima[1,3], K. Yamamoto[1], K. Ohnaka[2], R. Takayanagi[3], S. Kono[4].* 1) Division of Genome Analysis, Research Center for Genetic Information, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan; 2) Department of Advanced Medical Initiatives, Faculty of Medical Science, Kyushu University, Fukuoka, Japan; 3) Department of Medicine and Bioregulatory Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; 4) Department of Preventive Medicine, Faculty of Medical Science, Kyushu University, Fukuoka, Japan.

Serum high-density lipoprotein cholesterol (HDL-C) concentration is regulated by both genetic and life-style factors. Recent genome-wide association (GWA) studies have identified genetic variants related to HDL-C concentration. However, interaction between these variants and lifestyles has remained to be elucidated. We report here replication association analysis for the SNPs identified in the previous GWA studies, and SNP-lifestyle interaction analysis in the Japanese general population. We selected 15 SNPs of which MAF were over 0.1 in the HapMap JPT among the SNPs shown to be significantly associated with HDL-C concentration in GWA studies. The selected SNPs were located on the CETP, APO A5/A4/C3/A1, LIPC, LPL, LIPG, ABCA1, LCAT, ANGPTL4, APOB, GALNT2, NR1H3, MADD-FOLH1, FADS, MVK-MMAB, or chromosome 17p13.3 locus. We genotyped these SNPs in the Japanese ongoing cohort study (7,052 subjects) with TaqMan assay. First, we evaluated the association with HDL-C concentration adjusted for sex, age, BMI, alcohol consumption, smoking, and physical activity. The six SNPs, rs3764261 (CETP), rs662799 (APO A5/A4/C3/A1), rs1800588 (LIPC), rs10503669 (LPL), rs2156552 (LIPG), and rs4149268 (ABCA1) showed significant associations. The effect size of additive model was 9.6, 6.7, 5.0, 6.5, 3.7, and 2.6 mg/dl, and the P-value was $6.9x10^{-63}$, $2.1x10^{-45}$, $1.8x10^{-22}$, $7.0x10^{-19}$, $1.4x10^{-7}$, and $2.0x10^{-7}$, respectively. The remaining SNPs did not show significant associations. Second, we conducted the SNP-environment interaction analysis for these six SNPs. We found that rs662799 showed a significant interaction with physical activity in the condition of adjustment for sex, age, BMI, alcohol consumption, and smoking (P-value=0.008). Based on the result, we further analyzed the mean HDL-C concentration in each genotype and physical activity category. The differences of adjusted mean HDL-C concentration between the low and the high physical activity category were 0.6, 1.4, and 3.5 mg/dl in the risk allele homozygosity (G/G), heterozygosity (G/A), and non-risk allele homozygosity (A/A), respectively. In summary, we have confirmed the six loci significantly associated with HDL-C concentration in the Japanese general population cohort. The effect of the rs662799 (APO A5/A4/C3/A1) on HDL-C concentration may be modified by the physical activity. The risk allele G/G subjects may have an impaired adaptation to physical activity that could result in higher risk of atherosclerosis.

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The role of calcineurin pathway genes polymorphisms in cardiac parameters variability in normal subjects and patients with cardiovascular diseases. *O.A. Makeeva[1,2], V.M. Shipulin[3], O.G. Polovkova[1], A.A. Lezhnev[3], T.V. Zheykova[1], E.V. Kulish[4], K.V. Puzyrev[1], I.A. Goncharova[1], I.V. Tsimbal'uk[4], V.P. Puzyrev[1].* 1) Research Institute of Medical Genetics SB RAMS, Tomsk, Russia; 2) Research Institute of Complex Problems of Cardiovascular Diseases SB RAMS, Kemerovo, Russia; 3) Research Institute of Cardiology SB RAMS, Tomsk, Russia; 4) Siberian State Medical University, Tomsk, Russia.

Critical role of the calcineurin pathway (CP) in cardiac hypertrophy and heart failure had been demonstrated by experiments *in vitro* and studies of model organisms, though only few studies evaluated the role of CP genes polymorphisms in cardiac parameters variability and relation to different forms of cardiac remodeling. The **aim** of the study was to perform a systematic search for polymorphic variants in CP genes (*PPP3R1, PPP3CA, PPP3CB, GATA4*, and *NFATC4*) and to investigate their possible influence on myocardium characteristics in normal population and patients with cardiovascular diseases. **Methods**: A set of 30 polymorphisms with predicted functional significance, including those leading to amino-acid substitutions; variants in promoters; in predicted transfactor binding sites, and variants in conserved regions had been genotyped. Additionally, tagging SNPs and variants associated with cardiovascular phenotypes in previous researches were studied. A control group consisted of 287 unrelated subjects selected from general population according to normal echocardiographic parameters (1100 medical records were analyzed), patients with arterial hypertension (AH, n=250) and patients with ischemic heart disease (IHD, n=210) were enrolled for the study. Cardiac biopsy samples from 60 patients who undergo cardiac surgery were collected for CP genes expression profile study. **Results**: An association of the *PPP3R1* promoter insertion-deletion polymorphism (*5I/5D*) with left ventricular myocardium mass (LVMM), LVMM index, and LV walls thickness had been revealed in normal population. *5I/5D* variant was also associated with LVMM in patients with essential hypertension; the presence of *5I/5I* genotype was connected with larger echocardiographic indexes both in normal subjects and patients with hypertension. An association of the *GATA4* rs804271 with echocardiography characteristics and systolic blood pressure had been detected in healthy females; rs804271 was related with the presence of left ventricular hypertrophy (LVH) in essential hypertension group. *PPP3CA* tagSNP rs7696663 was associated with LVMMI and other cardiac parameters in patients with AH. *PPP3R1* tagSNP rs11126176 and *GATA4* rs10112596 and rs2898293 were associated the presence of LVH in essential hypertension sample. This preliminary data confirms the possible role of calcineurin pathway genes in cardiac remodeling and other clinically relevant phenotypes.

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Bayesian analysis of hepatic lipase (*LIPC*) genetic variation suggests different variants influence HDL-C level and HDL particle size. *P.E. Melton, K. Haack, T.D. Dyer, M.C. Mahaney, J.W. MacCluer, D.L. Rainwater, J. Blangero, L. Almasy, S.A. Cole.* Genetics Dept, Southwest Foundation for Biomedical Research, San Antonio, TX.

The gene for hepatic lipase (*LIPC*) is known to have a major role in lipoprotein metabolism and has been implicated as a regulator of HDL-C concentration and HDL particle size reduction through the reverse cholesterol transport pathway. However, the relationship between *LIPC* and HDL-C levels and HDL particle size phenotypes remains unclear. One *LIPC* promoter variant, -514C>T (rs1800588), has shown association with HDL-C and related phenotypes in a number of studies and is presumed to be functional. This study investigated the biological relationship between *LIPC* genetic variation and HDL-C and five HDL size measures, median diameter of particles containing apoA1 (A1), apoA2 (A2), unesterified cholesterol (UC), esterified cholesterol (EC), and ΔHDL, the difference in fractional absorbance patterns between large and small lipoprotein particles. We deep sequenced 28.7 kb coding and conserved non-coding regions of *LIPC* in 182 founders from 42 extended pedigrees and examined the resulting 625 single nucleotide polymorphism (SNP) genotypes in 1,336 Mexican American participants from the San Antonio Family Heart Study. Genetic association was assessed with measured genotype analysis (MGA) and SNPs with nominal p-values ($p < 0.1$) were used in Bayesian Quantitative Trait Nucleotide (BQTN) analyses. SNPs in high LD ($r2 > 0.9$) were grouped, with the single member with the lowest MGA p-value representing the isocorrelated redundant variant (IRV) set. BQTN identified 18 (five novel) potentially functional SNPs with substantial posterior probabilities (pps > 0.50) associated with one or more of the six investigated phenotypes. Seven SNPs demonstrated pps >0.95: two for HDL-C levels (rs1077834, rs16940299), two for A1 (rs375372, rs12914232), one for A2 and UC (rs11071387), and two for ΔHDL (rs485538, rs8023503). The SNP rs11071387 was associated with four size phenotypes (A1, A2, UC, EC). The promoter SNP, rs1077834, is in an IRV set with three other promoter SNPs, rs1800588, -763A>G (rs10077835) and -250G>A (rs2070895). Promoter variants did not demonstrate substantial pps with any of the five HDL-C size measures. Minor allele frequency ranged from 0.0008 for +106481 G>A (HDL-EC, HDL-UC) to 0.4596 for rs375372 (A1). This comprehensive investigation of *LIPC* genetic variation provides strong evidence that variants in addition to rs1800588 influence HDL-C levels and also suggests that different LIPC variants influence HDL-C levels and HDL particle size differentiation.

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Population diversity and GWAS-based genetic variants for BMI: The PAGE Study. *K.E. North[1, 12], M. Fesinmeyer[2], M.D. Ritchie[3], N. Francescini[1], P. Buzkova[4], U. Lim[5], M. Quibrera[1], M. Gross[6], K. Glenn[3], S. Buyske[7], C. Kooperberg[2], C.S. Carlson[2], R. Li[8], C.A. Haiman[9], L.R. Wilkens[5], L.L. Marchand[5], R.L. Prentice[2], L.H. Kuller[10], L. Hindorff[8], J. Manson[11], C. Chen[2], U. Peters[2].* 1) Dept Epidemiology, Univ North Carolina, Chapel Hill, NC; 2) Fred Hutchinson Cancer Research Center, Seattle, WA; 3) Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 4) Department of Biostatistics, University of Washington, Seattle, WA; 5) Cancer Research Center of Hawaii, Honolulu, HI; 6) Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN; 7) Department of Statistics & Biostatistics, Rutgers University, Piscataway, NJ; 8) Office of Population Genomics, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD; 9) Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA; 10) Department of Epidemiology, University of Pittsburgh, Pittsburgh, PA; 11) Department of Medicine, Harvard Medical School, Boston, MA; 12) Carolina Center for Genome Sciences, Univ North Carolina, Chapel Hill, NC.

The successes of genome-wide association studies (GWAS) in mapping loci that influence complex traits advance our understanding of genomic influences on common diseases. Translation of such knowledge into clinical and public health applications requires exploration of these associations in different ancestral groups. The NHGRI-supported 'Population Architecture using Genomics and Epidemiology (PAGE)' consortium of population-based studies accomplishes this by investigating the epidemiologic architecture of well-replicated genetic variants associated with complex traits. PAGE is comprised of CALiCo (Causal Variants Across the Life Course, a consortium of ARIC, CARDIA, CHS, and SHFS), EAGLE (Epidemiologic Architecture for Genes Linked to Environment, with participants from National Health and Nutrition Examination Surveys), Multiethnic Cohort, and Women's Health Initiative studies. We selected twenty putative body mass index (BMI) related SNPs identified by prior GWAS in European and European American populations, and genotyped each SNP in a diverse sample of up to ~70,000 subjects representing European American (N=39,421), African American (N=14,573), Mexican American (N=7,317), Asian and Pacific Islanders (N=3,236), and American Indian (N=7,317) individuals. We compared allele frequencies between ancestry groups and examined each SNP for departure from Hardy-Weinberg equilibrium within each subpopulation. The association between each SNP and BMI was analyzed stratified by PAGE site and self-identified race using additive genetic models and linear regression (mixed models for family relatedness), and adjusting for age and current smoking status specific for each sex strata. Results were combined across study but within ancestral groups using fixed effects meta-analysis techniques. Interestingly, some well established genetic variants did not replicate in any population we considered (for example, rs2815752 in NEGR1). However, several SNPs did replicate in most of the populations, for example rs7498665 in SH2B1 demonstrated a significant effect ($P < 0.05$) in all but the Mexican American subpopulation. Given known differences in allele frequencies and linkage disequilibrium patterns across ancestral groups, our findings suggest a complex architecture of BMI and the need for considering genetic, cultural, and environmental population differences in interpreting the increasing body of genetic associations emerging from well-replicated GWAS.

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Comparative genomics and functional analysis of coronary artery disease associated chromosome 9p21 region. *H. Ongen, T. Kyriakou, A. Goel, M. Farrall, H. Watkins, J. Peden.* Department of Cardiovascular Medicine, Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, Oxfordshire, United Kingdom.

Several independent genome wide association studies (GWAS) identified a 53 kb region on chromosome 9p21 that is associated with coronary artery disease (CAD), which is the most supported association to arise from CAD GWAS to date. Recent studies in mouse knockout models and human cell lines suggest that this region harbours elements that act in *cis*- and potentially have a regulatory role in the expression of neighbouring genes *CDKN2A* and *CDKN2B*. Phylogenetic footprinting and phylogenetic shadowing (a comparative genomics approach using only closely related sequences) have successfully identified conserved enhancer elements. Here we report phylogenetic footprinting and novel phylogenetic shadowing experiments designed to identify enhancer elements in this 9p21 CAD associated region. The information that would be gained by sequencing additional primates for the phylogenetic shadowing experiment was assessed by simulation of the sensitivity of detecting known functional elements as defined by the ENCODE project. These simulations ascertained that the addition of Colobus monkey, Owl monkey, and Dusky titi sequences to publicly available primate sequences increases accuracy. Orthologous bacterial artificial chromosome (BAC) clones for the 9p21 region from these three primates were sequenced using the 454 sequencing technology. 11 primate specific conserved elements (PSCEs), ranging from 80 bp to 431 bp, were predicted within the 9p21 region. Of these 11 PSCEs, 5 were prioritised for functional analysis based on putative histone modifications, DNase hypersensitive sites, and transcription factor binding sites as reported by the ENCODE project. In addition, another 8 mammalian conserved elements (MCEs) were identified within the publically available 34 species alignments due to their deep conservation and supporting functional evidence from ENCODE. Of these 8 MCEs, 4 have recently been independently assayed for functional activity and one was reported to be an enhancer. Ongoing work includes cloning the 5 PSCEs and 8 MCEs in luciferase reporter vectors so that their enhancer activity can be assayed in primary endothelial and smooth muscle cell cultures.

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A New Genetic Factor Influencing Pleoitropically Body Mass Index and Venous Thrombotic Risk Identified by Means of GWAS. *J.C. Souto[1], D.A. Tregouet[2], A. Buil[3], N. Saut[5], A. Martinez-Perez[3], M. Germain[2], S. Lopez[3], J. Fontcuberta[1], L. Almasy[4], P. Morange[5], J.M. Soria[3].* 1) Dept Hematology, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain; 2) INSERM, UMRS 937; Université Pierre et Marie Curie, Paris, France; 3) Unit of Genomics of Complex Diseases. Research Institute Hospital de la Santa Creu i Sant Pau, Barcelona, Spain; 4) Department of Population Genetics, Southwest Foundation for Biomedical Reseach, San Antonio, Texas, USA; 5) INSERM, UMRS 626, Université de la Mediterranée, Marseille, France.

Body mass index (BMI) is an independent risk factor for myocardial infarction, obesity and type 2 diabetes. In addition, recent data relate BMI and obesity with venous thromboembolic (VTE) risk. However, it is unknown if this relationship is due to common genetic causal factors. No one of the several human genetic loci associated with obesity or BMI have been involved in VTE risk. Methods: In order to identify genetic determinants of BMI, we conducted a genome-wide association study by analyzing 317000 single nucleotide polymorphisms (SNPs) in the GAIT study, composed of 398 individuals belonging to Spanish extended pedigrees with unexplained thrombophilia. Those SNPs showing significant p-values with BMI ($p<0.0001$) were replicated in the French cohorts of the MARTHA Project (419 patients with venous thrombosis and 1228 healthy controls) Results: Several SNPs in the same region of chromosome 1 associated with the BMI. The strong p-value was $p = 0.000007$. The same SNP showed a significant association with VTE in the French cohort ($p = 0.026$). A haplotype within this region also associated with thrombotic risk (OR 2.72, CI 1.7 - 4.2; $p = 0.00001$). Both, the SNP and the haplotype are located in a gene previously related to obesity. Conclusions: We have found a genetic determinant of the BMI that is pleiotropically related with venous thrombosis risk. The proposed research strategy (genomic search by means of a family study plus replication in a case/control study, using populations with different backgrounds) can be very helpful in the genetic analysis of many other complex traits and diseases. Our result suggests the existence of a common genetic basis between obesity and VTE, which would be clinically relevant.

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Interaction between Monoamine Oxidase A Gene Polymorphism and Childhood Emotional Abuse on Susceptibility to Early Atherosclerosis: A Twin Study. *J. Yang[1], V. Vaccarino[2], J. Zhao[1].* 1) Biostatistics and Epidemiology, University of Oklahoma HSC, Oklahoma City, OK; 2) Emory Univ, Atlanta, GA.

Background: A functional promoter polymorphism in MAOA has been implicated as a moderating factor in the relationship between childhood trauma and adult behavior or mental disorders, which often co-occur with cardiovascular disease (CVD). However, no previous study has examined the role of MAOA in preclinical atherosclerosis. Hypothesis: MAOA genotype is implicated in preclinical atherosclerosis and childhood trauma moderates the association between MAOA polymorphism and early atherosclerosis. Methods: We genotyped the MAOA polymorphism in a sample of 169 middle-aged male twin pairs drawn from the Vietnam Era Twin Registry. Childhood trauma, before age 18, was measured with the Early Trauma Inventory and included physical, emotional and sexual abuse and general trauma. Preclinical CVD was assessed by flow-mediated dilation (FMD) using ultrasound. Generalized estimating equation models were used to test main and interactive effects of MAOA genotype and each domain of childhood trauma on FMD, controlling for coronary risk factors. Results: General trauma was the most prevalent childhood trauma (27.5%), followed by physical abuse (21.1%), emotional abuse (17.5%) and sexual abuse (11.1%). For MAOA, five repeated sequences were identified. Based on differential transcription efficiency, twins were assigned to low activity group (2R, 3R or 5R), or high activity group (3.5R or 4R). There was no significant evidence for a main effect of MAOA genotype or childhood trauma on early atherosclerosis. However, we identified a significant interaction between MAOA genotype and emotional abuse on early atherosclerosis ($p = 0.02$), independent of multiple covariates. In the absence of emotional abuse, low activity alleles protected against subclinical CVD (mean FMD value: 6.24 in low activity group vs. 4.61 in high activity group), but the presence of emotional abuse reversed this association (mean FMD value: 4.17 in low activity group vs.5.26 in low activity group). Conclusion: This study provides the first evidence that an interaction between MAOA gene polymorphism and childhood emotional abuse influences the susceptibility for early atherosclerosis, independent of traditional cardiovascular risk factors.

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Genome-wide association study of intracranial aneurysm identifies new loci. *K. Yasuno[1,2], K. Bilguvar[1,2], R.P. Lifton[2,3], M. Gunel[1,2], The Genetics of Intracranial Aneurysm Consortium.* 1) Dept Neurosurgery and Neurobiology, Yale Univ Sch Med, New Haven, CT; 2) Dept Genetics, Yale Program on Neurogenetics, Yale Center for Human Genetics and Genomics, Yale Univ Sch Med, New Haven, CT; 3) Howard Hughes Medical Institute and Dept Internal Medicine, Yale Univ Sch Med, New Haven, CT.

Saccular intracranial aneurysms are balloon-like dilations of the intracranial arterial wall; their hemorrhage commonly results in severe neurologic impairment and death. We recently reported a genome-wide association study including 2,075 cases and 6,952 controls that identified 3 significant loci with odds ratios ranging from 1.24 to 1.36 (Bilguvar et al., Nat Genet 40, 1472-7, 2008). This analysis had limited power and explained only a small fraction of the risk of intracranial aneurysm. In order to increase the power to detect new intracranial aneurysm loci, we conducted a second genome-wide association study with discovery and replication cohorts from Europe and Japan comprising 5,891 cases and 14,181 controls with ~832,000 genotyped and imputed SNPs across discovery cohorts. We identified three new loci showing strong evidence for association with intracranial aneurysm in the combined data set, including intervals on 18q11.2 ($P=1.1\times10^{-12}$), on 13q13.1 ($P=2.5\times10^{-9}$) and on 10q24.32 ($P=1.2\times10^{-9}$). Odds ratios for these new loci were from 1.20 to 1.29. We also confirmed prior associations near SOX17 (8q11.23-q12.1; $P=1.3\times10^{-12}$) and CDKN2A/B (9p21.3; $P=1.5\times10^{-22}$). It is noteworthy that several putative risk genes play a role in cell-cycle progression, potentially affecting proliferation and senescence of progenitor cell populations that are responsible for vascular formation and repair. We will also present results from further follow-up study for 14 regions that showed less significant association with intracranial aneurysm in the discovery cohort.

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Ancestral missense mutations in TMEM43 cause arrhythmogenic right ventricular cardiomyopathy in outbred populations. *T. Young[1], A.F.M. Haywood[1], N. Merner[1], P. Syrris[3], V. Booth[2], J. Houston[1], W. McKenna[3], K.A. Hodgkinson[1].* 1) Faculty of Medicine, Memorial University, St. John's, NL, Canada; 2) Faculty of Science, Memorial University, St. John's, NL, Canada; 3) The Heart Hospital, 16-18 Westmoreland Street, London, United Kingdom.

Background: Arrhythmogenic right ventricular cardiomyopathy (ARVC/D) is considered a common cause of sudden death in the young. ARVC is genetically heterogeneous with 8 identified genes, most of them structural components of the cardiac desmosome. In 2008, we reported that an autosomal dominant form, ARVD5, was due to a missense mutation (S358L) in a highly conserved transmembrane domain of TMEM43, a gene of unknown function. Previous studies across 15 unrelated ARVD5 families of Newfoundland ancestry showed that the S358L variant resided on a shared (ancestral) haplotype and that the median age to death in untreated males was 40 years. The purpose of this study was to determine the role of TMEM43 mutations in outbred populations. Methods and Study Population: We recruited 5 patients from 2 clinical sites in Canada and 151 from the UK, and performed bidirectional, complete gene sequencing of TMEM43. We identified several amino acid substitutions in highly conserved domains of TMEM43. Results: One variant was a c.934C>T, R312W change in 4 clinically affected UK patients from three unrelated families. In one family, the variant segregated with subjects manifesting minor clinical anomalies and was absent in subjects with completely normal cardiac testing. The R312W variant was reported in 4/500 controls in The ARVD/C Genetics Variants Database (www.arvcdatabase). However, it is predicted to be deleterious using bioinformatics software, and like the founder S358L variant, lies within the highly conserved transmembrane domain of the TMEM43 protein. Interestingly, when we compared disease haplotypes between unrelated UK families, we discovered that the R312W variant resided on an identical haplotype suggesting that this is a potentially lethal ancestral mutation in the UK population. Conclusions: We speculate that a portion of sudden death in the population is due to recent, relatively rare, variably penetrant alleles in TMEM43. Pursuing family and population-based evidence improves our ability to distinguish lethal alleles from benign variants. This distinction is clinically relevant as implantable converter device (ICD) therapy for primary prevention in both sexes and secondary prevention in males significantly improves survival in S358L subjects. We are seeking other patients with a clinical diagnosis of ARVC or a related disorder to improve our ability to identify potentially lethal mutations using a similar approach.

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Epilogue: Association of APOCIII and APOAV gene with Coronary Artery Disease. *V. BHUPESH KUMAR[1], S. NAIK[2], V. KUMAR[3], SUBASH[4], S. DAYAKAR[1,5], M. D. SADHNANI[1].* 1) CLINICAL BIOCHEMISTRY, IGHGD, OSMANIA UNIVERSITY, HYDERABAD, ANDHRA PRADESH, INDIA. EMAIL: bhupeshkumar_v@rediff.com; 2) HEART INSTITUTE, APOLLO HEALTH CITY, JUBILEE HILLS, HYDERABAD, ANDHRA PRADESH, INDIA; 3) DEPARTMENT OF NEPHROLOGY, CHRISTIAN MEDICAL COLLEGE, VELLORE-632 004, TAMILNADU, INDIA; 4) DEPARTMENT OF CARDIOLOGY, DURGABAI DESHMUKH HOSPITAL AND RESEARCH CENTRE, ANDHRA MAHILA SABHA[AMS], UNIVERSITY ROAD, HYDERABAD- 500 044, ANDHRAPRADESH, INDIA; 5) APOLLO HOSPITALS EDUCATIONAL AND RESEARCH FOUNDATION, APOLLO HEALTH CITY, JUBILEE HILLS, HYDERABAD-500033, ANDHRAPRADESH, INDIA.

Background: Coronary artery Disease (CAD) in India is associated with high morbidity and mortality tends to be more severe and effects a younger age group compared to the west. The genes governing lipid transport and metabolism have always been considered as classical risk factors for CAD. Aim and Design of study: The aim of the study is to examine the association of polymorphisms in two Apolipoprotein genes APOCIII (C3238G), and APOAV (T1131C) in Myocardial infarction (MI) subjects compared with healthy volunteers. The SNPs were selected on the basis of their function and published evidence about their potential role in MI to their association with CAD. Methods: Genomic DNA is extracted from blood samples obtained of 150 MI subjects and 300 healthy volunteers following both inclusive and exclusive criteria. DNA samples were genotyped for C3238G, variant using PCR-RFLP assay and then DNA samples followed by genotyping T1131C variant using ARMS-PCR assay. Result and Conclusion: The C3238G SNP of APOCIII (S2 allele) tend to have high plasma Lipid concentrations was shown a risk factor in MI subjects when compared with healthy volunteers. The rare allele T1131C (CC allele) is also associated with elevated lipids levels. The Haplotypes analysis performed for APOAV and ApoCIII SNPs indicated significant differences (P.001) in distribution. Most significantly, a risk haploptype S2/C was obtained (P.001) and was associated with increased lipid. Key words: APOC3/A5- Apolipoprotein C3/A5, SNP- Single Nucleotide Polymorphism, ARMS-PCR- Amplification Refractory Mutation System.

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Model free Linkage Analysis of cIMT in the Jackson Heart Study. *S.G. Buxbaum[1], L. Ekunwe[1], E. Fox[2], D.F. Sarpong[1], G. Evans[3].* 1) Jackson Heart Study, Jackson State Univ, Jackson, MS; 2) Jackson Heart Study, University of Mississippi Medical Center, Jackson, MS; 3) Wake Forest University, Winston-Salem, NC.

The Jackson Heart Study is a longitudinal study of 5301 African Americans in the Jackson, MS metropolitan area. Nested within the JHS is a family study. While the targeted age bracket for the overall study population was 35-84 years, inclusion of adult relatives of the indexed families brought down the lower limit of the age bracket to ≥ 21 years. 374 autosomal microsatellite markers were used to conduct a linkage analysis of carotid intima-media thickness (cIMT) using data from 1091 full sibs and 305 half sibs.

Due to the prevalence of CVD among African Americans, furthering the understanding of the genetics underlying CVD is likely to be of benefit. A measure of subclinical atherosclerosis, the mean intima-media thickness of the carotid artery segments was determined by B-mode carotid ultrasonography.

Model free linkage analysis was conducted in the SIBPAL program in the S.A.G.E. package. Two IMT measures were analyzed: sum45_1, a maximum likelihood estimate of average far wall cIMT at the optimal angle of interrogation across the right and left common, bifurcation and internal carotid arteries and CCA45_1, a maximum likelihood estimate of average right and left common carotid far wall cIMT across circumferential views collected at anterior, lateral and posterior angles of interrogation. Both measures were estimated to be 29% heritable based on full sib correlations in FCOR in S.A.G.E..

Particularly strong evidence for linkage was found on chromosome 19p13. A multipoint analysis of CCA45_1 gave a LOD score of 5.7 and a singlepoint analysis gave a LOD of 5.1 at the same marker. At the same locus, sum45_1 also had a peak and a multipoint LOD score of 2.5 and a singlepoint LOD of 2.9.

Chromosome 3 also had significant linkage with sum45_1 (LOD=4.3) at 108 cM, but not with CCA45_1. Suggestive linkage was found on chromosome 12, but only with CCA45_1 (LOD=2.5). Singlepoint and multipoint LOD scores of 4 and higher were found on chromosome 18 using CCA45_1, while the highest LOD score for sum45_1 on chromosome 18 was 2.63 at the p arm telomere.

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A Novel Locus For Dilated Cardiomyopathy Maps To Chromosome 20q12. *G. Guo[1], C. Hayward[2], A. Keogh[2], P. Macdonald[2], D. Fatkin[1,2].* 1) Molecular Cardiology Program, Victor Chang Cardiac Research Institute, Sydney, NSW, Australia; 2) St Vincent's Hospital, Darlinghurst, NSW, Australia.

Dilated cardiomyopathy (DCM) is a heart muscle disorder characterized by dilation and contractile dysfunction of left and/or right ventricles that is a major cause of morbidity and mortality. Approximately 20-30% of cases of DCM have a positive family history suggesting a genetic etiology. Despite the considerable genetic heterogeneity demonstrated to date in familial DCM, the majority of families do not have mutations in any of the known disease genes. We have identified a three-generation Australian family (38 members, 11 affected) with autosomal dominant inheritance of DCM. A genome-wide (10 cM) linkage analysis was performed followed by 5 cM and fine mapping. A disease-causing interval was identified on chromosome 20 between markers D20S872 and D20S96 (~10 cM) by parametric and non-parametric analyses using ANALYZE and GENEHUNTER programs. The maximum LOD score 4.34 at $\theta = 0$ was shown at marker D20S107 by two-point analysis and a LOD score 4.35 was shown by multi-point analysis (penetrance = 0.9). Twenty candidate genes including ACSS2, APBA2BP, CTNNBL and STK4 in the interval have been screened, however a disease-causing mutation has not yet been identified. Elucidation of the molecular mechanisms underpinning familial DCM may provide a basis for new approaches to disease prevention and treatment.

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Quantitative Trait Loci for Brain Natriuretic Peptide Concentration among African Americans at the Jackson Heart Study. *S.K. Musani[1], S.G. Buxbaum[2], R.S. Vasan[3], A. Bidulescu[4], H.A. Taylor[1,2], E.R. Fox[1].* 1) University of Mississippi Medical Center, 2500 North State Street, Jackson MS 39216; 2) Jackson State University, Jackson MS; 3) Boston University, Boston MA; 4) Morehouse School of Medicine, Atlanta GA.

Statement of Purpose: The level of serum Brain Natriuretic Peptide (BNP) concentration is determined by cardiac pressure and volume overload. Further, BNP concentration has been found to be correlated with severity of heart failure. The purpose of this study is to investigate the extent of interindividual variation in plasma BNP levels among African Americans using microsatellite markers. Methods: We studied 1,300 Jackson Heart Study participants (mean age 55±12 years, 63% women) who underwent routine echocardiography and testing for serum BNP concentration. Sex specific multivariable models and Haseman-Elston regression were used to estimate polygenic heritability. We also performed multipoint marker linkage analysis using data from 402 microsatellite markers typed in 1,300 participants belonging to 264 families. Summary of Results: Age and echocardiographic variables respectively, accounted for 45% and 33% variation in log BNP concentration in men and women, respectively. We observed multivariable polygenic heritability of 0.237. Multipoint linkage analysis revealed regions of significant linkage to log BNP on chromosome 5q15-q21 (LOD score 3.73), and suggestive linkage on chromosomes 7q36 (LOD score 2.22) and distal 16p (LOD score 2.78). The significant QTL on chromosome 5q15-q21 is located near Proprotein convertase subtilin / kexin type 1 (PCSK1) gene that has been reported to be associated with body mass index. Conclusion: In this community-based sample, additive genetic effects explained moderate proportion of variation in brain natriuretic peptide levels. Additional analyses targeting significant linkage peak may provide more information about the genetic loci that regulate BNP levels in African Americans.

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Gene x environment interaction analysis reveals association of *APOE4* genotype with adiposity in children of lower cardiorespiratory fitness. *J. Ellis[1], A-L. Ponsonby[1,2], A. Pezic[1], E. Williamson[1], J. Cochrane[2], J. Dickinson[2], T. Dwyer[1,2].* 1) Murdoch Childrens Research Institute, Parkville, Victoria, Australia; 2) Menzies Research Institute, University of Tasmania, Hobart, Tasmania, Australia.

The plasma protein apolipoprotein E, encoded by the gene *APOE*, plays a well established role in lipid metabolism. There are three isoforms of ApoE, ε2, ε3 and ε4, which are encoded by three alleles, E2, E3, and E4. Evidence derived from animal models suggests that ApoE4 may be protective against increased adiposity, however this has not been thoroughly investigated in human populations. We examined the association of *APOE* genotype with BMI and other measures of adiposity, including truncal fat mass and waist circumference, among 8 year old children from the Tasmanian Infant Health Study. 290 children were genotyped for the E2, E3 and E4 alleles of *APOE*. As expected, overall *APOE* genotype was associated with lipid profile, especially low density lipoprotein cholesterol (LDL-C, $p < 0.001$). We also found that overall *APOE* genotype was associated with adiposity (eg BMI: $p = 0.01$). When we dichotomized the genotype groups into E2-containing and E4-containing, we found that the association with adiposity was specific to the E4 allele with adiposity lower in the E4-containing group (BMI: Mean difference -0.90 kg/m^2; 95% CI -1.51, -0.28; $p = 0.004$). Adjustment for potential confounders including age, sex, lipids, insulin and glucose did not alter the association. We then considered the role of sex, physical activity and cardiorespiratory fitness (CRF) as effect modifiers of the BMI-*APOE4* association. *APOE4* was associated with lower BMI only in those who were below the median for CRF (high fitness mean BMI difference 0.07 kg/m^2, 95% CI: -0.6, 0.7, $p = 0.83$; low fitness mean BMI difference -1.8 kg/m^2, 95% CI: -2.7, -0.8, $p < 0.001$ (adjusted for age and sex); interaction $p = 0.002$). We conclude that *APOE4* is related to lower childhood adiposity. *APOE* has not previously been identified as an adiposity determinant in humans. We demonstrated that consideration of the 'environment' in which the gene is expressed (in this case cardiorespiratory fitness level) may yield novel findings related to the genetic architecture of complex traits including adiposity.

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Exome sequencing in a large dislipidemic family identifies candidate genes for phospholipid transfer protein activity on chr. 19p. *E.A. Rosenthal[1], J. Ronald[1], R. Rajagopalan[1], G. Wolfbauer[5], J.J. Albers[4,5], J. Brunzell[6], M.J. Rieder[2], D.A. Nickerson[2], E.M. Wijsman[1,2,3], G.P. Jarvik[1,2].* 1) Division of Medical Genetics, Department of Medicine, Univ Washington, Seattle, WA; 2) Department of Genome Sciences, University of Washington, Seattle, WA; 3) Department of Biostatistics, University of Washington, Seattle, WA; 4) Department of Pathology,University of Washington, Seattle, WA; 5) Northwest Lipid Metabolism and Diabetes Research Laboratories, Department of Medicine, University of Washington, Seattle, WA; 6) Department of Medicine, Division of Metabolisn, Endocrinology, and Nutrition, University of Washington, Seattle, WA.

We combined next generation exome sequencing with quantitative trait linkage analysis in large families to identify genes for lipid traits. In contrast to simple Mendelian traits, where a simple model can be used to narrow candidate mutations to a single causal variant, complex oligogenic traits, such as lipid disorders, present additional challenges to gene discovery. Such challenges include multiple underlying genes, each with multiple alleles having different effect sizes, frequency distributions, and modes of inheritance. The use of large families is expected to reduce genetic heterogeneity, because fewer variants are expected to segregate within a family than in a population. Furthermore, large families allow for the estimation of the effect size of specific variants since the identical coding variant can be observed multiple times. Sequence data allows for simultaneous analysis of multiple linkage signals due to multiple traits in the same families. In this analysis, we focus on phospholipid transfer protein activity (PLTPa), a trait implicated in atherogenesis. We analyzed four large multigenerational pedigrees (F1-F4, N=466) ascertained for familial combined hyperlipidemia (FCHL), using multiallelic STRs, cardiovascular disease chip SNPs, and whole exome sequence data. Linkage analysis was carried out with the MCMC-based programs Loki, which employs an oligogenic model and can include SNPs as covariates, and with lm_markers from MORGAN, which provides parametric lod scores. Notably, we did not detect linkage to, nor find any exomic variation at, the *PLTP* structural locus on chr. 20. However, we detected linkage between PLTPa and the *LDLR* region of chr. 19 in family F1 (lodmax= 3.2). Exome sequences of 11 selected individuals in family F1 revealed 311 variant sites at 104 genes and 4 open reading frames under the linkage peak. Prioritization of these sites was based on cosegregation of the variant allele with PLTPa. Further prioritization of genes was based on biological factors including expression profile and GO terms; prioritization of variant sites within genes was based on evolutionary conservation score, mutation type, and predictive effect. Using this strategy, we identified mutations in three genes, excluding *LDLR*, for further analysis. Details of these variants will be presented.

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Hunting for Novel Dilated Cardiomyopathy Genes using Haplotype Sharing and Exome Sequencing. *R.J. Sinke[1], A. Pósafalvi[1], P.A. van der Zwaag[1], J. Bergsma[1], F. van Dijk[1], D.J. van Veldhuisen[2], K. van Spaendonck-Zwarts[1], G. te Meerman[1], R.M.W. Hofstra[1], M.P. van den Berg[2], J.P. van Tintelen[1], J.D.H. Jongbloed[1].* 1) Department of Genetics, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; 2) Department of Cardiology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands.

Background: Idiopathic Dilated cardiomyopathy (DCM) is a heritable, monogenic disorder in one-third of cases and is characterized by dilation and impaired contraction of the left ventricle. DCM is genetically highly heterogeneous and ≥40 genes are known to be involved. Mutations in these genes can be identified in around 30% of index-patients. To identify novel DCM genes we used an approach in which the Haplotype Sharing Test (HST) and exome sequencing were combined. Haplotype sharing has been shown to be a powerful tool for identifying candidate regions and disease-causing genes. Moreover, exome sequencing was shown to be effective in the identification of novel genes in monogenic disorders.

Methods: To localize novel DCM genes, 250K SNP genotyping was used to identify shared haplotypes between patients from a single pedigree. The HST was applied to (I) a family with 6 DCM patients and (II) a family with 3 DCM patients and 1 patient with reduced left ventricular function, yet not fulfilling formal DCM criteria. To subsequently identify the corresponding disease genes, exome sequencing using DNA of the probands of both families was performed.

Results: The HST revealed a shared haplotype of 71 cM on chromosome 15, containing ~600 genes in family I and a 46 cM haplotype on chromosome 9, containing ~475 genes in family II. Upon exclusion of known DCM genes within these regions, exome sequencing revealed several potentially pathogenic variants in both families. Carriership analysis in family members, healthy controls and mutation analysis in our DCM cohort is in progress.

Conclusions: We identified potentially pathogenic variants in candidate regions in two DCM families. Our results show that using a combined approach of the HST and exome sequencing is highly promising in identifying mutations in novel genes underlying inherited cardiomyopathies.

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Genome-wide Linkage and Positional Candidate Gene Study of Blood Pressure Response to Dietary Potassium Intervention: The GenSalt Study. *T.N. Kelly[1], J.E. Hixson[2], D.C. Rao[3], H. Mei[1], T.K. Rice[3], C.E. Jaquish[4], L.C. Shimmin[2], K. Schwander[3], C. Chen[1], D. Liu[5], J. Chen[6], C. Bormans[2], P. Shukla[2], N. Farhana[2], C. Stuart[2], P.K. Whelton[7], D. Gu[6], J. He[1,8].* 1) Dept Epidemiology, Tulane Univ, New Orleans, LA; 2) Dept Epidemiology, Univ of Texas, Houston, TX; 3) Div Biostatistics, Washington Univ, St. Louis, MO; 4) Division of Prevention and Population Sciences, NHLBI, Bethesda, MD; 5) National Laboratyory of Medical Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China; 6) Cardiovascular Institute and Fuwai Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, and Chinese National Center for Cardiovascular Disease Control and Research, Beijing, China; 7) Office of the President, Loyola University Health System and Medical Center, Maywood, IL; 8) Dept Medicine, Tulane Univ, New Orleans, LA.

Genetic determinants of blood pressure (BP) response to potassium, or potassium sensitivity, are largely unknown. We conducted a genome-wide linkage scan and positional candidate gene analysis to identify genetic determinants of potassium sensitivity. A total of 1,906 Han Chinese participants took part in a 7-day high-sodium followed by a 7-day high-sodium plus potassium dietary intervention. BP measurements were obtained at baseline and following each intervention using a random-zero sphygmomanometer. Significant linkage signals (LOD>3) for BP responses to potassium were detected at chromosomal regions 3q24-q26.1, 3q28, and 11q22.3-q24.3. Maximum multipoint LOD scores of 3.09 at 3q25.2 and 3.41 at 11q23.3 were observed for absolute diastolic (DBP) and mean arterial (MAP) responses, respectively. Linkage peaks of 3.56 at 3q25.1 and 3.01 at 11q23.3 for percent DBP response and 3.22 at 3q25.2, 3.01 at 3q28, and 4.48 at 11q23.3 for percent MAP response were also identified. AGTR1 SNP rs16860760 in the 3q24-q26.1 region was significantly associated with absolute and percent systolic (SBP) responses to potassium (p-values= 0.0008 and 0.0006, respectively). Absolute SBP responses (95% CI) for genotypes C/C, C/T, and T/T were: -3.71 (-4.02, -3.40), -2.62 (-3.38, -1.85), and 1.03 (-3.73, 5.79) mmHg, respectively; and percent responses (95% CI) were: -3.07 (-3.33, -2.80), -2.07 (-2.74, -1.41), and 0.90 (-3.20, 4.99), respectively. Similar trends were observed for DBP and MAP responses. Genetic regions on chromosomes 3 and 11 may harbor important susceptibility loci for potassium sensitivity. Furthermore, the AGTR1 gene was a significant predictor of BP responses to potassium intake.

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Low frequency and rare variant analysis of serum lipid levels. *K. Rehnström[1], A. Day-Williams[1], I. Surakka[2], J. Kettunen[2], T. Esko[3], C. Ladenvall[4], M. Mangino[5], A-K. Petersen[6], J. Eriksson[7], L. Groop[4], MR. Järvelin[8], A. Metspalu[3], O. Raitakari[9], V. Salomaa[10], T. Spector[5], E. Wichmann[6], L. Peltonen[1], E. Zeggini*[1], S. Ripatti*[2] on behalf of the ENGAGE consortium.* 1) Wellcome Trust Sanger Institute, Hinxton, Hinxton, United Kingdom; 2) Institute for Molecular Medicine Finland, FIMM, Helsinki, Finland; 3) Estonian Genome Project of University of Tartu, Tartu, Estonia; 4) Department of Clinical Sciences, Diabetes and Endocrinology, University Hospital Malmö, Lund University, Malmö, Sweden; 5) Department of Twin Research and Genetic Epidemiology, King's College London, St. Thomas' Hospital Campus, London, UK; 6) Institute of Medical Informatics, Biometry and Epidemiology, Ludwig-Maximilians-Universität, Munich, Germany; 7) University of Helsinki, Department of Public Health, Helsinki, Finland; 8) Department of Epidemiology and Public Health, Imperial College London, UK; 9) University of Turku and Turku University Hospital, Turku, Finland; 10) National Institute for Health and Welfare, Helsinki, Finland.

Numerous common genetic factors affecting serum lipid levels have been identified in GWAS. However, these variants only explain part of the heritability of these traits, and it is becoming increasingly clear that low frequency and rare variants also play a role in lipid traits. Here, we have used available genome-wide SNP data from 8 datasets from the ENGAGE consortium (DGI, Estonian biobank, Genmets, HBCS, KORA, Young Finns Study, NFBC 1966 and TwinsUK) cohorts totalling >17,000 individuals to examine the role of low frequency/rare variants (MAF<0.05) affecting blood lipid levels (HDL-C, LDL-C, total cholesterol (TC) and triglycerides (TG)). Low frequency variants are typically discarded from analysis because of low power to detect effects with single-point analysis. We followed a locus-specific approach and tested for an accumulation of low frequency variants within genes. Results were combined across cohorts using p value-based meta-analysis allowing for differences in the direction of effect. The number of genes with 3 or more rare variants per cohort resulting in meta-analysis p<0.0001 was 12, 15, 13 and 16 for TC, LDL-C, HDL-C and TG respectively. None of these loci have previously been identified in GWA studies of common variants. Unsurprisingly, there was overlap between genes with rare variants affecting TC and LDL-C, in keeping with hits from previous GWAS using common variants. The most significant result for LDL was obtained in the PVR gene on chr19 (p=4*10-6 for LDL-C and p=7*10-5 for TC) and AKAP6 (p=6*10-6 in LDL-C and p=9*10-6 in TC). The p-value threshold for genome-wide significance correcting for the 23192 tests performed is 2.2*10-6 leaving our results just outside genome-wide significance. The results need to be followed up by rigorous quality control of the genotyping quality of the variants contributing to the signals. Although GWAS platforms are strongly biased toward common SNPs, we show that they can also be used to identify association with low frequency/ rare variants. Data provided by large-scale sequencing studies will further help clarify the role of low frequency and rare variants affecting lipid traits. The next step of this study will be to pursue in silico replication. Further, a similar analysis will be performed in 1000genomes-imputed data.

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Evaluation of Targeted Enrichment Strategies for Next Generation Sequencing of Cardiomyopathy Associated Genes. *S. Dames[1], J.D. Durtschi[1], J. Stephens[1], B. Funke[3], H.L. Rehm[3], K.V. Voelkerding[1,2].* 1) ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, USA; 2) Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT, USA; 3) Department of Pathology, Harvard Medical School, Cambridge, MA, USA.

Background: Targeted multigene enrichment is a bottleneck for implementing next generation sequencing based diagnostic panels. Enrichment strategies can generally be classified as amplification or hybridization capture based. This study compares enrichment and sequencing results using long range PCR (LRPCR) and FEBIT (Lexington MA) array capture for genes associated with hypertrophic cardiomyopathy (HCM). Array capture technologies for targeted enrichment are an attractive alternative to PCR based methods since they streamline workflow, and nullify amplicon/allele drop out. **Methods**: 9 genes associated with HCM were enriched by LRPCR and FEBIT capture. A previously assayed control and 2 Coriell samples with short read archive (SRA) data were sequenced. A comparison of LRPCR amplicon normalization was also performed using gel purification and equimolar pooling versus SequalPrep (Invitrogen, Carlsbad CA). 76 base-length reads from Illumina GA IIX platform (San Diego CA) were analyzed using CLCbio (Cambridge MA) and DNASTAR (Madison WI) alignment software. RefSeq genes were used for sequence alignment of enriched libraries. Additional filtering of FEBIT sequencing data was performed by whole-genome alignment. **Results**: Analysis of the previously sequenced control (Illumina GAII; Sanger verified) reported no novel exonic variants between LRPCR enriched samples. Furthermore, no exonic false positives/negatives were found in any of the 9 genes. One gene associated with HCM, *MYH6*, was excluded from the panel due to homology with *MYH7* (51% gDNA; 80% mRNA). Analysis of the FEBIT sequence data showed co-capture of the *MYH6* gene, introducing false *MYH7* variants. Filtering of the FEBIT sequence data by whole genome alignment reduced the number of false variants observed in *MYH7*, but did not remove all false variants in other HCM genes not previously observed due to gDNA co-capture. The specificity of LRPCR for enrichment allowed for optimal alignment without the requirement of filtering, which dilutes coverage and may lead to aberrant variant calls. Analysis of SRA data for the Coriell samples is underway to determine variant correlation. **Conclusions**: FEBIT is an attractive technology but is limited by co-capture for HCM gene enrichment. LR-PCR offers greater target specificity, but is nontrivial in design, workflow, and implementation. We are extending this work using RainDance technology as an alternative enrichment strategy versus LRPCR with blinded HCM samples.

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Genome-wide association for anatomic coronary artery disease. *G.W. Beecham[1], K.L. Hamilton[1], P. Goldschmidt-Clermont[2], M.A. Pericak-Vance[1], D. Seo[1].* 1) Hussman Institute for Human Genomics, University of Miami, Miami, FL; 2) Miller School of Medicine, University of Miami, Miami, FL.

BACKGROUND Coronary heart disease is the leading cause of death in the United States, with atherosclerosis of the coronary arteries (CAD) being the major underlying etiology. Patients with CAD are more likely to suffer a myocardial infarction or sudden cardiac death. CAD is highly heritable, but there have been few genetic studies of anatomic CAD and the knowledge of CAD genetics is limited. To further determine the etiology of anatomic CAD, we have performed the first genome-wide association study of CAD, using our dataset of 2,000 cardiac catheterization patients. **METHODS** Each patient received cardiac catheterization and was assigned a CAD burden index which measures their overall anatomic CAD burden based on size and location of coronary lesions. Data were generated using the Affymetrix SNP array 6.0 platform for 900,000 SNPs. Extensive quality controls tests were performed to ensure the integrity of the data, including sample filters, SNP filters, and EIGENSTRAT methods to correct for population substructure. We tested for association using linear regression using the PLINK software package, and included smoking, cholesterol, blood pressure, age, sex, and two vectors describing the population substructure of the sample. **RESULTS** The first 1,050 samples are currently available for analysis. Among them, we detected strong association ($p < 0.00001$) at 9 SNPs on 6 different chromosomes, in 7 different genes. One SNP met a conservative Bonferroni threshold for genome-wide multiple testing correction (p-value = 0.05/782,000=6x10-8). This SNP was rs10206951 (p-value=2.6x10-8) on chromosome 2 near the CREB1 gene (cAMP responsive element binding protein). It is also located near the Kruppel-like factor 7 (KLF7) a gene previously associated with type 2 diabetes, and as such proves to be an interesting candidate. Another associated SNP, rs2786157 (7.3x10-7), located in the chitinase, acidic gene (CHIA), a gene that is expressed in pulmonary epithelial cells and is associated with asthma. The additional 1,000 samples are being genotyped and will be included in the final analysis. **CONCLUSION** These signals are being further investigated in a larger dataset. An additional 1,000 samples are currently being genotyped and will be used to confirm these signals. These data provide promising results that may lead to further insights into the genetics of anatomic coronary artery disease.

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A Genome-Wide Association Study Uncovers a QTL of Adiponectin on CDH13 Which Predicts Cardiometabolic Outcomes. *C. Chung[1], T. Lin[2,3], J. Chen[4,5], H. Chang[6], H. Leu[4], H. Ho[7], C. Ting[7], S. Sheu[2,3], W. Tsai[8], J. Chen[8], S. Lin[5], W. Pan[1].* 1) Inst Biomedical Sci, Academia Sinica, Taipei, Taiwan; 2) Division of Cardiology, Dept of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; 3) Dept of Internal Medicine, Faculty of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; 4) Cardiovascular Research Center, National Yang-Ming University, Taipei, Taiwan; 5) Dept of Medical Research and Education, Taipei Veterans General Hospital, Taipei, Taiwan; 6) Center of General Education, Hsuan Chuang University, Hsinchu, Taiwan; 7) Taichung Veterans General Hospital, Taichung, Taiwan; 8) College of Medicine, National Cheng Kung University, Tainan, Taiwan.

The plasma adiponectin levels, a potential upstream and internal facet of metabolic diseases and cardiovascular diseases, have a reasonably high heritability. it has not been carefully examined upon whether other novel genes may influence the variation of adiponectin level and the roles of these genetic variants on subsequent clinical outcomes. Therefore, we aimed not only to identify novel variants modulating plasma adiponectin but also investigate whether these variants are associated with adiponectin related metabolic traits and cardiovascular diseases. We conducted a genome-wide association study (GWAS) on adiponectin levels by using genotypic information derived from the Illumina HumanHap550 SNP chip in 382 young-onset hypertensive (YOH) subjects to identify the quantitative trait loci associated with the adiponectin levels. The culpable SNP variants for lowered adiponectin were then tested for their association with risk of metabolic syndrome, type 2 diabetes mellitus(T2DM), and stroke in an independent community-based prospective cohort, the CardioVascular Disease risk FACtors Two-township Study (CVDFACTS)(n=3350). The SNP (rs4783244) most significantly associated with adiponectin levels was located in the intron 1of CDH13 gene in the first stage (p value=1.42x10-8). We further replicated and confirmed the association between rs4783244 and plasma adiponectin levels in an additional 559 YOH subjects (p value=6.87x10-17). This same risk allele at CDH13 was also associated with higher risks of metabolic syndrome, T2DM (in male) (OR=3.25,p=0.02) ,and stroke (OR=2.13; p= 0.002). These findings may broaden our understanding of the mechanisms modulating adiponectin levels and their roles in the development of cardiometabolic diseases.

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Genetics of Coronary Atherosclerotic Plaque Rupture and Myocardial Infarction. *J.F. Ferguson[1], M. Li[3], J. He[3], A.N. Qasim[1], M.S Burnett[4], J.M. Devaney[4], S.L. DerOhannessian[1,2], C.W. Knouff[5], J.R. Thompson[6], A.F.R. Stewart[7], T.L. Assimes[8], J. Barnard[9], P.S. Wild[10], H. Alllayee[11], D. Absher[14], L. Chen[8], A.S. Hall[16,17], T. Quertermous[8], S. Blankenberg[10], S.L. Hazen[15], R. Roberts[7], R. McPherson[7], S. Kathiresan[18,19,20,21,22], V. Mooser[5], H. Hakonarson[23], N.J. Samani[12,13], S.E. Epstein[4], D.J. Rader[1,2], M.P. Reilly[1,2], Wellcome Trust Case Control Consortium.* 1) The Cardiovascular Institute, University of Pennsylvania, Philadelphia, PA 19104, USA; 2) The Institute for Translational Medicine and Therapeutics, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA; 3) Biostatistics and Epidemiology, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA; 4) Cardiovascular Research Institute, Medstar Health Research Institute, Washington Hospital Center, Washington, DC 20010, USA; 5) Genetics Division and Drug Discovery, GlaxoSmithKline, King of Prussia, Pennsylvania 19406, USA; 6) Department of Health Sciences, University of Leicester, Leicester LE1 7RH, UK; 7) Department of Medicine, University of Ottawa Heart Institute, Ottawa K1Y4W7, Canada; 8) Department of Medicine, Stanford University School of Medicine, Stanford, California 94305, USA; 9) Department of Quantitative Health Sciences, The Cleveland Clinic Foundation, Cleveland, Ohio 44195, USA; 10) Department of Medicine II, University Medical Center Mainz, Mainz 55131, Germany; 11) Department of Preventive Medicine, Institute for Genetic Medicine, USC Keck School of Medicine, Los Angeles, California 90033, USA; 12) Department of Cardiovascular Sciences, University of Leicester, Leicester LE3 9QP, UK; 13) Leicester National Institute for Health Research Biomedical Research Unit in Cardiovascular Disease, Glenfield Hospital, Leicester, LE3 9QP, UK; 14) HudsonAlpha Institute for Biotechnology, Huntsville, Alabama 35806, USA; 15) The Center for Cardiovascular Diagnostics & Prevention, Cleveland Clinic, Cleveland, Ohio 44195, USA; 16) Leeds Institute of Genetics Health and Therapeutics (LIGHT), University of Leeds, Leeds, UK; 17) Multidisciplinary Cardiovascular Research Centre (MCRC), University of Leeds, Leeds, UK; 18) Cardiovascular Research Center and Cardiology Division, Massachusetts General Hospital, Boston, Massachusetts 02114, USA; 19) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA, USA, 02114; 20) Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115, USA; 21) Department of Medicine, Harvard Medical School, Boston, Massachusetts 02115, USA; 22) Program in Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, Massachusetts02142, USA; 23) The Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania 19104, USA.

Introduction: While the presence of coronary artery disease (CAD), as measured by angiography, is present in the majority of myocardial infarction (MI) cases, there are many individuals who do not develop MI despite having considerable coronary atherosclerosis. We hypothesized that some genetic risk for plaque rupture and MI is distinct from that which relates to development of atherosclerosis. Methods: We performed a meta-analysis across eight genome-wide (~2.3 million genotyped and imputed SNPs) association studies (GWAS) of CAD in European ancestry participants who were phenotyped through coronary angiography. In order to identify loci that predispose to MI in the setting of angiographic CAD (AngCAD; at least one coronary artery with >50% stenosis), we compared patients with AngCAD and MI (AngCADMI+, N=5,783) to those with AngCAD but no MI (AngCADMI-, N= 3,644). Using the DAVID Functional Annotation Tool, we examined enrichment for certain functional themes for genes with SNPs with suggestive association (P<0.0001) with AngCADMI+ compared to all genes on the Affymetrix 6.0 array. Results: Our top finding (described elsewhere) for association with AngCADMI+ was for SNPs at the ABO locus, a glycotransferase gene. Using DAVID, we observed enrichment for certain functional themes, including cell adhesion (enrichment score = 1.81, p=0.01) and immunoglobulins (enrichment score = 1.7, p= 0.0001), as well as fibronectins (enrichment score = 2.13, p=0.0001), and glycoproteins and genes with N-linked glycosylation sites (enrichment score 1.59, p=0.008). Loci (e.g, including 9p21) and pathways (e.g., lipid and fatty acid biosynthetic processes) previously implicated in CAD by GWAS were not enriched in AngCADMI+ patients. Conclusion: Genetic predisposition to MI in the setting of atherosclerosis may be distinct from that associated with the development of atherosclerosis. Genes that encode proteins involved in cell-cell adhesion and glycomic modifications may modulate plaque rupture and MI. These findings suggest potential novel therapeutic avenues for prevention and treatment of plaque rupture and MI.

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Eight genetic loci associated with variation in lipoprotein-associated phospholipase A2 (Lp-PLA2) mass and activity and CHD: Meta-analysis of genome-wide association studies from five community-based studies. *H. Grallert[1], JC. Bis[2], A. Dehghan[3,4], M. Barbalic[5], C. Lu[6], J. Baumert[1], RB. Schnabel[6], K. Rice[7], JCM. Witteman[3,4], RP. Tracy[8,9], W. Koenig[10], EJ. Benjamin[6], CM. Ballantyne[11] on behalf of the CHARGE inflammation working group.* 1) Institute of Epidemiology, Helmholtz Zentrum Munchen, Neuherberg, Germany; 2) Cardiovascular Health Research Unit and Department of Medicine, University of Washington, Seattle, WA; 3) Department of Epidemiology, Erasmus University Medical Center, Rotterdam, The Netherlands; 4) Member of the Netherlands Consortium on Healthy Aging; 5) Genetics Center, University of Texas Health Science Center, Houston, Texas, USA; 6) National Heart, Lung, and Blood Institute's and Boston University's Framingham Heart Study, Framingham, MA; 7) Department of Biostatistics, University of Washington, Seattle, WA, USA; 8) Department of Pathology, University of Vermont College of Medicine, Burlington, VT; 9) Department of Biochemistry, University of Vermont College of Medicine, Burlington, VT; 10) Department of Internal Medicine II-Cardiology, University of Ulm Medical Center, Ulm, Germany; 11) Section of Atherosclerosis and Vascular Medicine, Baylor College of Medicine, Houston, Texas.

Background: Lipoprotein-associated phospholipase A2 (Lp-PLA2), is an enzyme that generates pro-inflammatory and pro-atherogenic compounds in the arterial vascular wall, Lp-PLA2 may represent a therapeutic target in coronary heart disease (CHD). Methods: We sought to investigate genetic loci related to Lp-PLA2 mass or activity by conducting meta-analyses of genome-wide association (GWA) findings from 5 population-based studies as part of the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium, comprising 13,664 subjects. Results: In meta-analysis, two loci (PLA2G7, CETP) were associated with Lp-PLA2 mass, with rs1805017 in PLA2G7 as strongest signal ($p=2.4\times10^{-23}$, log Lp-PLA2 difference per allele (beta): 0.043). Six loci were associated with Lp-PLA2 activity (PLA2G7, APOC1, CELSR2, LDLR2, ZNF259, SCARB1), among which the strongest signals were at rs4420638, near the APOE-APOC1-APOC4-APOC2 cluster ($p=4.9\times10^{-30}$, log Lp-PLA2 difference per allele (beta): -0.054). There were no significant gene-environment interactions of these 8 top SNPs and age, sex, body mass index or smoking status in relation to LP-PLA2 mass or activity. Moreover, in an additional analysis, four loci (APOC1, CELSR2, LDLR, ZNF259) were significantly associated with cardiovascular outcome (APOC1: $p=9.2\times10^{-11}$, beta= 0.301). Conclusions: We report the first genome-wide meta-analysis for SNPs associated with Lp-PLA2 activity and mass. Whereas levels of Lp-PLA2 mass and activity were associated with the gene coding for this protein, Lp-PLA2 activity was also strongly associated with genetic variants related to low-density lipoprotein cholesterol levels and CHD. Further studies are needed to assess whether and how Lp-PLA2 concentrations are causally involved in atherogenesis.

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Genome-wide association study of Kawasaki disease. *J. Kim[1], K. Kim[2], Y. Park[1], I. Park[3], J. Lee[1], Korean Kawasaki Disease Genetics Consortium.* 1) Asan Institute for Life Sciences, University of Ulsan College of Medicine, Seoul, Korea; 2) Genome Research Center, Asan Medical Center, Seoul, Korea; 3) Department of Pediatrics, University of Ulsan College of Medicine, Asan Medical Center, Seoul, Korea.

Kawasaki disease (KD) is an acute self-limited vasculitis of infants and children, manifested by fever and signs of mucocutaneous inflammation. Coronary artery aneurysms develop in approximately 15 to 25% of untreated and 3 to 5% of treated children and may lead to myocardiac infarction, ischemic heart disease or sudden death. Although its etiology is largely unknown, epidemiologic data suggest the importance of genetic factors in susceptibility to KD. To identify genetic variants influencing KD susceptibility, we performed a genome-wide association study (GWAS) in 186 Korean KD patients and 600 healthy controls. Eighteen and twenty-five genomic regions with one or more sequence variants were associated with KD and KD with coronary artery lesions (CALs), respectively ($P < 1 * 10^{-5}$). Of these, only one locus was replicated in 266 children with KD and 600 normal controls (rs527409, odds ratio (OR) = 2.90, 95% confidence interval (95% CI) = 1.85 - 4.54, $P_{combined} = 1.46 * 10^{-6}$) and another one locus was replicated in 86 KD patients with CALs and 600 controls (rs7604693, OR = 2.70, 95% CI = 1.77 - 4.12, $P_{combined} = 2.00 * 10^{-6}$). We also performed replication study in Taiwanese population. Further fine-mapping of the candidate genes is needed to identify causative variants for KD susceptibility.

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Genome-wide association study to identify the susceptible genes for Kawasaki disease. *T.J. Lai[1, 2], M.W. Lin[3], C.H. Lin[4, 6], S.F. Tsai[4, 6], K.S. Hsieh[5], K.P. Weng[5], C.C. Shih[1, 7], L.P. Ger[2], C.J. Tseng[1, 2].* 1) Institute of Clinical Medicine, National Yang-Ming University, Taipei, Taiwan; 2) Department of Medical Education and Research, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan; 3) Institute of Public Health and Department of Public Health, National Yang-Ming University, Taipei, Taiwan; 4) Genome Research Center, National Yang-Ming University, Taipei, Taiwan; 5) Department of Paediatric Cardiology, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan; 6) National Health Research Institutes, Miaoli, Taiwan; 7) Division of Cardiovascular Surgery, Taipei Veterans General Hospital, Taipei, Taiwan.

Kawasaki disease (KD) is the most common cause of pediatric acquired heart disease of an unknown etiology. Epidemiologic data suggest that KD is triggered by unidentified infections in genetically susceptible children. To identify the susceptible genes involved in KD in Han-Chinese of Taiwan population, we performed a genome-wide association study (GWAS) with a total of 112 KD cases and 58 healthy controls. All cases were diagnosed using the criteria for KD according to the Japanese Kawasaki Disease Research Committee and recruited at the Kaohsiung Veterans General Hospital (KVGH), Taiwan. Controls were recruited from two junior high schools in the KSVGH community. The GWAS was used to screen 346,110 SNPs in 86 cases and 10 controls by use of the Illumina HumanHap370 BeadChip and 1,016,423 SNPs in 26 cases and 48 controls by use of the Illumina HumanOmnil BeadChip. The SNPs with following three criterions were excluded from analyses: (1) call rate<95%, (2) missing data rate>5% for SNPs with a minor allele frequency<1%, and (3) p-value of Hardy-Weinberg disequilibrium test$<10^{-7}$. In addition, only 206,176 overlapping SNPs of the two GeneChips were further analyzed by use of both allelic and recessive-dominant models. We initially identified 26 SNPs/loci ($10^{-10}<P<10^{-3}$), which contain novel and known SNPs/loci. Imputation of the non-overlapping SNPs in two GeneChips for further analyses and a SNP-replication study by Sequenom-based genotyping assay are in preparation for the coming 60th Annual ASHG Meeting.

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Meta-analysis of European and South Asian genome-wide association studies of coronary disease. *J.F. Peden[1], J.C. Hopewell[2], D. Saleheen[3,4], J.C. Chambers[8], R. Clarke[2], R. Collins[2], J. Danesh[3], P. Deloukas[6], P. Elliott[8], M. Farrall[1], J. Hager[5], A. Hamsten[7], J. Kooner[9], M. Lathrop[5], S. Parish[2], H. Watkins[1,10], C4D consortium.* 1) Cardiovascular Medicine, Wellcome Trust Center for Human Genetics, University of Oxford, Roosevelt Drive, Oxford OX3 7BN, United Kingdom; 2) CTSU Richard Doll Building Old Road Campus Roosevelt Drive Oxford OX3 7LF United Kingdom; 3) Department of Public Health and Primary Care University of Cambridge Cambridge, CB1 8RN United Kingdom; 4) Center for Non-Communicable Diseases, Karachi, Pakistan; 5) Centre National de Génotypage 2 rue Gaston Crémieux CP 5721, 91 057 Evry Cedex France; 6) The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, UK; 7) Atherosclerosis Unit, King Gustaf V Research Institute, Karolinska Institutet Stockholm Sweden; 8) Department of Epidemiology and Public Health, Imperial College London, London W2 1PG, UK; 9) Hammersmith Hospital, National Heart and Lung Institute, Imperial College London, London W12 0NN, UK; 10) Department of Cardiovascular Medicine, University of Oxford, Level 6, West Wing, John Radcliffe Hospital, Oxford, OX3 9DU, United Kingdom.

Genome-wide association studies in coronary artery disease (CAD) have so far focussed on populations of European ancestry and have detected common variants with odds ratio of about 1.12 or greater. The variants discovered to date explain a small proportion of the predicted genetic risk. We report a prospective meta-analysis of four large unpublished genome-wide association studies of CAD susceptibility.

The discovery stage comprised four participating GWAS studies with a total of 15,418 cases and 15,067 controls matched for country of origin, all typed on high-density Illumina arrays: PROCARDIS (5720 European cases), HPS (2704 European cases), PROMIS (4253 South Asian cases) and LOLIPOP (2741 South Asian cases). After quality control, data for 574,911 SNPs with frequency ≥1% were entered into a fixed effects meta-analysis to combine the results of the individual studies for all overlapping SNPs. Heterogeneity between studies was assessed using standard techniques. The primary analysis examined the associations of SNPs with CAD in: (i) all four studies in combination; (ii) the two European Studies; and (iii) the two South Asian studies.

Previously reported susceptibility loci were confirmed (e.g. 9p21.3: $p<10^{-28}$; 1p13.3: $p<10^{-11}$). Although examples were identified where there were significant differences in haplotype frequency and/or effect size, there was little evidence for either European or Asian specific associations. Thus we did not find evidence for fundamental differences in genetic susceptibility to explain the excess risk of CAD in South Asian populations.

Potential novel associations from the meta-analysis were tested by genotyping 50 SNPs from 35 loci in an independent replication sample comprising about 19,000 cases and 14,000 controls, predominantly of European origin. A number of novel associations of genome-wide significance were identified, some with associated expression QTLs in a panel of relevant tissues. These findings implicate pathways not previously identified through GWAS findings. The effect sizes of the novel variants were all small, suggesting that a large number of small effect common variants contribute to CAD susceptibility.

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Genome-wide association study of coronary artery disease in East Asian populations. *Y. Shin[1], J. Lee[1], Y. Kim[1], K. Kim[1], C. Hong[1], D. Shin[4], K. Park[5], D. Yoon[11], S. Moon[1], B. Lee[3], M. Yokota[6], H. Asano[7], M. Nakatochi[8], T. Matsubara[9], H. Kitajima[10], K. Yamamoto[10], H. Park[2], H. Kim[12], B. Han[1], H. Kim[5], Y. Jang[4], J. Park[3], J. Lee[1].* 1) Center for Genome Science, Korea National Institute of Health, KCDC, Seoul, 122-701, Korea; 2) Division of Cardiovascular Diseases, Center for Biomedical Sciences, Korea National Institute of Health, KCDC, Seoul, 122-701, Korea; 3) Division of Cardiology, Samsung Medical Center, Seoul, 135-710, Korea; 4) Cardiology Division, Department of Internal Medicine, Cardiovascular Genome Center, Yonsei University College of Medicine, Seoul,120-752, Korea; 5) Department of Internal Medicine, Seoul National University Hospital, Seoul 110-744, Korea; 6) Department of Genome Science, Aichi-Gakuin University, School of Dentistry, Nagoya, Japan; 7) Department of Cardiology, Chiousha Iwakura Hospital , Japan; 8) Department of Biotechnology, Nagoya University School of Engineering, Nagoya, Japan; 9) Department of Internal Medicine, Aichi-Gakuin University, School of Dentistry, Nagoya, Japan; 10) Research Center for Genetic Information, Medical Institute of Bioregulation, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan; 11) Interdisciplinary Program in Bioinformatics, Seoul National University, Seoul, Korea; 12) Department of Biochemistry, School of medicine, Ewha Womans University, Seoul. Korea.

Coronary artery disease (CAD) is the most common form of heart disease and the leading cause of death worldwide. Multiple genetic factors and environmental exposures have been shown to affect pathogenesis of coronary artery disease (CAD) together. The etiology contributing to CAD pathogenesis are different between Asian and European populations. Recently genetic variants contributing to CAD have been identified through Genome-wide association (GWA) studies in the European population. In this study, we conducted a two-stage GWA analysis of CAD to identify Asian-specific genetic variants influencing CAD in East Asian populations. In the discovery stage, we carried out SNP genotyping for 2,123 cases of individuals with CAD and 2,690 gender-matched controls of healthy individuals using Affymetrix Genome-wide Human SNP array 6.0 platform. The logistic regression model (1 d.f.) with adjustment for age and gender was used to analyze the association. In the replication stage, 16 SNPs were tested in 812 cases of CAD and 4,422 gender-matched controls of Japanese population using TaqMan assay. After combining the GWAS and replication samples for 16 SNPs, we identified three new loci associated with CAD at 12q24, 13q12 and 4q12.

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Genomic strategies to identify functional noncoding sequences in loci associated with cardiovascular diseases. *J.M. Westlund, S. Smemo, I. Aneas, N.J. Sakabe, M.A. Nobrega.* University of Chicago, Chicago, IL.

Genome-wide Association Studies (GWAS) routinely identify noncoding loci associated with complex traits, raising the possibility that variation in long-range cis-regulatory elements might lead to disease risk. Technologies to identify and experimentally validate these putative long-range regulatory sequences are, therefore, key steps in the follow-up of GWAS. We carried out a strategy to identify regulatory variation associated with increased risk to cardiovascular diseases in loci emerging from GWAS. Using ChIP-seq, we profiled the chromatin states and binding sites for a number of transcription factors and transcription co-factors in mouse hearts, generating a comprehensive transcription regulation map in an organ that lacks cell types amenable to routine culture in vitro. Using a combination of comparative genomics, open chromatin marks and binding sites of transcription factors and co-activators, we uncovered a number of candidate cis-regulatory sequences in loci associated with various cardiovascular diseases, such as SCN5A, SCN10A, TBX5, TBX3, PITX1, KCNQ etc. These putative regulatory sequences either harbor SNPs associated with cardiovascular traits or are in LD with associated SNPs. To test the regulatory potential of these candidate sequences, in vivo, we cloned each DNA sequence in a GFP reporter vector, injected these constructs in zebrafish eggs and evaluated the resulting transgenic zebrafish for GFP expression in the heart. Our data demonstrates that several of our candidate regulatory sequences possess in vivo heart specific enhancer properties. Combined, our results describe a strategy for the functional follow-up of GWAS implicating noncoding loci in disease etiology and identify functional noncoding sequences in loci associated with cardiovascular disorders.

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A genome-wide gene-based association study identifies SLC4A4, WWOX and COMMD7 as hypertension susceptibility genes in a Han Chinese population. *H.-C. Yang[1], L.-J. Liang[1,3], K.-M. Chiang[2,4], G. Thomas[6], B. Tomlinson[7], S. Cherny[8], Y. Gu[8], T.-H. Lam[9], J.-W. Chen[5], W.-H. Pan[2].* 1) Institute of Statistical Science, Academia Sinica, Taipei, Taiwan; 2) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 3) Graduate Institute of Biomedical Electronics and Bioinformatics, National Taiwan University, Taipei, Taiwan; 4) School of Public Health, National Medical Defense Center, Taipei, Taiwan; 5) National Yang-Ming University School of Medicine and Taipei Veterans General Hospital, Taipei, Taiwan; 6) Public Health, Epidemiology and Biostatistics, School of Health and Population Sciences, University of Birmingham, Birmingham, UK; 7) Department of Medicine and Therapeutics, Chinese University of Hong Kong, Hong Kong; 8) Genome Research Centre, Hong Kong University, Hong Kong; 9) School of Public Health, Hong Kong University, Hong Kong.

Hypertension [OMIN #145500] is a complex disorder with high prevalence and social impact all over the world, especially in industrialized regions. This study conducts the first genome-wide gene-based association scan for hypertension in a Han Chinese population. By analyzing genome-wide SNP data of ~400 matched pairs of young-onset hypertensive patients and normotensive controls genotyped with the Illumina HumanHap550-Duo BeadChip, 100 susceptibility genes of hypertension are identified and then validated by permutation tests. In addition to differential allelic distributions, 17 of the 100 genes also exhibit differentially expressed distributions in case and control groups. These genes provide a good molecular signature for a classification of hypertensive patients and normotensive controls. Among the 17 genes, SLC4A4 encoding the Electrogenic Sodium Bicarbonate Cotransporter 1 Protein and WWOX encoding the WW Domain-Containing Protein are not only identified by our gene-based association scan and gene expression analysis but also replicated by a gene-based analysis of the Hong Kong Hypertension Study. Previous mouse experiments have suggested that SLC4A4 is associated to decreased body weight/size and abnormal ion homeostasis and WWOX is associated to hypoglycemia and hyperphosphatemia. In addition, a novel hypertension gene COMMD7 encoding COMM Domain-Containing Protein 7 is replicated by a gene-based analysis of the WTCCC Hypertension Study. Identification of these genes benefits to enrich a collection of hypertension genes thereby to unravel complex development etiology of hypertension in Han Chinese populations.

737/W

Admixture mapping analysis of blood pressure identifies a genetic locus associated with blood pressure in the CARe consortium. *X. Zhu[1], S. Kang[1], E. Fox[2], J. Young[3], N. Franceschini[4], B. Tayo[5], A. Adeyemo[6], Y. Sun[7], Y. Li[1], A. Morrison[8], C. Newton-Cheh[9], K. Liu[10], S. Ganesh[11], A. Kutlar, V. Ramachandran[12], A. Dreisbach,S. Wyatt[13], J. Polak[14], W. Palmas, R. Fabsitz[15], R. Townsend[16], D. Dries, J. Glessner, C. Chiang[9], T. Mosley, S. Kardia[7], D. Curb, J. Hirschhorn[9], C. Rotimi[6], A. Reiner[16], C. Eaton[17], J. Rotter[18], R. Cooper[5], B. Keating[19], S. Readline[1], A. Chakaravati[20], D. Levy[21], the NHLBI CARe BPHTN working group.* 1) School of Medicine, Case Western Reserve Univ, Cleveland, OH; 2) Department of Medicine,-University of Mississippi Medical Center,Jackson, MS; 3) Department of of Medicine and Epidemiology The Johns Hopkins University Baltimore, MD; 4) Department of Epidemiology University of North Carolina Chapel Hill ,Chapel Hill, NC; 5) Preventive Medicine & Epidemiology Loyola University Medical Center, Maywood, IL; 6) Center for Research on Genomics and Global Health National Human Genome Research Institute Bethesda, MD; 7) University of Michigan School of Public Health, Department of Epidemiology,Ann Arbor, MI; 8) Human Genetics Center University of Texas at Houston,Houston, Texas; 9) Harvard Medical School Broad Institute of Harvard and MIT; 10) Department of Preventive Medicine Northwestern University Medical School,Chicago, IL; 11) Division of Cardiovascular Medicine University of Michigan Health System, Ann Arbor, MI; 12) Boston University School of Medicine,Framingham, MA; 13) Division of Nephrology, University of MS Medical Center,Jackson, MS; 14) Department of Radiology Tufts University School of Medicine,Jamaica Plain MA; 15) Division of Prevention and Population Sciences National Heart, Lung, and Blood Institute, Bethesda, MD; 16) School of Public Health, University of Washington; 17) Memorial Hospital of Rhode Island, Pawtucket, RI; 18) Division of Medical Genetics, CSMC, Los Angeles, CA; 19) School of Medicine, University of Pennsylvania, Philadelphia; 20) Johns Hopkins University School of Medicine Baltimore, MD; 21) Center for Population Studies National Heart, Lung, & Blood Institute, Framingham, MA.

We performed an admixture mapping analysis for systolic and diastolic blood pressure (SBP, DBP) followed by association analysis in 6302 unrelated African-Americans in the CARe consortium. We identified 5 genome regions (P <0.001) harboring genetic variants contributing to inter- individual blood pressure variation. In follow-up association analysis in these 5 regions, , we identified 4 loci associated with SBP and one associated with DBP which were significant after correcting for multiple comparisons (P £5×105). Further analysis suggested that there are 9 independent SNPs contributing to the phenotypic variation observed in the admixture mapping analysis. These 9 SNPs were carried forward for replication analysis in multiple large independent African-American samples (Women's Health Initiative, Maywood, GENOA and HUFS) as well as one Nigerian sample, with a total sample size of 12,153. Meta-analysis of the replication cohorts identified evidence of a novel variant on chromosome 5 as being associated with SBP and DBP (P £0.0015). The genes near the variant include SUB1, PDZD2, NPR3, MTMR12. Our results suggested admixture mapping can be fruitful for detecting genetic variants underpinning complex traits such as blood pressure.

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Association of Potassium Channel Genes, KCNK3 and KCNK9, with Blood Pressure and Serum Levels of Potassium and Aldosterone. *J. Jung[1], G. Eckert[2], W. Tu[2], JH. Pratt[2].* 1) Med & Molec Gen, Indiana Univ Sch Med, Indianapolis, IN; 2) Dept. of Medicine, Indiana Univ Sch Med, Indianapolis, IN.

The epithelial sodium channel (ENaC) in the kidney's distal nephron is the final and perhaps the most important site for reabsorption of sodium and for establishing levels of blood pressure (BP). The principal regulator of ENaC is aldosterone which is secreted by the adrenal zona glomerlosa (ZG) under the synergistic influences of angiotensin II and potassium. We performed association studies for two genes highly expressed in ZG, KCNK3 and KCNK9, that encode for the potassium channels TASK-1 and TASK-3, respectively. Both were shown in mouse knock-out models to restrain aldosterone secretion. Subjects (n=286; 127 males and 159 females) were from a cohort of healthy African Americans (AA) adolescents with mean ages of 15 years old (range of age: 5-25 yr). BP was measured semi-annually for up to 20 years; levels of potassium and aldosterone as well as the ratio of aldosterone to renin (ARR) were measured cross-sectionally. Seventy-four tag SNPs for KCNK9 and 20 for KCNK3 were selected using Tagger with R2 =80% and MAF >10%. Population-based association studies identified 10 SNPs that associated with BP (4 with p<0.01; 6 with p<0.05) and of these 4 SNPs (rs888345, rs2545462, rs3824281, rs3780037) associated with serum K (p=0.006). rs888345 also associated with serum aldosterone and ARR (p=0.003 and 0.01, respectively). No significant associations with KCNK3 were observed. The findings suggest that variations in TASK-3 affect sodium retention and in turn BP in young and healthy AA, a group at increased risk for salt-sensitive hypertension.

739/W

A large mutation screen in the German LIANCO cohort identified *SREBF1* and *SREBF2* as hypercholesterolemia-associated genes. *N. Plume[1, 2, 3], E. Milz[1, 2, 3], I. Gouni-Berthold[4], W. Krone[4], H. Berthold[5], B. Wollnik[1, 2, 3].* 1) Institute of Human Genetics, University Hospital Cologne, Cologne, Germany; 2) Center for Molecular Medicine Cologne (CMMC), University of Cologne, Cologne, Germany; 3) Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Germany; 4) Department of Internal Medicine II, University of Cologne, Cologne, Germany; 5) Lipid Clinic at the Interdisciplinary Metabolism Center, Charite University Medicine Berlin, Virchow Clinic Campus, Berlin, Germany.

Familial hypercholesterolemia (FH) is a highly prevalent autosomal dominant disorder and the high cholesterol level in affected individuals is a major risk factor for coronary artery disease (CAD) and myocardial infarction. We initiated a molecular study of 200 cases from the German LIANCO (Lipid-Analytic-Cologne) cohort with the aim (i) to determine the *LDLR* mutation spectrum (ii) to identify molecular modifiers of cholesterol levels in *LDLR*-caused FH, and (iii) to find novel FH causing genes. Sequencing of *LDLR* revealed a mutational spectrum of known (34) and novel (7) heterozygous mutations, predominantly missense, but also nonsense and splice-site mutations as well as small deletions and insertions. Using MLPA we found 4 large deletions within the *LDLR* gene. Interestingly, one heterozygous mutation, c.1-188C>T, was identified within the predicted *LDLR* promoter region we could clearly demonstrate a reduced ability for transcriptional activation. Only one patient carried the p.R3500Q hot-spot mutation in *ApoB100*, whereas no alterations were found in *PCSK9* and *SCARB1*. We also tested the nonsense SNPs rs10491178 in *ABCA10* and rs328 in *LPL* in all mutation positive patients but could not observe a clear genotype-phenotype correlation. In order to identify novel hypercholesterolemia associated genes, we sequenced the transcription factors *SREBF1* and *SREBF2* for mutations in our mutation-negative patients. Interestingly, we found one novel nucleotide substitution in both, *SREBF1* and *-2*, respectively. The c.G2435A substitution is located in exon 13 of *SREBF1* and is predicted to cause an amino acid change from arginine to glutamine at position 812. The putative mutation in *SREBF2* was found in exon 14 (c.G2554A) and changing the glycine at position 852 to arginine. Both alterations were not found in 200 control chromosomes. We are currently investigating the functional effect of both mutations via Luciferase Activity Assay, Cleavage Efficiency Assay and Co-Immunoprecipitation of wild-type and mutant SREBF1 and SREBF2 with SCAP. In summary, we could show that *LDLR* mutations are present in the German LIANCO cohort. Moreover, we identified *SREBF1* and *SREBF2* as putative novel hypercholesterolemia causing genes. Our data indicate that transcriptional activation of *LDLR* is essential for normal cholesterol levels and that mutations in the *LDLR* promoter as well as the transcriptional regulators SREBF1 and SREBF2 underlie hypercholesterolemia.

740/W

Candidate gene association study of frailty in older community dwelling women. *Y-Y. Ho, Q-L. Xue, A.M. Mattenini, W. Yao, A. Chakravarti, D.E. Arking, L.P. Fried, J.D. Walston, M.D. Fallin.* Johns Hopkins University, Baltimore, MD.

Frailty is a late-life syndrome of vulnerability to adverse health outcomes that is characterized by muscle weakness and increased inflammatory pathway activation. Evidence suggests that these variations may play important roles in frailty susceptibility. We hypothesized that variations in genes related to inflammation and muscle maintenance associate with frailty in older adults, and tested this hypothesis in candidate gene association study in older community dwelling women. Subjects were participants in a cohort study on the development of disability, aged 70-79, and Caucasian. Frailty defined as a three-level outcome was assessed using a validated assessment method that includes grip strength, walking speed, physical activity, weight loss, and fatigue. Genotypes were obtained for 1364 SNP markers across 134 candidate genes related to inflammation and muscle biology using the Illumina Genotyping platform. Likelihood ratio tests were performed for each SNP adjusting for age using multinomial logistic regression model. Individual SNPs within MTR (rs10925235, rs4659725, rs1770449, rs2297967, rs10802569, rs1050993), CASP8 (rs3769827, rs6747918, rs2037815), FN1 (rs7567647), RIPK1 (rs9405191) loci were associated with frailty before adjustment for multiple comparisons (p value < 3 x10-3). These genes are related to DNA methylation, apoptosis, and inflammation biology. Although no conclusions can be drawn for this data given small number of genotypes, and as the SNPs from the top 20 hits do not exceed the genome wide significant p value of 0.0004, these genotypes may provide further insights into the pathways that may underlie the development of frailty and late-life decline.

741/W

Association analysis of coronary artery disease loci with the Metabochip array. *P. Deloukas[1], K. Stirrups[1], S. Potter[1], S. Hunt[1], S. Edkins[1], C. Langford[1], P.S. Braund[2], A. Balmforth[3], A.S. Hall[3], M.I. McCarthy[4], G. Dedousis[5], M. Sandu[1,6], N.J. Samani[2], Wellcome Trust case Control Consortium.* 1) Wellcome Trust Sanger Inst, Cambridge, UK; 2) University of Leicester, Leicester, UK; 3) Leeds Institute of Genetics, Health and Therapeutics, Leeds General Infirmary, Leeds, UK; 4) WTCHG, University of Oxford, Oxford, UK; 5) Harokopio University, Athens, Greece; 6) Department of Public Health & Primary Care, Strangeways Research Laboratory, University of Cambridge, UK.

Following an initial round of GWAS, large scale meta-analyses of common disease phenotypes including coronary artery disease (CAD) have led to the identification of multiple loci reaching genome wide significance (p 5 10^{-8}). In parallel, such analyses yielded additional loci with moderate evidence of association. A custom iSELECT array harbouring ~196,000 markers, the Metabochip, was designed to undertake both fine mapping in loci of confirmed association and extensive replication of the weaker signals for several cardiometabolic traits. For CAD, marker content (~23,000 SNPs) was supplied from a meta-analysis of over 22,000 CAD cases and 67,000 controls of European descent (CARDIOGRAM). Markers for fine mapping were selected from dbSNP and the 1000 Genomes (August 2009). We have genotyped over 6500 CAD cases and 8000 controls with the metabochip array so far. The presence of a large fraction of low frequency variants (3 - 0.1%) on the metabochip along-side common ones complicates genotype calling. We analysed an initial set of 3000 cases (BHF collection) and 5000 UK controls using three calling algorithms GenCall, Illuminus and GenoSNP. Close to 93% of all SNPs have a call rate ≥99%, any calling algorithm, with circa 55,000 markers being non polymorphic or having an AF < 0.1%. Most SNPs (93%) are over 99.8% concordant among all genotype calling algorithms. After data QC, tests of self association in both the case and control series found no significant background association for markers with AF ≥1.5%. Preliminary analysis of the 3000 CAD cases vs 5000 controls indentified an excess of association signals with over 180 having p < 10^{-5}. As expected most of these SNPs fall within the regions of confirmed association with 11 such loci providing initial insights to fine mapping. The strongest signal was obtained in the ANRIL locus on chromosome 9p21 with the lead SNP been rs10757278 (p < 6.4 10^{-5}). At least one locus, ADAMTS7 on chromosome 15 has evidence of two independent signals - r^2 with the lead SNP <0.2. In five of the 11 loci the emerging lead SNP was novel derived from the 1000 genomes data set. In addition to expanding analysis to all of the above cohorts to increase power we are typing 2000 CAD patients and 2000 controls from South Asia to explore transethnic fine mapping. An international effort to analyse over 20,000 CAD cases and 40,000 controls is underway testing replication of the weaker signals identified in Cardiogram.

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Characterization of a new dominant Marfan-like phenotype by deep whole genome sequencing. *S. Scherer[1,3,5], D. Milewicz[2], S. LeMaire[4,5], I. Volguina[4,5], L. Palmero[4,5], M. Bainbridge[1,5], J. Reid[1,5], D. Muzny[1,5], J. Coselli[4,5], R. Gibbs[1,3,5].* 1) Human Genome Sequencing Center; 2) Division of Medical Genetics, University of Texas Health Science Center at Houston Houston, TX; 3) Department of Molecular and Human Genetics; 4) Texas Heart Institute at St. Luke's Episcopal Hospital; 5) Baylor College of Medicine Houston, TX.

Various defects in the transforming growth factor-beta (TGF-β) signaling pathway give rise to a number of well-described phenotypes, including hereditary hemorrhagic telangiectasia (HHT), Marfan syndrome (MFS), Loeys-Dietz syndrome (LDS), and familial thoracic aortic aneurysms and dissections (FTAAD). We recently demonstrated the utility of using whole genome sequencing (WGS) to identify causative allelic variants in recessive Charcotte Marie Tooth disease [Lupski et al. 2010 NEJM]. Here, we extend these methodologies to determine the mutation underlying a unique, dominantly inherited vascular phenotype. Deep, WGS SOLiD sequencing was used to characterize the genome of a Caucasian female proband that presented with a dilated aorta and underwent aortic root and arch replacement at age 17. The proband has features of MFS (arched palate, arachnodactyly, a positive thumb sign and mild scoliosis), LDS (dilatation and tortuousity of large arteries) and HHT (arteriovenous malformations requiring treatment and atypical telangiectasias). Sequencing of the known genes for these disorders failed to reveal any disease-causing mutations. The proband's mother also underwent aortic root replacement surgery and displays the same phenotype as her daughter and similarly affected two first cousins; there is no evidence of consanguinity in the family. Preliminary light coverage sequencing identified a heterozygous nonsense mutation in exon 62 of the FBN2 gene, which was validated and present in all affected family members. Analysis of deep sequencing results, variant validation and genotyping data are ongoing as we seek to extend these protocols to the clinical setting.

743/W

Targeted capture and massively parallel sequencing in pediatric cardiomyopathy: development of novel diagnostics. *M. Tariq[1], T. Lee[1], P. Putnam[2], S. Kindel[1], C. Jamison[2], M. Keddache[3], SM. Ware[1,3].* 1) Division of Molecular Cardiovascular Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 2) Division of Biomedical Informatics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 3) Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

Pediatric cardiomyopathy is a genetically heterogeneous disease associated with significant morbidity. Although identification of cause is important for management, therapy, and screening of at risk family members, molecular diagnosis has been difficult due to the large number of causative genes, high rate of private mutations, and cost of testing. The aim of this study was to determine the utility of array-based targeted sequence capture coupled to massively parallel sequencing for diagnosis of pediatric cardiomyopathy. A custom 385K NimbleGen sequence capture array containing 110 genes and providing 99.9% coverage of the exons of interest was used to screen 3 patients with cardiomyopathy. The sensitivity and specificity of the custom array was >99% as determined by comparison to a subset of 31 genes subjected to long range PCR-based next-generation sequencing, Sanger sequencing validation of all missense variants, and genotyping using the Illumina Infinium Omni1 array. On the custom array, 99.73% of the total targeted regions were captured and sequenced at >10X coverage, allowing reliable variant calling in all patients. Variant analysis identified 146 non-synonymous missense variants and 6 indels in 26 genes including 43 missense and 1 indel in patient 1, 41 missense and 3 indels in patient 2, and 62 missense and 2 indels in patient 3. The functional pathogenicity of all the non-synonymous variants were analyzed through the bioinformatic tools Polyphen, SIFT and PANTHER. This study demonstrates the digenic or oligogenic pattern of inheritance in pediatric cardiomyopathy. Comparison of the performance of targeted sequence capture vs. PCR-based enrichment methods demonstrate that former is more sensitive and efficient although more costly. This study attests to the importance, robustness and performance of array-based sequence capture technology followed by massively parallel sequencing as a promising and comprehensive tool for the genetic screening of cardiomyopathies.

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ANRIL, the non coding RNA in the Coronary Artery Disease associated 9p21 locus has trans-effects on gene expression and a potential regulatory role. *T. Kyriakou, A. Goel, H. Ongen, J. Peden, H. Watkins.* Department of Cardiovascular Medicine,Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom.

Genome-wide association studies have identified a 53kb haplotype block in the 9p21 region associated with risk of diverse cardiovascular diseases. This locus overlaps with ANRIL, a non coding RNA gene of unknown function and genomic architecture. Using RT-PCR and RACE-PCR we identified alternative transcription start and end sites and multiple alternative spliced transcripts differentially expressed between different tissues and cell lines. siRNA oligonucleotides were designed against a constitutively expressed exon and against the last exon of all transcripts identified . siRNA oligonucleotides were transfected in a primary endothelial cell model (Human Umbilical Vein Endothelial Cells). Total RNA from transfected cells was used to assess global changes in gene expression using microarray technology. Analysis of changes in global expression levels was performed using PARTEK software and after a false discovery rate (FDR) correction of 0.05 approximately 200 genes were perturbed more than 50% and almost 90 genes had more than 2-fold altered levels of expression after the knock-out when compared with controls. The majority of the top 20 affected genes are involved in cell cycle regulation. Interestingly, CDKN2B, a neighboring gene that overlaps with the first intron of ANRIL and previously associated with expression levels of ANRIL was found to be significantly upregulated. In contrast, it has previously been reported that expression levels of CDKN2B were found to be drastically down regulated in a mouse model when the 9p21 syntenic region was knocked out. Our data demonstrate that ANRIL has a role to play in regulating the expression of number of different genes in different cellular pathways and does so by acting in trans.

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Fatty acid desaturase 1 (FADS1) transcripts are associated with insulin sensitivity and are controlled by a local expression quantitative trait locus (eQTL). *J.N. Hellwege[1], S.K. Das[2], N.K. Sharma[2], A.K. Mondal[2], S.C. Elbein[2].* 1) Molecular Genetics and Genomics, Wake Forest University, Winston-Salem, NC; 2) Internal Medicine- Endocrinology, Wake Forest University Health Sciences, Winston-Salem, NC.

Fatty acid desaturase 1 (FADS1) is a delta-5 desaturase involved in the essential omega-3 and omega-6 fatty acid metabolic pathways. SNPs in the FADS1 gene have been shown previously to be associated with polyunsaturated fatty acids (rs174547, rs174537, rs174548), fatty acid serum levels (rs174546), total cholesterol levels (rs174548), and recently fasting glucose levels and possibly abnormal insulin secretion. However, the function of these noncoding variants on FADS1 transcript levels, or the role of these transcript levels has not been examined. We examined FADS1 transcript levels in adipose and muscle from 62 individuals chosen for extremes of insulin sensitivity (31 insulin resistant, 31 insulin sensitive). FADS1 was 1.59 fold reduced in insulin resistant muscle (q value 0.88%) and 1.67-fold reduced in insulin resistant adipose (q value 0.96%). Intronic SNP rs174550 was associated with FADS1 transcript levels in two different liver studies ($p<0.0001$), but did not reach significance ($p<0.001$) in either muscle or adipose from these 62 individuals. To further explore the role of GWAS associated SNPs in transcript expression, we genotyped SNPs rs174548 and rs174550 in 498 individuals including 153 who provided subcutaneous adipose and 146 with skeletal muscle biopsy samples. The two SNPs were in strong linkage disequilibrium in Caucasians ($r^2 = 0.89$) but not in African Americans ($r^2 = 0.015$). SNP rs174550 was associated with FADS1 expression in skeletal muscle ($p = 0.0002$, dominant model), even with adjustment for age, gender, and ethnicity. However, neither SNP was associated with insulin sensitivity among the 498 individuals with detailed assessments by frequently sampled intravenous glucose tolerance test. Among individuals with biopsies, adipose FADS1 transcript levels were negatively correlated with AUC insulin levels from the oral glucose tolerance test ($p = 0.014$). We show that FADS1 expression in both muscle and adipose tissue is associated with insulin sensitivity and that intronic SNP rs174550 is associated with FADS1 expression in both liver and skeletal muscle. FADS1 has multiple splice forms that are now under evaluation, and may explain tissue specific differences. Markedly different allele frequencies in African Americans and Caucasians suggest that FADS1 may be primarily a European risk locus, acting as a local eQTL in liver and muscle.

746/W

Investigating the association of MGP promoter polymorphisms with coronary artery disease and their effect on gene expression. *M. Keramatipour[1], P. Ahmadi[1], M. Abiri[1], S. Sadeghian[2], M. Pedram[1].* 1) Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran; 2) Department of Cardiology, Tehran Heart Center Hospital, Tehran University of Medical Sciences, Amirabad St., Tehran, Iran.

Coronary Artery Disease (CAD) is a major cause of death worldwide including Iran. Positive family history among patient points to the significance of genetic elements in the risk of CAD. Two single nucleotide polymorphisms (SNPs) at -7 (rs1800801) & -138 (rs1800802) positions on Matrix- Gla Protein (MGP) gene have been suggested to play a role in susceptibility towards CAD. Such a role is possibly due to the effect of these SNPs on the level of gene expression. This study aims to investigate the association of these SNPs with CAD in Iranian population and their possible effect on MGP gene promoter activity in vitro. To achieve these goals, a population based genetic association study is being conducted on appropriate groups of patients and controls on the basis of their clinical assessments and angiographic reports. Analysis of the preliminary data obtained from genotyping 150 cases and 150 controls shows an excess of A allele at position -7 and T allele at position -138 among patients. The observed differences are not significant at this point, but genotyping of proposed sample size is not complete yet. On the other hand, four appropriate vectors that express GFP under the control of different haplotypes of MGP promoter have been constructed. To assess the effects of haplotypes on promoter activity, vectors were transfected into the Hek293 cells and relative expression of GFP was quantified. Analysis of data using Student's T-test, revealed that G-7A SNP has a significant effect on promoter activity in cultured cells. Presence of nucleotide A in -7 position resulted in 12% increase in promoter activity ($p<0.002$). T-138C SNP did not show a significant change in promoter activity ($p>0.7$). The maximum mean activity of the promoter was seen with A-T haplotype while the minimum activity appeared by G-T haplotype. By the way, statistically the most significant difference in promoter activity is seen between vectors containing A-C and G-T haplotypes ($p<0.0005$). less significant differences in promoter activity were shown between A-T and G-T haplotypes as well as A-T and G-C haplotypes ($p<0.05$).

747/W

A common variant of beta3-adrenergic receptor gene and pregnancy-induced hypertention in Japan. *G. Kobashi[1,5], H. Yamada[2], A. Hata[3], K. Ohta[1,3,4], H. Minakami[4], N. Sakuragi[4], H. Tamashiro[5], S. Fujimoto[6], Hokkaido PIH Epidemiological Study Group.* 1) Research Center for Charged Particle Therapy, National Institute of Radiological Sciences, Chiba, Japan; 2) Dept. of Obstetrics and Gynecology, Kobe University Graduate School of Medicine; 3) Dept. of Public Health, Chiba University Graduate School of Medicine; 4) Dept. of Obstetrics and Gynecology, Hokkaido University Graduate School of Medicine; 5) Dept. of Global Health and Epidemiology, Hokkaido University Graduate School of Medicine; 6) Sapporo Maternity-Women's Hospital.

Pregnancy-induced hypertention (PIH), major cause of maternal morbidity and mortality, is considered to be a multifactorial disease resulting from an interaction among several factors including lipolysis and obesity. Beta3-adrenergic receptor (B3AR) is mainly expressed in brown and white adipose tissues and is considered to be responsible for thermogenesis, lipolysis, insulin sensitivity and body weight gain, and was reported to be associated with non-insulin dependent diabetes mellitus. Recently, an association between a common variant, Trp64Arg of the B3AR gene and manifestation of PIH was suggested in its pathogenesis, however, it has been yet confirmed. In the present study, we genotyped and analyzed the variant and other genetic and environment risk factors in order to confirm the association in the Japanese subjects. Seventy-nine Japanese primiparous patients with PIH including preeclampsia, protein uric type of PIH, were matched with 158 Japanese normal pregnant controls according to age and parity. Informed consent for the study was obtained from every subject. Genotypings of variants including Trp64Arg of the B3AR gene were carried out using PCR-RFLP methods after extraction of genomic DNA from 1.0 ml of whole blood samples. Differences were statistically analyzed by the chi-square test (degree of freedom=1). Fisher's exact test was used when an observed number was £ 5. In the allelic frequencies of Arg64, a mutant type of the B3AR gene, there were no significant differences between the cases and controls (15.8% in PIH and 16.1% in controls). Furthermore, no significant differences were found in the subgroup analyses using the other PIH risk factors such as prepregnancy body mass index, family history of hypertension, M235T of angiotensinogen gene, Glu298Asp of the endothelial nitoric oxide synthase gene and some lifestyle factors. The present result suggests that the Trp64Arg variant of the B3AR gene is not associated with PIH in the Japanese women.

748/W

Evaluation of three molecular mechanisms for altered *MMAB* transcript level at a locus associated with high density lipoprotein cholesterol (HDL-C). *M.P. Fogarty[1], M.L. Bucholovich[1], K.J. Gaulton[1], Y. Li[1,2], K.L. Mohlke[1].* 1) Department of Genetics, University of North Carolina, Chapel Hill, NC; 2) Department of Biostatistics, University of North Carolina, Chapel Hill, NC.

Liver eQTL studies and our hepatocyte RNA and protein expression imbalance studies showed that an HDL-C-associated locus on chromosome 12 is also associated with higher levels of *MMAB*. Our goal is to determine the underlying SNP(s) and molecular mechanisms affecting *MMAB* transcript level. We hypothesize that a SNP(s) acting at this locus likely has a subtle effect (~20%) on *MMAB* transcript synthesis or stability and we are exploring 3 potential mechanisms for this effect. First, we tested whether HDL-C associated SNP rs877710, located in the *MMAB* 3'UTR, affects targeting by miR-564. The G allele of rs877710 is predicted to weaken the target site for miR-564 and thus increase *MMAB* expression levels. Using a dual luciferase reporter vector and 293T cells, we demonstrated that in the presence of miR-564, the G allele of rs877710 demonstrated a 16% weaker repression of luciferase activity compared to the C allele (P=.002), suggesting preferential binding to the C allele. Additional studies will test whether miR-564 results in decreased endogenous *MMAB* expression in liver cells. Second, to assess whether a regulatory SNP acts in *cis* to affect transcript synthesis, we tested SNPs for allele-specific enhancer activity in HepG2 cells. We used linkage disequilibrium data obtained from HapMap and the 1000 Genomes Project to identify 63 SNPs in r^2>.7 (CEU) with highly HDL-C associated SNP rs2338104, and we identified 3 associated SNPs located in potential regulatory elements based on evidence of open chromatin, histone modifications and predicted transcription factor binding. Preliminary results showed that of 57 SNPs screened to date, six leading SNPs display >1.5-fold allele-specific enhancer activity in the direction expected. Additional experiments are needed to verify the magnitude and significance of allele-specific differences. Third, rs2287180 (r^2=.22 with rs2338104) disrupts a predicted splicing enhancer and has been implicated in alternative splicing of *MMAB* leading to decreased expression in the expected direction. rs2287180 genotypes in our human hepatocyte cohort (n=89) will be used to evaluate the relative contribution of this decrease on overall *MMAB* transcript level. Taken together, these results provide suggestive evidence for more than one mechanism that may alter *MMAB* transcript level. Identification of a molecular mechanism for altered *MMAB* expression may guide functional studies to determine how variants at this locus influence HDL-C.

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Differential gene expression and enhancer use are correlated with asymmetries in epigenetic modification within the developing heart. *S. Smemo, I. Aneas, N. Sakabe, M. Nobrega.* Human Genetics, University of Chicago, Chicago, IL.

The mammalian heart is heterogeneous in form and function, and understanding those differences has been at the center of cardiac research for centuries. In modern times this has been carried out at the genetic level, with a multitude of differences in gene expression, including many genes encoding transcription factors, being described and correlated with chamber identity, structure, function and disease. Nonetheless, it is not fully resolved how these transcriptional asymmetries, evident at even the very early stages of morphogenesis, are established and maintained. One hypothesis, pursued here, is that chromatin accessibility is modulated independently within the developing heart via epigenetic modification, thereby potentiating or excluding expression of specific genes. To study this we have performed transcriptional profiling by RNA-seq on isolated left and right ventricles of embryonic mice and correlated the findings with ChIP-seq against H3K27me3, a mark of closed chromatin associated with gene repression. Furthermore, by performing ChIP-seq in left and right ventricles for H3K4me1, a chromatin modification associated with enhancers, and p300, a chromatin-binding coactivator found at active enhancers, we have extended this hypothesis to include differential usage of transcriptional enhancers.

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Association study, KCNE1, polymorphisms, non-familial atrial fibrillation(AF), potassium channel. *A.R. Park[1], D.-J. Shin[1,2], H. J. Hwang[3], N.-H. Son[1], K.-B. Lee[1], J.-W. Im[1], E.-S. Shin[4], J.-E. Lee[4], H.-N. Pak[3], M.-H. Lee[3], S.-S. Kim[3], Y. Jang[1,3].* 1) Yonsei Cardiovascular Genome Center, Seoul, Seodaemun-ku, Korea; 2) Yonsei University Research Institute of Science for Aging, Seoul, Korea; 3) Division of Cardiology, Yonsei University College of Medicine, Seoul, Korea; 4) DNA Link, Inc, Seoul, Korea.

Slowly activating delayed-rectifier potassium currents in the heart are produced by a complex protein with alpha and beta subunits composed of the potassium voltage-gated channel KQT-like subfamily, member 1 (KCNQ1) and the potassium voltage-gated channel Iks-related family, member 1 (KCNE1), respectively. Recent studies have been proposed to play a mechanistic role in non-familial Atrial fibrillation (AF). We preformed a case-control study to evaluate the presently controversial question of whether KCNE1 gene polymorphisms are associated with AF in Koreans. We studied a sample population of 754 Koreans, comprising of 400 controls and 354 cases with AF, which were recruited from Cardiovascular Genome Center in Korea. We analyzed 8 single nucleotide polymorphisms (SNPs) of KCNE1 genes (rs2834485, rs3453, rs13050198 and rs41314067, rs1805127, rs2236608, rs2834502, rs4817669). All subjects were genotyped for these polymorphisms by single base primer extension assay using the SNaPShot assay. The distribution of allele for rs2834485 and rs2834502 was significantly different between the patients with AF and the controls in female group (P=0.0197) and male group (P=0.0379), respectively. Logistic regression analysis represented that KCNE1 rs2834485 T carriers were significantly associated with AF under a dominant model (CT+TT/CC; OR, 0.535; confidence interval (CI), 0.313-0.913; P=0.0219), rs2834502 A carriers were significantly associated with AF under a dominant model (AA+AG/GG; OR, 0.661; confidence interval (CI), 0.447-0.977; P=0.0379). In haplotype analysis, the dominant model of haplotype AAG and recessive model of haplotype AA was shown to predisposing effect significantly in females (OR, 2.318; confidence interval (CI), 1.138-4.721; P=0.0205) and males (OR, 1.541; confidence interval (CI), 1.040-2.285; P=0.0313), whereas haplotype CAA-GATAA decreased the risk of AF by 46% (OR, 0.536; confidence interval (CI), 0.288-0.997; P=0.0491) under a dominant model in female subjects. These findings suggest that KCNE1 gene polymorphisms are associated with non-familial Atrial fibrillation in Koreans. Further investigations should be performed to analyze association of these polymorphisms with non-familial Atrial fibrillation in other genes and conducted to evaluate the functional role of these genes as a genetic contributor.

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A common polymorphism in the NOTCH1 gene is associated with calcific aortic valve stenosis. *V. Ducharme[1], N. Gaudreault[1], P. Pibarot[1], P. Mathieu[1], Y. Bossé[1,2].* 1) Centre de recherche Institut universitaire de cardiologie et de pneumologie de Québec, Laval University, Quebec, Canada; 2) Laval University Hospital Research Center (CRCHUL), Quebec, Canada.

Calcific aortic valve stenosis (AVS) is the most frequently acquired valvular heart disease affecting 1-2% of the population older than age 65 years. It is characterized by pathological remodeling and calcification of the aortic valve cusps, which eventually lead to left ventricular outflow track obstruction. Genetic studies in few multi-generation families have provided compelling evidence that functional DNA variants at the NOTCH1 locus result in aortic valve anomalies and severe valve calcification. Whether genetic variants in that gene contribute to AVS in the population at large still remain to be determined. A total of 14 genetic variants surrounding the NOTCH1 gene were genotyped in 467 patients with severe AVS and tricuspid valve morphology. Genotyping was carried out using the Illumina BeadXpress platform. SNPs were selected to document the previously rare mutations associated with AVS, but also to capture the common genetic variation within this locus. Allele frequencies of common SNPs for patients with AVS were compared to a share control group (Illumina iControlDB, n=3294). The mutation R1107X, previously associated with AVS in an autosomal-dominant mode of inheritance with complete penetrance, was detected in one patient. The mutations R1279H and V2285I were detected in 19 and 15 heterozygotes, respectively. In contrast, two additional missense mutations (T596M and R938Q) as well as a frameshift mutation (H1504del) were not detected in our AVS population. A common polymorphism (rs13290979) located in intron 2 was significantly associated with AVS (minor allele frequencies: 32% cases and 38% controls, p = 0.001). This study suggests that multiple rare variants and a common polymorphism in the NOTCH1 locus confer susceptibility to AVS. The prevalence of the rare variants in healthy controls and replication of the common variant in an independent population warrant further investigation.

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Fine mapping of loci associated with low-density lipoprotein cholesterol (LDL-C) via candidate gene sequencing and large scale genotyping. *A. Mulas[1], B. Li[2], C. Sidore[1,2], H.M. Kang[2], S. Sanna[1], S. Najjar[3], E. Lakkata[3], M. Uda[1], F. Cucca[1], D. Schlessinger[4], G.R. Abecasis[2], R. Nagarja[4], SardiNIA project.* 1) Istituto di Neurogenetica e Neurofarmacologia, CNR, Monserrato, 09042 (CA), Italy; 2) Center for Statistical Genetics, Ann Arbor, University of Michigan, MI, USA; 3) Laboratory of Cardiovascular Science, Intramural Research Program, National Institute on Aging, National Institutes of Health, Baltimore, USA; 4) Laboratory of Genetics, National Institute on Aging, Baltimore, MD, USA.

Lipoprotein levels are a risk factor for cardiovascular diseases, the leading cause of morbidity and mortality in developed countries. Several studies have shown that low-density lipoprotein cholesterol (LDL-C) is associated with increased risk of coronary artery disease, a connection that is emphasized by the fact that all genetic variants associated with increased LDL-C also increase the risk of coronary artery disease. Our previous genome-wide association studies of >8,000 subjects identified a number of loci associated with LDL-C levels, including both previously reported and newly implicated loci. To further understand the genetic contributions of both common and rare variants to the LDL-C level, we sequenced exons and flanking regions of 9 genes at associated loci in 256 unrelated Sardinian individuals with either extremely low or high LDL-C levels, along with 120 HapMap samples. Among all variants identified, 71% (81/121) nonsynonymous and 56.3% (40/71) synonymous mutations have frequencies below 1%. In addition, two frame shift (in *APOB*) and two truncation mutations (in *PCSK9*) were identified. Comparisons between high LDL-C and low LDL-C groups showed that rare coding variants are enriched in one of the two groups for a set of genes (*APOB*, *LDLR*, *PCSK9*, *SORT1*). To increase the power to detect associations, we imputed all the variants detected by sequencing in a subset of the cohort (N=4,305) and successively genotyped those in the entire SardiNIA sample (N=5,543).Among all tested variants, 11 showed a pvalue $<10^{-4}$, including six that have not previously been associated with LDL-C variation, as for example a very rare non-synonymous variant on the *LDLR* gene (MAF 0.005), that explains 0.5% of the total variance of LDL-C levels in Sardinia ($p<10^{-6}$). Evidence for association typically increased as we moved from the sequence of extreme individuals to imputation of a subset of the cohort to direct genotyping of the entire sample. For example, at the APOE-005012 (MAF=0.037) non-synonymous mutation association started at $2x10^{-4}$, increased to $2x10^{-8}$ after imputation, and to $4x10^{-33}$ when using the complete set of genotypes. Overall, these results identify a novel set of common and rare variants in LDL associated genes, and suggest that sequencing coding regions in extreme individuals followed by large scale imputation and genotyping are efficient designs for detecting potentially causative variants and increase the variance explained for these traits.

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Is E-selectin Gene S128R Polymorphism Associated with Incidence or Severity of Kasawaki Disease? *S. Toshihiko, K. Hideo, N. Kazuhisa, M. Hideki, M. Hiroyuki.* Nagasaki University Hospital, nagasaki-shi nagasaki-ken, Japan.

Background: Kawasaki disease (KD) is an acute systemic vasculitic syndrome in childhood of unknown etiology, and development of coronary arterial lesions (CAL) is the most critical prognostic factor of KD. E-selectin, which intermediates between vascular endothelial cells and inflammatory cells, has been shown to increase in blood during the acute stage of KD. Interestingly, p.S128R polymorphism (rs5361) of E-selectin gene has been associated with the incidence of adulthood coronary artery diseases (e.g., myocardial infarction) in which vasculitic process is recognized as an important etiological factor. Therefore, we hypothesized that the polymorphism of E-selectin gene might be associated with incidence of KD or development of CAL in KD. Methods: Genomic DNA was extracted from peripheral blood of 176 patients with KD (61 with and 115 without CAL) and 305 controls. E-selectin genotypes was determined by melting curve method using intercalating dye with real-time PCR. The genotype distribution was assessed using the Chi-square test. Experimental procedures were approved by the Committee for the Ethical Issues on Human Genome and Gene Analysis in Nagasaki University. Results: R allele frequencies were 0.0454 and 0.0344 in KD and control, respectively (OR 1.33 [95% CI, 0.68 - 2.59]). On the other hand, R allele frequencies in KD with and without CAL were 0.0410 and 0.0478, respectively (OR 0.85 [95% CI, 0.28 - 2.50]). Discussion: This study was unable to prove association of p.S128R polymorphism of E-selectin gene with the incidence of KD or development of CAL in KD, although we cannot rule out the possibility that the sample size was not large enough to detect existent but subtle association. Therefore, further studies are warranted to identify single nucleotide polymorphisms (SNPs) in genes that are associated with both coronary artery diseases in adulthood and KD in childhood, including another SNP in E-selectin gene, G98T in 5'UTR area (rs1805193). (Collaborators: K. Ikeda, T. Ohno, T. Hara [Kyushu University], S. Nishimura, M. Zaitsu, Y. Hamasaki [Saga University]).

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Sex and age differential genetic effect of chromosome 9p21 on atherosclerosis. *P.C. Tsai[1], H.F. Lin[2,3], R.T. Lin[2,3], G.T. Khor[2], S.H. Juo[1,4].* 1) Department of Medical Research, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; 2) Department of Neurology, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; 3) Department of Neurology, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; 4) Department of Medical Genetics, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan.

Purpose: Chromosome 9p21 has recently been shown to be a risk region for a broad range of vascular diseases. Carotid intima-media thickness (IMT) and plaque are independent predictors for vascular diseases. We aimed to test for the association between 9p21 and stroke, MI and subclinical phenotypes (IMT and plaque) in a Chinese population residing in Taiwan. **Methods:** We enrolled 687 stroke patients, 425 MI patients and 1377 stroke- and MI-free volunteers as health controls. Carotid segment-specific IMT and plaques were examined among the 1083 control subjects. Three commonly used SNPs (rs1333040, rs2383207, and rs1333049) were genotyped and genetic effects were tested for each SNP and haplotype. Further subgroup analysis was performed to explore sex-specific and age-specific association. Permutation was used to correct for multiple testing. **Results:** Multivariate permutation analyses demonstrated that carriers of the T allele of SNP rs1333040 was significantly associated with thicker common carotid artery (CCA) IMT (p = 0.042) and MI (p=0.045), but not for stroke (p=0.280). The risk G allele of SNP rs2383207 was associated with higher ICA IMT (p = 0.010) and MI (p=0.002), but not for stroke (p=0.097). Carriers of the C allele of SNP rs1333049 was found to be significantly associated with thicker ICA IMT (p = 0.026) and the greater risk for the presence of carotid plaque (OR=1.55 for heterozygous carriers; OR= 1.65 for homozygous carriers). Haplotype analysis showed a global p value of 0.265 for stroke, 0.032 for MI, 0.015 for ICA IMT, and 0.067 for the presence of carotid plaque. Further analyses showed the significant association for IMT and plaque only existed in men, and for MI only in young subjects (less than 65 years old). **Conclusions:** Chromosome 9p21 had a significant association with carotid atherosclerosis and myocardial infarction but not stroke. Furthermore, such genetic effect was in a gender-specific and age-specific manner in the Chinese population.

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Stem Cell Regulatory Genes Associated with the Racial Differences in the Promotion of Coronary Artery Disease by Tobacco Smokers. *L.R. Yanek[1], D.M. Becker[1], L.C. Becker[1], R.C. Levitt[2,3,4].* 1) Johns Hopkins Medical Institutions, Baltimore, MD; 2) Department of Anesthesiology, Miller School of Medicine, University of Miami, Miami, FL; 3) Hussman Institute of Human Genomics, Miller School of Medicine, University of Miami, Miami, FL; 4) Miami Veterans Healthcare System, Miami, FL.

Background: Coronary artery disease (CAD) is clustered strongly in families and associated with risk factors, including tobacco smoke exposure. Recent evidence suggests that biologic variability in stem cell mediated vascular repair may play an important role in CAD. We hypothesize that functional polymorphisms in stem cell regulatory genes underlie the promotion of CAD by tobacco smoke in susceptible individuals. To investigate this, we analyzed for association between CAD and a panel of 7 candidate genes involved in the regulation of stem senescence that may affect stem cell mediated vascular repair. **Methods:** The GeneSTAR study population was ascertained from families via a proband who experienced an early onset (prior to age 60) cardiovascular event, and included apparently healthy siblings (n=1140; 49% Blacks; 51% Whites) who completed baseline screening. Incident CAD was assessed by follow-up questionnaires and confirmed by medical records. Self-reported smoking status was confirmed by exhaled CO. Genotype data were generated using the Illumina Human 1M Beadchip; 116 SNPs in Blacks and 106 SNPs in Whites were analyzed. Data integrity was ensured with extensive quality controls including sample and SNP filters. Racial groups were analyzed separately to control for population substructure. Mixed effects models implemented in SAS were used to conduct candidate gene association analyses under an additive model. Analyses were adjusted for age, sex, smoking, eigenvector(s) for within race population stratification, and familial clustering and a SNP*smoking interaction term was included in the models. **Results:** Preliminary analyses indicate significant differences between racial groups and associations between CAD with SNPs in stem cell regulatory genes. WNT2 (rs10487362, P=0.0007), WNT2B (rs12138754, P<0.0004), and MDM2 (rs3730613, P<0.001) were associated with CAD in Blacks. Only WNT2B (rs3790604, P<0.04) and WNT10A (rs10177996, P<0.05) were potentially associated with CAD in Whites. A SNP*smoking interaction was found for CDKN1A (rs3176321, P<0.004 Whites), and HMGA2 (rs7967476, P<0.004 Blacks). **Conclusions:** These data indicate for the first time that the stem cell regulatory genes may be implicated in the promotion of incident CAD by tobacco smoke. Associations may differ by racial background. These findings suggest that smoking may have its impact on CAD by altering stem cell mediated vascular repair.

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Allele-specific regulatory activity of variants associated with human high-density lipoprotein cholesterol level at the *GALNT2* locus. *T.S. Roman[1], A.F. Marvelle[1], K.J. Gaulton[1], M.P. Fogarty[1], A.J. Gonzalez[1], Y. Li[1,2], K.L. Mohlke[1].* 1) Department of Genetics, University of North Carolina, Chapel Hill, NC; 2) Department of Biostatistics, University of North Carolina, Chapel Hill, NC.

Genome-wide association studies have identified SNPs within intron 1 of the *GALNT2* gene that are strongly associated with human high-density lipoprotein cholesterol (HDL-C) levels, although the molecular mechanism responsible for this association remains unknown. *GALNT2* encodes an N-acetylgalactosaminyltransferase important for O-linked oligosaccharide biosynthesis, and may influence HDL-C level by modifying proteins involved in cholesterol transfer or processing. SNPs in strong linkage disequilibrium (r^2>.8, HapMap and 1000 Genomes Project CEU) with the reported HDL-C -associated SNPs span ~15 kb of intron sequence, and resequencing of the *GALNT2* exons did not identify other variants likely to explain the association signal. We hypothesize that one or more of the intron 1 variants have an allele-specific regulatory effect on gene expression. Eight variants are located in regions with evidence of regulatory function based on open chromatin and histone modification data generated in HepG2 cells. We used dual-luciferase assays to test these variants as possible allele-specific enhancers of transcriptional activity in HepG2 cells. A 780-bp region containing one haplotype showed a 15-fold increase in transcriptional activity compared to a control vector containing only a minimal promoter. An alternate haplotype showed a 3-fold further increase in enhancer activity. This increase was observed for alleles associated with increased HDL-C level. To determine which of the four variants in the haplotype might be responsible for this increased activity, we created additional haplotypes by site-directed mutagenesis. Preliminary results suggest that at least two of the SNPs (rs4846913 and rs2144300) contribute significantly and additively to differential transcriptional activity. Electrophoretic mobility shift assays are being used to evaluate evidence of differential protein binding at these SNPs. Notably, the direction of allele-specific haplotype enhancer activity we observed in HepG2 cells contrasts a recent report that *Galnt2* knockdown in mouse liver increased plasma HDL-C levels, suggesting that rs4846913 and rs2144300 do not act directly to influence *GALNT2* expression. Successful identification of the molecular mechanism underlying the genome-wide association signal would provide insight into the variants' role in *GALNT2* activity, and may lead to a greater understanding of the biological processes that help regulate human HDL-C metabolism.

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A de novo terminal deletion of 10q26.3 associated with poor attention, developmental delay, and ataxia with normal cerebellum on MRI. *C.L. Goldsmith[1], A. Doja[2], K.M. Boycott[1].* 1) Genetics, Children's Hospital of Eastern Ontario, Ottawa, Ontario, Canada; 2) Neurology, Children's Hospital of Eastern Ontario, Ottawa, Ontario, Canada.

The phenotype associated with terminal deletions of 10q is variable but reported features include developmental delay/learning disability/mild to moderate mental retardation, speech and language delay, poor attention, strabismus and distinctive facial features in the context of normal growth and good health. We report a 6 year-old girl who presented to the neurogenetics clinic at the age of 3 years of age with global developmental delay, poor coordination, and ataxia. MRI showed no abnormality of the posterior fossa. Standard investigations for ataxia were unrevealing. A high-resolution (105 K) comparative genomic hybridization microarray identified a deletion of the terminal 4.6 Mb of the long arm of chromosome 10, within cytogenetic band 10q26.3. This finding was confirmed by FISH analysis. The parents were tested and this is a de novo change. There are approximately 40 genes in the deleted interval. A small number of children reported with deletions in this region have poor balance/wide-based gait (AJMG 2009; 149A:669-680) and further investigation of two of these children demonstrated vestibular anomalies. These two children had an overlapping deletion which included the HMX2 and HMX3 genes and haploinsufficiency was proposed as the mechanism based on previous reports that Hmx2/Hmx3 knockout mice have vestibular anomalies. The deletion in the patient reported here is distal and does not include these two genes; CT of the inner ear is pending. Our findings suggest that there may be other genes involved in coordination/gait/ataxia in 10qter.

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Down syndrome with bilateral absence of cochlear nerves, a previously unreported association. *N. Leonard[1], T. Uwiera[2], A. Kanigan[3], R. Bhargava[3].* 1) Department of Medical Genetics, University of Alberta, Edmonton, AB, Canada; 2) Department of Surgery, University of Alberta, Edmonton, AB, Canada; 3) Department of Radiology, University of Alberta, Edmonton, AB, Canada.

Individuals with Down syndrome are prone to hearing loss, most often conductive in nature. Sensorineural hearing loss, usually mild-moderate in severity, is seen in 4-20% of cases. We report a unique case of a child with Down syndrome with bilateral absence of the cochlear nerves, a previously unreported association. Following parental concerns raised over possible hearing loss, a 6 month old boy with Trisomy 21 had an audiologic assessment that identified bilateral profound sensorineural hearing loss. Auditory brainstem responses confirmed lack of responses. Otoacoustic emissions were absent in both ears and tympanometry was normal. A workup began to determine cochlear implantation candidacy. A high resolution CT scan showed significantly narrowed cochlear apertures raising the concern of absence of cochlear nerves bilaterally. There were normal appearing cochleae. The lateral semicircular canals and vestibules were mildly enlarged. The middle ear was normal. Additional imaging on MRI demonstrated the absence of the cochlear branches of the vestibulocochlear nerves bilaterally. The pregnancy was uncomplicated except for delivery by cesarean section for maternal hypertension for the 43 year old gravida 1 mother. He had treated hypothyroidism and no health concerns. In particular there were no concerns for diabetes, infection, hyperbilirubinemia, hypoxia, prematurity, pigmentation, olfaction or vision. Physical exam was in keeping with Down syndrome. There was no family history of consanguinity, neuropathies, hearing loss or health concerns. The family opted to decline genetic testing for hearing loss. In the literature, histopathology and radiology reports indicate numerous middle ear abnormalities that can be seen in Down syndrome. For inner ear abnormalities, there are reports of inner ear dysplasia in individuals with Down syndrome, the most common feature being generalized hypoplasia of the inner ear structures. There are reports of cochlear nerve canal hypoplasia and internal auditory canal stenosis or duplication, but no case of absent cochlear nerves (Blaser 2006). To date, this is the first report of an individual with Down syndrome having absence of the cochlear nerves. Profound sensorineural hearing loss in a child with Down syndrome should prompt MRI imaging to look for absence of the cochlear nerves as cochlear implantation is contraindicated in this setting. Ref: Blaser S, et al. Laryngoscope. 2006 Dec;116(12):2113-9.

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Clinical phenotypes and genetics of various subtypes of polymicrogyria and evidence for a novel locus for bilateral perisylvian polymicrogyria narrowed to 2p16.1-p16.3. *D. Amrom[1], A. Poduri[2], B. Dan[3], N. Deconinck[3], C. Christophe[4], B. Pichon[5], F. Dubeau[6], D. Tampieri[7], F. Andermann[8], W.B. Dobyns[9], C.A. Walsh[10], E. Andermann[11].* 1) Neurogenetics Unit, Montreal Neurological Hospital and Institute (MNH/I); Department of Neurology & Neurosurgery, McGill University, Montreal, Canada; 2) Division of Epilepsy and Clinical Neurophysiology, Department of Neurology, Children's Hospital Boston, Boston, MA; 3) Pediatric Neurology Unit, Hôpital Universitaire des Enfants Reine Fabiola (HUDERF) Université Libre de Bruxelles, Brussels, Belgium; 4) Department of Neuroimaging, Hôpital Universitaire des Enfants Reine Fabiola (HUDERF); Université Libre de Bruxelles, Brussels, Belgium; 5) Department of Medical Genetics, Hôpital Erasme; Université Libre de Bruxelles, Brussels, Belgium; 6) Epilepsy Service and Seizure Clinic, Montreal Neurological Hospital and Institute; Department of Neurology & Neurosurgery, McGill University, Montreal, Canada; 7) Department of Diagnostic & Interventional Neuroradiology, Montreal Neurological Hospital and Institute; Department of Radiology, McGill University, Montreal, Canada; 8) Epilepsy Service and Seizure Clinic, Montreal Neurological Hospital and Institute; Departments of Neurology & Neurosurgery and Pediatrics, McGill University, Montreal, Canada; 9) Departments of Human Genetics, Neurology and Pediatrics, University of Chicago, Chicago, Illinois; 10) Division of Genetics and Manton Center for Orphan Disease Research, Children's Hospital Boston; Howard Hughes Medical Institute; and Harvard Medical School, Boston, MA; 11) Neurogenetics Unit, Montreal Neurological Hospital and Institute; Departments of Neurology & Neurosurgery and Human Genetics, McGill University, Montreal, Canada.

BACKGROUND: Polymicrogyria (PMG) is a malformation of brain cortical development. Common clinical features include seizures, developmental delay, oromotor dysfunction and motor disabilities. PMG is a clinically and etiologically heterogenous condition. It can be attributable to environmental causes, single gene disorders with various patterns of inheritance, or chromosomal rearrangements, usually sporadic. AIMS: To report the clinical phenotypes and genetic data of several subtypes of PMG and establish genotype-phenotype correlations. METHODS: Search of our brain malformation databases at the MNH/I and HUDERF hospitals, and inclusion of all types of PMG, except those associated with schizencephaly and confirmed congenital CMV/toxoplasmosis infections; detailed review of medical records; karyotype and FISH 22q11; CGH and/or SNP microarray of genomic DNA. RESULTS: We enrolled 24 patients: 11 symmetric bilateral perisylvian polymicrogyria (BPP), 2 asymmetrical BPP, 4 unilateral right PMG, 3 unilateral left PMG, and 4 bilateral posterior PMG. A 22q11 deletion was found in one patient with unilateral right PMG. The brain MRI showed an associated large contralateral frontal heterotopia. He presented with congenital mitral valve stenosis, congenital left hemiparesis, facial dysmorphism, and moderate mental retardation. A 2p13.3-p16.3 duplication was found in a patient with symmetric BPP. He presented with neonatal global hypotonia, feeding difficulties, and delayed psychomotor development. At the age of 10 years, physical examination revealed mild facial dysmorphic signs, mental retardation, severe language delay, attention deficit helped by methylphenidate, and growth deficiency treated with growth hormone. CONCLUSIONS: The inheritance of PMG is heterogeneous. Our observations confirm that PMG, including right-sided PMG, is associated with deletion 22q11 (DiGeorge) syndrome; however, the association of a large contralateral frontal heterotopia is unusual. Altogether this suggests asymmetrical gene(s) expression between the hemispheres. Phenotypic comparison with previously published patients harboring other types of proximal 2p duplications shows a subgroup of patients with BPP sharing a common locus narrowed to 2p16.1-p16.3.

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47,XX,iso(18p) Case History. *j. martinez[1], i. briceno[1,2], m. brigmon[1], a. venegas[2].* 1) Universidad de La Sabana, Bogota, Colombia; 2) Pontificia Universidad Javeriana, cra 7 numero 40-52.

Sixteen year old patient with, moderate mental non progressive deficiency and attending a school for the mentally handicap, a friendly and loquacious personality, no relevant diseases history, menarche at thirteen years, during the physical examination the only signs were: a thoracic kyphosis of 50 degrees and a lumbar lordosis of 8 degrees and hypoplasia of the fourth and fifth toes of the right foot. Kariotype revealed 47 XX+mar Microarray DNA analysis by Affimetrix SNP 6.0: showed that the marker corresponds to an isochromosome eighteen from p 11.21 a pter. Diagnosis: 47, XX iso 18p. Conclusion: This case is evidence that additional short arm of Chromosome 18 produces a mild clinical manifestation in contrast to the short arm of chromosome 18.

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A Heterozygous 3q24-q25 Deletion in a Patient with the C Syndrome. *A. Nakatomi[1], T. Kondou[2], A. Mori[2], O. Shimokawa[4], Y. Sasaki[4], N. Harada[4], T. Shimoji[3], M. Fukuda[2], T. Matsumoto[1], H. Moriuchi[1].* 1) Dept Pediatrics, Nagasaki Univ, Nagasaki city, Japan; 2) Division of Developmental Disabilities,The MISAKAENOSONO MUTSUMI,Isahaya city,Japan; 3) Okinawa Prefectural Nanbu Medical Center & Childrens Medical Center,Okinawa,Japan; 4) Cytogenetic Testing 2nd.Group,Molecular Genetic Testing Dept,Clinical Laboratory Center,Mitsubishi Chemical Medience Corporation,Nagasaki city,Japan.

The C (Opitz Trigonocephaly) syndrome (OMIM #211750) is characterized by severe mental retardation, trigonocephaly and such characteristic facies as broad dental alveolar ridges, epicanthal folds, broad depressed nasal bridge and abnormal external ears. Brain and heart anomalies have also been associated with it. Mutations of CD96, located on 3q13.13, have been reported in a form of the C syndrome (Kaname et al, 2007). Furthermore, cytogenetic abnormalities of chromosome 3 or 4 have been found in patients with the C or C-like syndrome (Preus et al, 1975; Sargent et al, 1985; McGaughran et al, 2000; de Ravel et al, 2009), suggesting genetic heterogeneity for the C syndrome. We here present a C syndrome patient with brain anomaly who had an interstitial 3q deletion, del(3)(q24q25.3). The patient, a 6-year-old girl, was born by unrelated parents after uneventful pregnancy and delivery. There was no significant family history. Poor weight gain and mild motor retardation were noted during infancy. Her verbal development had been remarkably delayed: she had not spoken even a word until now. Since 3 years of age, she had been awake at night, screaming and hitting her head against the wall, and had been clinging to specific sounds, mimicking autistic spectrum disorder. She had trigonocephaly, broad dental alveolar ridges, long philtrum and epicanthus. Neurologically, ataxic gait and hyperreflexia of the lower limbs were noted. The blood cell counts and biochemical examinations revealed no abnormality. Brain MRI detected hypoplasia of cerebellum (Dandy-Walker variant) and brain stem and delayed myelination of periventricular white matter. CD96 gene analysis revealed no mutation. Chromosomal and genomic copy number analyses with high resolution SNP array interpreted the karyotype of the patient as 46,XX,del(3)(q24q25.3).-arr3q24q25.31(148,242,205-158,030,540)×1. According to the Genome Browser of database of genomic variants (build 36 assembly: http://projects.tcag.ca/cgi-bin/variation/gbrowse/hg18/), the 9.7-Mb deletion contains 57 RefSeq genes including the following OMIM disease genes, ZIC4, ZIC1, USH3A, MME, AGTR1, CP, P2RY12, SLC33A1, GMPS and HPS3, none of which has never been associated with the C syndrome. Interestingly, ZIC1 and ZIC4 genes were responsible to Dandy-Walker malformation. Further investigation of the deleted area is warranted to clarify the genetic basis of the C syndrome phenotypes and brain anomalies including Dandy-Walker malformation.

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17q21.2ter microduplication and 20p13ter deletion in a dysmorphic girl with microcephaly, abnormal cerebellum and absence-like events. *C. Vinkler[1,2], R. Shuvalov[2], T. Lerman-Sagie[2], S. Kivity[2], M. Michelson-Kerman[1,2], D. Lev[1,2].* 1) Inst Med Gen, Wolfson Med Ctr, Holon, Israel; 2) Metabolic- Neuro-genetic Clinic Wolfson Medical Center, Holon, Israel.

Partial duplication of the terminal portion of the long arm of chromosome 17, is a rare but well recognized disorder. The syndrome is characterized by variable degrees of mental retardation, growth retardation, microcephaly, a large spectrum of dysmorphic features including, frontal bossing, flat nasal bridge, bulbous nose, long philtrum, wide mouth, thin upper lip, cleft palate, low-set ears, short neck, and anomalies of the limbs, heart, CNS and the urogenital system. . We describe a girl with 17q21.2ter duplication and monosomy 20p13ter who presented with a complex clinical manifestations including significant brain malformation and absence-like events. She is the third child of healthy consanguineous parents of Georgian- Jewish origin. She had a brother who was born with multiple congenital anomalies who died at the age of three weeks and another 10y old healthy brother. The mother had two spontaneous abortions . At the age of 19 m she had severe developmental delay and growth retardation. On examination she had microcephaly and dysmorphic features including, frontal bossing, flat nasal bridge, bulbous nose, long philtrum, thin upper lip, bifid uvula, low-set ears, short neck, low posterior hairline and proximal limb shortness. Right after birth, she had PFO and PDA that closed spontaneously. Brain MRI revealed a short and thick corpus callosum, enlarged forth ventricle, dysplasia of the cerebellar vermis and abnormal structure of the cerebellum. At the age of three years, her parents complained that she had up to 30 absance-like events per day. There was no correlation with EEG changes neither when awake nor during sleep. Microarray CGH showed a 15Mb17q21.2ter duplication and 600kb 20p13ter deletion. Her mother was found later to carry a balanced translocation 46,XXt(17;20)(q21;p13). CNS involvement in patients with 17q duplication has been previously described. Structural changes involving both the vermis and the cerebellar hemispheres may well be associated with 17q duplication in this child. However, the absence-like events have not been described yet in this disorder. They may represent epilepsy without EEG changes because of its deep hemispheric origin (e.g. inferior mesiofrontal).They may be either a new feature in this syndrome or associated with the partial monosomy 20p found in this case.

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Ring Chromosome 21 and Cleft Palate a Case Report and Possible Candidate Gene for Orofacial Clefting Phenotype. *I. Briceno, M. Brigmon, J.C. Martinez.* Faculty of Medicine, Universidad de La Sabana, Chia, Colombia.

We report a case of a feminine child who is the product of a second controlled pregnancy ended preterm (36 weeks) by cesarean delivery, who also incurred a premature rupture of membranes. At birth, the child weighed 2100g, height of 47cm; she was diagnosed early with an atrial septal defect that required several hospital stays until surgical correction at 4 years of age. During her care in pediatrics, a genetic evaluation was solicited because of the following phenotypical findings: brachycephaly, oblique palpebral fissures, poor language skills, and small size. During the clinical genetic evaluation, the positive genetic findings were as follows: flat occiput, bilateral epicanthus, bilateral palpebral fissures, broad nasal bridge, microcephaly, hypertelorism, surgical correction of cleft palate, extremities with bilateral syndactyly in II and III toe, and bilateral shortening of the V finger. Parent comments that language skills had improved with cleft palate correction and the patient received myringotomy in the right ear to help hearing loss and language development. Our case report also had generalized seizures until about 2 years of age and the necessity of surgical correction of thoracic scoliosis at 12 years. Also, We preformed a G band karyotype that found a ring chromosome 21. After a copy number variation based on whole genome analysis we found a chromosomal deletion of 21q 22.2-gter which could be a possible candidate gene for the orofacial clefting phenotype. After an exhaustive literature review, we found that this is only the 2nd case report of ring chromosome 21 with craniofacial abnormalities such as cleft palate and a unique case for study.

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GENETIC CHROMOSOMIC (turner, klinefelter, criduchat, down), ENZIMATIC/METABOLIC (Mucopolysacharidossis, Duchenne Muscular dystrophy, Osteogenesis Imperfecta, williams) AND MULTIFACTORIAL (Goldenhar, Beckwith Wiedemann, Ectodermic Dysplasia, Cornelia de Lange, Albinism) SYNDROMES. MULTIDISCIPLINARY BI-ANNUAL MEETING AT THE HOSPITAL PARA EL NINO POBLANO, MEXICO. *L. Hurtado-Hernandez[1], J.M. Aparicio-Rodriguez[2,3,4,5,6], S.A. Garcia-Damian[3], J.P. Rangel-Vargas[4], M.G. Sol-Gamboa[3], C.G. Gonzalez-Ayon[4], H.J. Rivera-Prado[4], S. Hernandez-Reyes[6], N.S. Gomez-Gonzalez[3], D.S. Gonzalez-Mendoza[5], B. Teutle-Coyotecatl[3], D. Joachin-Suarez[3], A.C. Martinez-Sanchez[4], S. Colombres-Garcia[6], C. Hernández-Contreras[4], C.B. Escobar-Canches[5], M.C. Beltrán-Vargas[4], D.C. Illan-Gallardo[4], C.A. Fuentes-Chavez[6], A. Delfin-Roji[5], G. Cisneros-Navarro[4], R.M. Arellano-Santos[3], C.A. Alonso Hernández[4], M.R. Nolasco Delint[4], R.P. Romero-Jaka[3], R.C. Sandoval-Cobarrubias[5], M.J. Valdes-Galvan[6].* 1) Cytogenetics; 2) Genetics, Hospital para el Nino Poblano,; 3) Pediatric Estomatology; 4) Ortodontics; 5) Integral, Benemerita Universidad Auronoma de Puebla; 6) Biomedicine, Universidad de las Americas, Puebla, pue., Mexico.

INTRODUCTION. A genetic disorder is caused by abnormalities in genes or chromosomes. While some diseases, such as goldenhar are due in part to genetic disorders, they can also be caused by environmental factors. Most disorders are quite rare and affect one person. A single gene disorder is the result of a single mutated gene. There are about 4000 human diseases caused by single gene defects. Single gene disorders can be passed on to subsequent generations, however, may affect inheritance patterns. The mendelian alteration have been reported as recessive, dominant and X-linked inheritance. A great variety of genetic and non genetic disorders have been described in this study, such as Cornelia de Lange, Dextrocardia, Kabuki, Duchenne Muscular Dystrophy (DMD), Achondroplasia, Mucopolysacharidossis, Albinism, Osteogenesis Imperfecta, Neurofibromatosis, Down, Criduchat, Turner, Klinefelter, Cystic Fibrosis, Marfan, Fetal Alcohol, Moebius, Ectodermic dysplasia, Goldenhar, Beckwith wiedemann, Williams, Arthogryposis, Epiolia. MATERIAL AND METHODS. A wide study was performed in this hospital since 1992 to 2010, where cytogenetic (6480 Caryotypes performed), Iontoforesis and metabolic studies (MS) among others have been performed, in order to obtain a diagnosis, for a better treatment. 2370 metabolic studies performed, where 102 clinical cases were associated to different inborn errors of metabolism (IEM). CONCLUSIONS. The IEM due to metabolic error for a protein or enzyme absence were observe a similar incidence (4.3%) a that already reported. In relation to chromosome aberration a great variety of mutation were observed, however the most frequent chromosome aberrations as down, turner and klinefelter syndromes among others have been invited for a multidisciplinary meeting during the year, especially for Genetic Counseling. For example, achondroplasia is typically considered a dominant disorder, but children with two genes for achondroplasia have a severe skeletal disorder that achondroplasics could be viewed as carriers, similar as DMD where molecular studies are performed to females (mother and sisters) since it is a X-linked disease. The porpoise of these meetings are to obtain an earlier multidisciplinary medical evaluation for a genetic diagnosis, and a better medical and therapeutic treatment to offer a better quality of life for the different patients according to their genetic disease.

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Genetic Dosage Compensation in a Family with Velo-cardio-facial/ DiGeorge/22q11.2 Deletion Syndrome. *A.A. Alkalay[1], T. Guo[2], C. Montagna[2], C. Digilio[3], B. Marino[4], B. Dallapiccola[5], B. Morrow[2].* 1) Obstetrics and Gynecology, Albert Einstein College of Medicine/Montefiore Medical Center, Bronx, NY; 2) Department of Genetics, Albert Einstein College of Medicine, Bronx, NY; 3) Medical Genetics, Bambino Gesù Hospital, Rome, Italy; 4) Department of Pediatrics, La Sapienza University of Rome, Rome, Italy; 5) Department of Medicine, Viale Regina Margherita, Rome, Italy.

Cytogenetic studies of a male child carrying the 22q11.2 deletion common in patients with velo-cardio-facial/DiGeorge syndrome revealed an unexpected rearrangement of the 22q11.2 region in his normal appearing mother. The mother carries a 3 Mb deletion on one copy and a reciprocal, similar sized duplication on the other copy of chromosome 22q11.2 as revealed by fluorescence in situ hybridization and array comparative genome hybridization analysis. The most parsimonious mechanism for the rearrangement is a mitotic non-allelic homologous recombination event in a cell in the early embryo soon after fertilization. The normal phenotype of the mother can be explained by the theory of genetic dosage compensation. This is the second documented case of such an event for this or any genomic disorder. This finding helps to reinforce this phenomenon in a human model, and has significant implications for genetic counseling for future children.

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BRAF Gene Mutation Associated With Intraoral Giant Cell Granulomas. *S. Beiraghi, V. Leon-Salazar, A.K. Thurmes, W.S. Looman, G.C. Anderson.* University of Minnesota School of Dentistry, Minneapolis, MN.

Cardio-Facio-Cutaneus Syndrome (CFCS; MIM 11550) is part of five syndromes with overlapped phenotype caused by mutations in genes encoding various components of the Ras/Mitogen-Activated Protein Kinase (RAS/MAPK) pathway. CFCS is characterized by a heterogeneous autosomal dominant transmission pattern, a distinctive facial dimorphism, mental retardation, global developmental delay, heart defects, and ectodermal abnormalities. Although not rare, there are few reports about multiple giant cell lesions (MGCL) associated with mutations in the RAS/MAPK pathway. Here we report CFCS with intraoral MGCL and xantho granuloma lesions in a patient with BRAF gene mutation. A 5.8 year-old boy born to nonconsaguineous parents at 36 weeks gestation with polycythemia and multiple congenital anomalies including macrocephaly, submucous cleft palate, stenotic ear canals, left ptosis and exotropia, supravalvular pulmonary stenosis, bilateral cryptorchidism, dysplastic right kidney and hydronephrosis. Significant growth and development delays, including short stature, expressive and receptive language delays, and conductive hearing loss, chronic generalized eczema, especially in the facial area, chronic ear infection, plagiocephaly, lymphedema, xantogranulomas, and bilateral expansible jaw cysts (MGCL) were part of the phenotype. Genetic testing revealed that he carries one copy of a sequence variant (1406 G>A) in the BRAF gene. Since the RAS/MAPK genes has been associated with dysregulation in oncogenesis and the presence of Xantho granulomas, this patient is tested every 3 months for leukemia.

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Solitary median maxillary central incisor (SMMCI) syndrome in a pediatric patient. A case report, Hospital para el Niño Poblano, México. *D. Sanchez-Lopez[1], J.M. Aparicio-Rodriguez[2], N.G. Hernandez-Trejo[1], L. Bueno-Garcia[1].* 1) Odontopediatrics; 2) Medical Genetics , Hospital para el Niño Poblano, Puebla, Puebla, Mexico.

INTRODUCTION. Solitary median maxillary central incisor syndrome known as SMMCI, is a rare and complex disorder consisting of different mainly midline defects of development. This is secondary to vary intra uterine factor(s) between the 35th-38th day(s) from conception. It's incidence is about 1:50,000 live births. It has been suggested that missense mutation in the SHH gene (I111F) at 7q36 may be associated with SMMCI. This syndrome tooth differs from the normal central incisor, in that the crown form is symmetric; it develops and erupts precisely in the midline of the maxillary dental arch in both primary and permanent dentitions. It has been also observed nasal malformation (choanal atresia, midnasal stenosis or congenital pyriform aperture stenosis) associated with SMMCI. The presence of an SMMCI tooth can be part of a nervous central system anomaly like holoprosencephaly, severe to mild mental retardation, congenital heart disease, cleft lip and/or palate and less frequently, microcephaly, hypopituitarism, hypothyroidism, hypotelorism, strabismus, esophageal and duodenal atresia, cervical hemivertebrae and scoliosis, kidney malformation and ambiguous genitalia. MATERIAL, METHODS AND CASE REPORT. A 3 years old female patient was evaluated at the departments of estomatology and genetics, she is the 5th child of a normal family. A 35 years old mother and 40 years old father. The patient was evaluated at ortopedics to discard an osseous dysplasia. However due to the short stature of the patient, was sent to endocrinology for Human Hormone Evaluation. Hematury and proteinury was observed in urine laboratory sample, a kidney ultrasound was requested to discard kidney malformation. A caryotype was requested by genetics since different chromosomes aberrations had been reported, such as triple XXX syndrome, 7q-, 18p- and 22q. CONCLUSION. Diagnosis should be made by eight months of age, but can be made at birth and even prenatally at 18-22 weeks from the routine mid-trimester ultrasound scan. Medical management depends upon the different individual anomalies present. Short stature may require growth hormone (GH) therapy, as the case in this study, which will start GH treatment. Same as neurology with early rehabilitation therapy if necessary. The department of odontopediatrics will provide a multidisiciplinary treatment with orthodontic, prosthodontic and oral surgical treatment, to ensure a better quality of life for the patient.

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Clinical heterogeneity in a family with a small microduplication of chromosome 22q11.2. *J.D Weisfeld-Adams[1,2], I.K. Gadi[3], L. Mehta[1].* 1) Gen & Genomic Sci, Mount Sinai Med Ctr, New York, NY; 2) Department of Pediatrics, Mount Sinai Med Ctr, New York, NY; 3) LabCorp, Center for Molecular Biology and Pathology, Research Triangle Park, Durham, NC.

The chromosome 22q11.2 region is commonly involved in nonallelic homologous recombination (NAHR) events. Microduplications of 22q11.2, usually involving a 3 Mb or 1.5 Mb region, are thought to be approximately half as common as the microdeletion syndrome involving the same genomic region. Clinical features decribed in affected individuals are variable and show overlap with the 22q11.2 microdeletion syndrome. Previously reported clinical features associated with larger microduplications in this region have included dysmorphic facial appearance, velopharyngeal insufficiency, congenital heart disease, and immunologic defects. We report an 8 month old boy who presented for genetics evaluation because of microcephaly and mild developmental delay. Prenatal history was unremarkable, with spontaneous delivery at term. Head circumference at birth was 31.5 cm (<3rd centile) and at 8 months was 41.7 cm (<3rd centile). Other findings included hypertelorism, left-sided cryptorchidism and developmental dysplasia of the left hip. At 8 months, gross motor development was delayed with inability to sit unsupported. Array CGH (Affymetrix Genome-Wide Human SNP Array 6.0) revealed a novel 438 Kb interstitial duplication at 22q11.21, involving TBX1. The same duplication was confirmed by FISH in the patient's mother and a sister. The mother is described as having a history of anxiety disorder, but had normal head size and was a college graduate. The sister had delayed motor milestones, walking at 22 months. She is described as having normal development at 2 years of age. None of the three carriers of the duplication has any documented cardiac issues, renal anomalies or any significant medical problems. This family demonstrates the clinical heterogeneity observed in microduplications of 22q11.2, and further illustrates the difficulty in providing prognostic information and accurate genetic counseling to families where this finding is detected. The described microduplication is the smallest reported in the literature to date.

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Clinical Reports and Further Delineation of the 14q32 Deletion Syndrome. *E.L. Youngs[1], J.A. Hellings[1], M.J. Dasouki[2], M.G. Butler[1].* 1) Departments of Psychiatry & Behavioral Sciences and Pediatrics, University of Kansas Medical Center, Kansas City, KS 66160; 2) Department of Pediatrics, University of Kansas Medical Center, Kansas City, KS 66160.

Individuals with deletions of the chromosome 14q32 region are rare. The focus of our report is to describe additional subjects with de novo 14q32 deletions and compare with 11 individuals ranging in age from 1 day to 33 years described in the literature with proximal and distal breakpoints within the 14q32 band. We report two subjects (8 month old female and 18 year 6 month old female) with 14q32 deletions using chromosomal microarray hybridization to delineate the cytogenetic findings. Our older female presented at birth with a small appearing jaw, large tongue, high-arched palate with a bifid uvula, horizontal nystagmus, optic nerve hypoplasia, hypotonia and a 14q32 deletion. Bilateral sensorineural hearing loss was detected by 2 years of age along with developmental delay. Strabismus was corrected at 5 years of age. Hyperactivity and behavioral problems (OCD, depression, anxiety, impulsivity) were noted by 8 years of age. At 18 years of age, her growth parameters were within normal range. Her face was long and narrow with a high forehead, downslanting palpebral fissures, blepharophimosis, epicanthal folds, a flattened, wide nasal bridge, short nose with anteverted nares and a long appearing pointed chin. She had a bifid uvula, high-arched palate and digital findings. Her full scale IQ was 74; verbal IQ was 71 and performance IQ was 81 (WAIS-III) at 18 years. Her 4.16 Mb deletion (102.21 to 106.37 Mb from pter) extending from 14q32.32 to qter identified by microarray analysis contained approximately 50 genes. Our second female presented at 8 months with severe hypotonia and failure to thrive, global developmental delay, dysmorphic facial features, congenital heart disease and a cat-like cry. Her 6.1 Mb deletion of the 14q32.2-q32.31 region occurred from 95.58 to 101.77 Mb from pter. The following list of commonly occurring physical anomalies reported in subjects with the 14q32 deletion including our subjects in order of frequency is: broad philtrum (8/9 subjects), broad and flat nasal bridge (8/9), thin upper lip (5/6), hypotonia (9/11), telecanthus (8/11), high-arched palate (8/11), blepharophimosis (6/9), malformed helices (5/8), small mouth (5/9), pointed chin (5/10), strabismus (5/10) and downslanting palpebral fissures (4/9). Individuals with ring chromosome 14 were excluded in our analysis as these subjects frequently present with seizures, visual problems and retinal abnormalities not generally seen in the 14q32 deletion subjects.

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Deletion 3q22.3q23: Blepharophimosis-ptosis-epicanthus inversus syndrome (BPES) and spastic diplegia, speech delay and penile skin anomalies. *S. Zahanova[1], B. Labieniec[2], H. Verdin[3], B.F. Meaney[4], J.-C. Wang[5], E. De Baere[3], M.J.M. Nowaczyk[4,5].* 1) DeGroote School of Medicine, McMaster University, Hamilton, Ontario, Canada; 2) School of Medicine, Jagiellonian University, Krakow, Poland; 3) Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium; 4) Dept. Pediatrics, McMaster University, Hamilton, Ontario, Canada; 5) Dept. Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada.

BPES is an autosomal dominant disorder affecting craniofacial development and presenting with specific eyelid abnormalities. Premature ovarian failure and internal genital anomalies are found in a subgroup of female patients. BPES is caused by mutations of the *FOXL2* gene located at 3q23; only 10% of BPES cases are caused by interstitial deletions of 3q. We report a boy who presented with classic facial features of BPES, who also had a webbed penis noted at birth. There was no separation of the skin on ventral aspect of the penis and the scrotum; the skin was fused. At age two he presented with speech delay, and articulation problems persisted to after age 4. The patient also had gross motor delay and spastic diplegia; upper motor neuron dysfunction was documented. Molecular testing for hereditary spastic paraplegias SPG 3A, 4, 6, 7, 8, 17 and 31 were negative; brain and spine MRIs were normal. His karyotype was 46,XY,del(3)q(22.3-23), and BAC probe RP11-54801 was deleted on FISH analysis. Whole-genome oligonucleotide array demonstrated a 4.7 Mb deletion at chr3:139,354,104-144,013,999. MLPA also confirmed the *FOXL2* deletion, and showed that the deletion spans the *ATR* gene; this is a relatively large deletion. The genital anomalies observed in our patient have not been reported in male patients with BPES, although premature ovarian failure and internal genital anomalies are observed in females with BPES type I. Spasticity and developmental delay, observed in a number of BPES deletion patients, in addition to the dystonia seen in our patient, may be due to haploinsufficiency of *MRPS22,* a skeletal muscle mitochondrial protein, located in proximity to *FOXL2*. It has also been hypothesized that mental retardation and growth delay in BPES patients with large deletions is due to haploinsufficiency for *ATR*, a gene associated with autosomal recessive Seckel Syndrome. Although an unrelated genetic condition or an alternate disease mechanism cannot be ruled out in our patient, to date we do not have an adequate explanation for his neurologic presentation and the penile anomalies.

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Third case of limb reduction defect in a patient with Jacobsen 11q distal deletion syndrome. *B. Hall.* University of Kentucky, Lexington, KY.

Finger and toe abnormalities such as clinodactyly, syndactyly, and short fingers are common in Jacobsen's syndrome. Limb deficiency has been reported only twice and in both instances the deficiency was distal, unilateral, and involved a single limb. One case was in a 34 year-old male with a missing distal right forearm with 4 nubbin-like digits attached to the remaining forearm. The other instance involved a 37 week gestation female who was missing the anterior half of her left foot. I report a third case of Jacobsen's syndrome to more firmly establish this limb reduction defect association. The girl was a 41 week gestation product of an uncomplicated pregnancy who was born to a 33 year-old G2P2 mother. The child had normal birth parameters and mild thrombocytopenia (60,000-80,000 platelets) for the first twenty-four hours. She had a hypoplastic right hand with nubbins for fingers 3 and 4 and hypoplastic 2nd and 5th fingers. Additional abnormalities included multiple thoracic hemivertebra, VSD, and accessory left nipple. On standard karyotype she had deletion 11q23.3-qter, but on ARRAY the deletion was 11q24.2-qter. The two literature cases showed deletions in the same region although one was a terminal mosaic deletion involving 11q24.1 and the second an 11q24-qter deletion. All 3 cases showed unilateral, distal, and single limb reduction defects. Only one of the two literature cases showed any prolonged thrombocytopenia. Over 80% of Jacobsen patients have thrombocytopenia and some researchers have theorized it to be the causative factor in the limb reduction defects via fetal hemorrhage/ vascular disruption. The gene (s) for the thrombocytopenia is at 11q24.3. At this point no limb development gene has been located in the 11q24-pter region to explain the limb reduction defects. In any regard limb abnormalities including limb deficiencies are common in terminal 11q deletions.

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Natural history of Down syndrome patients in Japan: a questionnaire investigation. *T. Kondoh[1], K. Morifuji[4], T. Matsumoto[4], H. Nakane[5], E. Tsukada[3], M. Fukuda[1], M. Doi[2], H. Motomura[2], S. Honda[6], H. Moriuchi[2].* 1) Dept Pediatrics, Misakaenosono Mutsumi, Isahaya, Japan; 2) Dept Pediatrics, Nagasaki University Hospital, Nagasaki, Japan; 3) Genetic Counseling Unit, Nagasaki University Hospital, Nagasaki, Japan; 4) Dept Nursing, Nagasaki University School of Health Science, Nagasaki, Japan; 5) Dept of Occupational Therapy, Nagasaki University School of Health Science, Nagasaki, Japan; 6) Dept Public Health, Nagasaki University School of Medicine, Nagasaki,Japan.

[Background] Down syndrome (DS) is the most common chromosomal aberration. Its incidence has been increasing from one of every 932 live births in 1975 to one of every 583 live births in 2005, and its life expectancy has been elongated in Japan. Nevertheless, the natural history of Japanese DS patients remains obscure. [Materials and Methods] Study subjects were all DS patients who were sixteen years or older who lived in Nagasaki Prefecture. A small proportion included those in neighboring prefectures. A total of 1,300 questionnaires were delivered to their families and institutional caregivers. Questionnaire investigation was performed in 2-fold: one for the families to clarify chronological changes as well as current conditions, and the other for caregivers to assess current conditions supplementing the former. [Results and Discussion] We collected 551 questionnaires from 269 families and 282 caregivers. Based on this investigation and Nagasaki prefectural census data, morbidity of DS was 0.47 in 1,000 persons aged 16-64 years and the average life span was 57.8 years old. While younger DS patients live along with their families at home, the proportion of those who live in institutes increased with age: 55% in early 30's and 100% in late 50's and 60's. Only a minority in young age groups could talk fluently, and difficulties in language function increased with age: approximately 30% of DS patients in 40's had lost verbal communication skill. As for daily activities, those who needed help all the time were less than 10% in the late 10's and early 20's, but increased to around 30% in 30's and to around 40% in 50's and 60's. Among DS patients aged 27-44 years, there was little difference in the ability to move between those at home and those in institutes; however, the latter had more difficulties in verbal communication and daily activity than the former. Most DS patients appeared to be the most active physically and intellectually before and around 20 years old. Physiological aging signs started to develop in the late 20's. 75% of DS patients consulted with hospitals. 23% of DS patients had operations. Major psychiatric problems included disturbance of skills in daily lives, defects of memory, general mental disability, language dysfunction, personality disorders, depression, delusion, panic disorder and anxiety disorder. Further investigations are warranted to clarify characteristics of the natural history of Japanese DS patients.

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Novel Clinical Manifestations in Pallister-Killian Syndrome: Comprehensive Evaluation of 26 Affected Individuals and Review of all Previously Reported Cases. *A. Wilkens, L. Conlin, M. Kaur, L. Campbell, M. Deardorff, M. Jackson, N. Spinner, I. Krantz.* Children's Hospital of Philadelphia, Philadelphia, PA.

Pallister-Killian Syndrome (PKS) (OMIM # 601803) is a rare, multi-system developmental disorder caused by tetrasomy 12p that exhibits tissue-limited mosaicism. The spectrum of clinical manifestations in PKS is wide and although almost universally described as a severe disorder with multiple congenital anomalies and severe to profound cognitive involvement, recent reports have described a milder phenotype. We report the clinical findings of 26 individuals with PKS who were all evaluated at family meetings of the PKS Foundation, or during a clinical genetics evaluation at The Children's Hospital of Philadelphia. All subjects were enrolled under an IRB-approved protocol of informed consent. Consented individuals filled out a medical history survey and all available records obtained were reviewed by the genetics team. Dysmorphologic examinations were performed on all subjects and neurological and developmental examinations were conducted on a subset. These cases were then compared to the 152 cases of PKS reported in the medical literature. Index and reference cases without cytogenetic confirmation or with variant chromosomal abnormalities involving 12p were excluded. Several novel clinical characteristics of our cohort were identified. Among our patient cohort, several previously unreported clinical features were observed including a high prevalence of dermatoglyphic whorls, philtral skin extending into vermilion border of the upper lip and posterior ear pits. In addition, our patients demonstrated greater variability in cognitive outcomes compared to patients reported in the literature. Advanced molecular SNP and CGH array-based techniques which can detect low levels of mosaicism in blood without the requirement of a skin biopsy has improved diagnostic capabilities, even in the absence of a suspected PKS clinical diagnosis. However not all patients can be diagnosed using blood-based testing and recognition of the clinical phenotype is critical. Such higher resolution molecular technologies have also aided in the identification of patients with variable 12p chromosomal rearrangements in probands with overlapping PKS phenotypes, which has allowed for the delineation of a PKS critical region within chromosome 12p. This report expands and defines the clinical phenotype of PKS, highlights the highly variable expressivity of this disorder, and discusses the impact of array-based cytogenetic analysis on diagnosis and counseling.

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Deletion 3p syndrome and phenotypic change with age: an adult case report. *N. Davalos[1,4], I.J. Garcia-Gonzalez[1], V.M. Anguiano-Alvarez[1], J.L. Villarreal-Salgado[2], G.H. Galicia-Hernandez[1], C.A. Galvan-Diaz[3], A.M. Franco-Martinez[1], A.R. Rincón-Sanchez[5].* 1) Inst Human Genetics, Univ Guadalajara, Guadalajara, Mexico; 2) Hospital Regional de Zona "Valentín Gómez Farías", ISSSTE, Cirugia reconstructiva, Zapopan, Mexico; 3) Centro De Investigación Biomédica De Occidente IMSS, Guadalajara, Mexico; 4) Hospital Regional de Zona "Valentín Gómez Farías", ISSSTE Servicio de Genética, Zapopan, Mexico; 5) Instituto de Enfermedades Crónico Degenerativas, CUCS, UdeG;, Guadalajara, Mexico.

Introduction: The deletion of the short arm of the chromosome 3 is a rare disorder characterized by growth failure, psychomotor and mental retardation and craniofacial anomalies and less commonly others cardiac, renal and gastrointestinal malformations. Case report: The propositus is male reported at the age of 20 months for the presence of congenital malformation and hypotonia. The karyotype was 46,XY,del(3p26). Currently he is 18 years old and exhibits evidence of phenotype changes no characteristics of the syndrome at pediatric ages. Physical examination revealed a height of 167 cm, weight of 47 kg. He is normocephalic with triangular face, malformed ears, low frontal hairline; low nasal base and bridge, absent and long philtrum, full inferior lip, narrow and high arched palate; hypotrophyc limbs, hands with cutaneous syndactyly and bilateral clinodactyly in the fifth finger. Neurological examination is normal. Discusion: The deletion 3p syndrome it has a characteristic phenotype, nevertheless a few adult cases were reported. The patient shows some physical findings not compatible with the syndrome and could be age-related. Other are more associated with the extension of the deletion. Conclusion: This syndrome presents a strong connection between the severity of the disease and the portion of the deletion. The previous statement justifies the importance of delimit the affected chromosomic area with molecular characterization and the phenotype-genotype correlation. We also consider the existence of a phenotypic variability that is related with the age in this syndrome.

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A *de novo* deletion of 12p12.1 partially overlapping the *SOX5* gene in a patient with developmental delay and hypotonia. *M. Gorre[1], J. Kim[1], S. Gunn[1], W. Burdo-Hartman[2], K. Hovanes[1].* 1) CombiMatrix Diagnostics, Irvine, CA; 2) Spectrum Health, Grand Rapids, MI.

Here we describe a 20 month old female referred to the neurodevelopmental clinic due to global developmental delays. Her mother reports that she sat at 6 months of age, crawled at 8-9 months of age, and has only progressed to standing and cruising now. She only says a few consonant sounds. She holds her hand in fisted position with her left thumb is in a cortical position. Her rate of weight gain has been slow as well. MRI showed her to have bilateral optic nerve hypoplasia and a small pars intermedia cyst in the pituitary. She was born at term and weighed 7 pounds and 6 ounces and there was no alcohol, drug or tobacco use during the pregnancy. Her mother has a past history of having a stillbirth and a miscarriage. The family history is significant for a maternal uncle who had Friedreich's ataxia and died at about 40 years of age and her mother who had speech delay as a child. Her weight is 9.52 kg (305th%), length is 85.1 cm (75-90th%), head circumference is 45.2 cm (10th%), and her weight for length is at the 3rd%. Her head is normocephalic and fontanels are closed and sutures are approximated. Pupils are equal round and reactive to light and extraocular muscles are intact. Ears are well formed, nose is bulbous, and teeth are normal. Neck is supple without lymphadenopathy, lungs are clear, heart has a regular rate and rhythm, abdomen is soft, and bowel sounds are normal. Extremity examination reveals long tapering fingers with a cortical left thumb. Neurologically, she has decreased strength and muscle mass. Her tone is decreased except at her left thumb. She can bear some weight on her legs and she was interactive and gave eye contact. Oligonucleotide array comparative genomic hybridization (aCGH) revealed the patient to have a 1 Mb deletion of 12p12.1. A search for genes in the deleted region revealed partial deletion of a single annotated gene -- the *Sry-related HMG box 5* (*SOX5*) gene, a member of the Sox D subfamily of genes. *SOX5* codes for a transcription factor whose long transcript is expressed in the brain and cartilage during embryonic development in mice and humans and is thought to play a role in neuronal and skeletal development. Failure to detect the same deletion in parental samples suggests that this is a de novo event. This is the first reported case of a de novo deletion affecting the *SOX5* gene in a patient with developmental delay and hypotonia suggesting a potential genotype/phenotype correlation.

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Ambiguous genitalia in a patient with a genetic diagnosis, 46,XX/46,XY chimera: A Case Report at the Hospital Para el Niño Poblano, Mexico. *F. Cuellar-López[1], J.M. Aparicio-Rodriguez[2,6], M.L. Hurtado-Hernandez[3], C. Carrillo-Gonzalez[4], M. Barriento-Perez[4], H. Tovar-Gonzalez[5].* 1) Pediatric Urology; 2) Genetics; 3) Cytogenetics; 4) Endocrinology; 5) Psychology, Hosp Para el Nino Poblano, Puebla, Mexico; 6) Estomatology, Benemerita Universidad Autonoma de Pueblña, Mexico.

INTRODUCTION. The phenotypic spectrum of 46,XX/46,XY chimeric cytogenetics results is variable. It ranges from normal male or female genitalia to different degrees of ambiguous genitalia. Chimerism results from the mixure of two different zygotes in a single embryo, whereas mosaicism results from a mitotic error in a single zygote. Here, we report on a case of chimerism (46,XX/46,XY) in a pediatric patient, Clinical examination revealed ambiguous genitalia, female external genitalia with a small tissue similar to a small penis. During cytogenetic diagnosisit was observed the presence of 46,XX and 46,XY cell lines in 47%/53% respectively. Diferent cases of chimerism have been reported by Hunter et al., 1982; Freiberg et al., 1988; Pinhas-Hamiel et al., 2002; Simon-Bouy et al., 2003; Chen et al., 2005. In 1962, Gartler et al. described the first case of chimerism. Since then, about 30 cases have been reported in the literature. MATERIAL AND METHODS. After undergoing cytogenetic studies in a pediatric patient referred to medical genetics, two different cells lines (46,XX [47%] and 46,XY [53%]) were observed in blood cell culture. Karyotyping after G banding was performed according to standard procedures on cultured blood cells, venous blood sampling and peripheral blood lymphocytes. 100 hundred metaphases were studied in order to estimate the percentage of each cell line. DNA was extracted from peripheral blood cells. RESULTS. Karyotypes obtained confirmed the presence of 2 cell lines from 100 cells examined (46,XX [47] and 46,XY [43]). A multidisciplinary medical meeting was performed to discuss whether the patient could be surgically transformed as female or male with a genitalia modification. It was concluded taking in considerations the cell line percentage and the best sexual future function for the patient, that it could be better to perform a surgery for a female rather than male. It was discussed with the family whom agreed on this decision. The earlier cytogenetic and urologic diagnosis is very important in these patients in order to obtain the best quality of life. REFERENCES. 1. Danon M, Friedman SC: Pediatric endocrinology: a clinical guide. Ambiguous genitalia, micropenis, hypospadias, and cryptorchidism. 3rd ed; Marcel Dekker; New York; 1996, 281-304. 2. Freiberg AS, Blumberg B, et al. XX/XY chimerism encountered during prenatal diagnosis. Prenat Diagn 1988; 8: 423-426.

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De Novo Deletion and Duplication of the _SMC1A_ gene at Xp11.2. *ES. Goh[1], G. De Veber[2], DJ. Stavropoulos[3], D. Chitayat[1].* 1) Division of Clinical & Metabolic Genetics, The Hospital for Sick Children, Toronto, ON, Canada; 2) Division of Neurology, The Hospital for Sick Children, Toronto, ON, Canada; 3) Cytogenetics Laboratory, Department of Paediatric Laboratory Medicine, The Hospital for Sick Children, Toronto, ON Canada.

Microarray analysis has become part of clinical practice in screening patients with mental retardation and or congenital anomalies. However, Buysse *et al.*, showed that in a population of 703 patients with mental retardation and/or congenital anomalies who had undergone 44K oligonucleotide array, 11% was found to have causal copy number variants, while 21% showed changes of unknown significance. Thus, information obtained by microarray analysis often presents a challenge in interpretation.

We report two probands with multiple congenital anomalies and findings of copy number variation in the Xp11.2 region. Case 1 is a female with facial dysmorphism including hypertelorism, hypoplastic supraorbital ridges, left cleft lip and palate, bilateral overlapping 2nd and 3rd digits with camptodactyly, hypotonia and poor suck. At 2 months of age she developed intractable seizures which resulted in her death. She had a *de novo* deletion at Xp11.2 including a partial deletion of exons 19-25 in the *SMC1A* gene. Case 2 is a male born with missing right fibula, short bent tibia with subcutaneous spur, equinus right foot with limitation of ankle joint movement and oligodactyly with 3 toes (great toe, 2nd and 3rd). The left lower limb was unremarkable. He has a maternally inherited duplication of Xp11.2 in the same region. The *SMC1A* (OMIM 300040) gene is located at Xp11.2 and is reported to escape X-inactivation. It is associated with a variant of Cornelia de Lange (CdLS) which is characterized by mild facial features such as a prominent nose with lack of synophrys or arched eyebrows, mild developmental delay and absence of major congenital structural anomalies or limb reduction defects (Deardorff *et al.* 2007). We compare the recent literature on the cases of *SMC1A*-associated CdLS as well as copy number variation at Xp11.2. To our knowledge, case 1 represents the largest deletion known affecting *SMC1A* only and case 2 is the smallest duplication of the Xp11.22, including the *SMC1A* gene reported in the literature. We postulate based on the phenotype of these two cases that the pathogenic mechanism of *SMC1A* is not due to a gene dosage effect but rather due to defects in dominant negative function of the gene.

•Buysse K, Delle Chiaie B, Van Coster R *et al. Eur J Med Genet.* 52:398-403.(2009)

•Deardorff M, Kaur M, Yaeger D *et al. Am J Hum Genet.* 80:485-494.(2007).

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Neurocognitive spectrum in boys with the fragile X premutation. *S.W. Harris[1,2], A. Schneider[1], N. Russo[3], C. McKown[3], D. Alderson[3], M. Lipton[3], P. Hagerman[1,4], F. Tassone[1,4], R.J. Hagerman[1,2].* 1) MIND Institute, Univ of California Davis, Sacramento, CA; 2) Department of Pediatrics, University of California Davis, Sacramento, CA; 3) Rush NeuroBehavioral Center, Skokie, IL; 4) Department of Biochemistry and Molecular Medicine, University of California Davis, Davis, CA.

Background/Purpose

We are conducting a study regarding profiles of cognitive involvement in young males with the fragile X premutation, and are interested in the neucognitive profile of these children, including autism features, memory, and social-emotional functioning.

Methods

To date we have done a comprehensive neurocognitive assessment with 10 boys between 8 and 15 years of age (mean= 11.2 + 2.1) who have the fragile X premutation. We assessed cognitive ability using a Wechsler intelligence scale (either WISC-IV or WASI), and we also used the Autism Diagnostic Observation Schedule (ADOS) to assess characteristics of autism spectrum disorders. Additional measures were completed to assess social-emotional and theory of mind abilities, including subtests from the CASL (pragmatic judgment), NEPSY-II (theory of mind and affect recognition), and the TOPS (problem solving), as well as memory abilities. We also completed several research measures to assess social-emotional functioning, including computerized measures of affect processing of both faces and postures. Molecular data is being collected on each subject as well, including mRNA and protein (utilizing the updated ELISA method).

Results

The subjects assessed showed a wide range of cognitive abilities. Full scale IQ (FSIQ) scores ranged from 51 to 141 (mean= 93 + 25) and classification utilizing the ADOS showed that several of the subjects were classified as within the autism spectrum. Interestingly, two of the subjects were diagnosed with Asperger's syndrome, and this has not been reported previously in boys with the premutation. We will also report on results of memory and social-emotional functioning of the subjects assessed to date, including results from some of the standardized measures and research paradigms used to assess these children. We will also present data on correlations between these clinical measures and the molecular data for these subjects.

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Whole genome FAIRE analysis reveals regions of chromatin fluctuations in Angelman syndrome deletion versus 15q interstitial duplication autism. *X. Wang[1], N. Urraca[2], W. Taylor[3], L.T. Reiter[1,2].* 1) Dept Anatomy and Neurobiology, UTHSC, Memphis, TN; 2) Dept Neurology, UTHSC, Memphis, TN; 3) Molecular Resource Center, UTHSC, Memphis, TN.

The q arm of human chromosome 15 contains several low copy repeat (LCR) regions which predispose segment 15q11.2-q13 to non homologous allelic recombination events resulting in copy number changes for the intervening genes. Maternally inherited deletion events at this locus result in the neurological disorder Angelman syndrome (AS) when they include the human *UBE3A* gene, while the reciprocal interstitial duplications of the locus surrounding *UBE3A* give rise to milder phenotypes that include autism when maternally derived. To provide a framework from which to initiate the identification of transcripts that show variable expression in the presence of decreased (AS) or increased (dup15) levels of UBE3A we used lymphoblast cell lines from a class II AS deletion and a class II int dup15 subject for analysis by whole genome Formaldehyde-Assisted Isolation of Regulatory Elements (FAIRE). We identified signals on all chromosomes that showed enriched FAIRE signals indicative of increased regional gene expression and a decrease in chromatin condensation. We scanned 10kb segments for regions where the average FAIRE signal showed opposing patterns in AS deletion vs dup15 cell lines. We found 189 regions where FAIRE signals were enriched in one direction or the other depending on the deletion or duplication on 15q. Some regions undergoing changes in chromatin conformation included autism associated loci on 1q21.1 and 16p13.1. We also found signal differences in genes: 1) *ASTN1* (1q25.2) a neural migration gene; 2) *NFX2* (Xq22.1) an FMRP interacting protein that regulates Nxf1 mRNA stability in neuronal cells and; 3) *CNTNAP2* (7q35-q36) a gene encoding Caspr2, a member of the neurexin family linked to autism with language impairment. The number of pericentromeric reads on chromosomes 1, 2, 6 and 10 was over represented in FAIRE treated dup15, but unchanged in these same regions in AS deletion. Although this could be an artifact generated by the repetitive nature of these regions, it could also indicate a general loss of chromatin compaction in 15 duplication subjects. These regions may contain genes that are regulated at the chromatin level by upstream changes in *UBE3A* expression and are therefore good candidates for expression studies to identify molecular changes that result in both AS and int dup15 autism. The combination of gene expression data and promoter region specific FAIRE signal analysis may reveal new gene networks involved in both AS and autism.

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MECP2 Duplication with Left Ventricular Apical Trabecular Cardiomyopathy. *C.A. Bay[1], C. Cottrill[2], N. Soares[3], B.D. Hall[1], T. Bonilla[1], A. Rutherford[1].* 1) Clinical/Biochemical Gen, University of Kentucky, Lexington, KY; 2) Community Pediatric Cardiology, University of Kentucky, Lexington, KY; 3) Developmental Behavioral Pediatrics, University of Kentucky, Lexington, KY.

We have observed a 15 year old caucasian male followed since childhood with severe cognitive delays, and ataxia. Dysmorphic features include epicanthal folds and flat malar bones. Family tree is consistent with X-linked recessive inheritance. Previous karyotype, Angelman studies, and Fragile X testing were negative. Cardiovascular monitoring documented arrhythmias which responded to medication. The finding of left ventricular apical trabecular cardiomyopathy prompted reappraisal and consideration of Barth syndrome. He did not have neutropenia. Sequencing of TAZ gene did not identify a mutation. Chromosomal Microarray documented a duplication within Xq28. Duplication size is a minimum of 0.890 Mb, and includes both MECP2 gene, and FLNA, but does not include TAZ. We believe this patient, who is clinicallly consistent with MECP2 duplication, to be the first reported MECP2 duplication patient with left ventricular trabecular cardiomyopathy. Recent reports have documented cardiovascular abnormalities in patients with an Xq28 duplication including both MECP2 and FLNA, but those abnormalities did not include left ventricular apical trabecular cardiomyopathy. This type of cardiomyopathy can be seen in Barth syndrome, also found at Xq28. We suggest that cardiovascular abnormalities may be an important clinical feature of MECP2 duplication. Left ventricular apical trabecular cardiomyopathy could represent a broader spectrum of the observed cardiovascular features when the Xq28 duplication includes MECP2 as well as FLNA. Alternatively, in our patient Tafazzin function might be impaired due to epigenetic factors associated with the proximity of the TAZ gene to the duplicated Xq28 segment.

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Identification of pathogenic copy number variants and of a novel single-gene deletion on syndromic obesity. *C.S. D'Angelo, I. Kohl, C.P. Koiffmann.* Department of Genetics and Evolutionary Biology, Institute of Biosciences, University of São Paulo, Rua do Matão 277, sala 204/209 CEP 05508-900, São Paulo, SP, Brazil, Financial Support: FAPESP, CEPID-FAPESP, CNPq.

The impact over the ascertainment of rare copy number variants (CNVs) in cohorts with extreme phenotypes in addition to congenital malformations and/or developmental delay (DD) has been recently appreciated with the discovery of deletions at 16p11.2 as a new highly penetrant form of human obesity. In an ongoing research on syndromic obesity we sought for pathogenic CNVs in patients with negative testing for PWS with array genomic hybridization (AGH). We found several CNVs of *de novo* occurrence: a deletion of chromosomes 2p25.3, 6q16.1q21, 7q22.1q22.3, 12q15q21.1 and Xp22.13p22.12, a duplication of chromosomes 14q11.2 and Xq28, and an unbalanced translocation between chromosomes 3p26.3 and 11q22.3. Obesity in monosomy 6q16 is related to the hemizygosis of *SIM1*. Several presumed obesity-associated genes were mapped to the genomic intervals of the remaining CNVs. To evaluate the contribution of these CNVs to syndromic obesity, we developed a synthetic MLPA probemix containing probes to each of these genomic intervals. With the same purpose, we synthesized another probemix based on the observation of rare CNVs that were recurrent in patients with obesity-associated phenotypes listed on the DECIPHER. Besides, we performed 100 K Affymetrix GeneChip® array in combination with CNAT (v4.0) and CNAG (v3.0) on a select group of 11 patients with obesity and negative testing for Smith-Magenis syndrome. Thus far, we have found two unrelated patients out of 38 screened by MLPA with *de novo* deletions at 2q37, and apparently pathogenic CNVs in three of the patients tested with SNP arrays: a *de novo* single-gene deletion at 14q12 (~600 kb), a *de novo* duplication at 12q21.32q23.1 (~12 Mb), and a duplication at 20q11.1q11.2 (~2 Mb). The deletions at 2q37 encompass at least 4 genes assigned as candidates for obesity in 2q37 monosomy syndrome. The deletion at 14q12 covers the entire coding region of *PRKD1*. This gene is usually deleted in patients with 14q12 microdeletion syndrome. No benign CNVs have been assigned to *PRKD1* and we found two patients with DD and overlapping deletions within this gene on the DECIPHER. The remaining CNVs affect several genes with important signaling functions, such as *DUSP6* and *DUSP15*. The use of AGH has increased the recognition of new syndromic forms of obesity, and is a potential source for understanding the etiology of common obesity as well. We present our preliminary data towards the identification of novel obesity-associated genomic *loci*.

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Paternal UPD14 diagnosed by whole genome array: clinical and radiological features. *B.E. Mucha-Le Ny[1], J.M. Kalish[1], N.B. Spinner[2], E.H. Zackai[1], L.K. Conlin[2].* 1) Division of Human Genetics, The Children's Hospital of Philadelphia, Philadelphia, PA; 2) Department of Pathology and Laboratory Medicine, The Children's Hospital of Philadelphia, Philadelphia, PA.

The introduction of single nucleotide polymorphism (SNP) array analysis allows the direct diagnosis of uniparental disomy (UPD) even when UPD is not initially suspected. We report two patients referred for array analysis, who were diagnosed with paternal uniparental disomy of chromosome 14. Patient 1 presented at birth with bony abnormalities including "coat hanger" ribs and bony spurs emanating from the mental protuberance of the mandible and calcaneus. She died at 8 weeks of age secondary to respiratory distress. Patient 2 had a milder phenotype with only mild respiratory distress exacerbated by upper airway infections. Whereas the chest was only slightly bell-shaped on X-ray imaging, she exhibited similar bony spurs as patient 1 on the mandible and the calcaneus. Patient 1 had isodisomy of chromosome 14 based on homozygosity for the entire chromosome 14 on SNP array analysis. She had a normal 46,XX karyotype. Parental genotyping confirmed paternal isodisomy and these findings together indicate that UPD 14 arose by monosomy rescue. Presumably, this occurred secondary to maternal non-disjunction resulting in a nullisomic egg with subsequent duplication of the paternal chromosome 14. SNP array analysis of patient 2 revealed mosaic trisomy 14 in 20-25% of peripheral blood cells which was confirmed by chromosomal analysis. The presence of runs of homozygosity near the centromere and telomere indicated uniparental disomy in the diploid cell line which parental genotyping revealed was paternal. The uniparental disomy in this patient is both iso- and heterodisomic. In this case, UPD arose by trisomy rescue presumably secondary to nondisjunction in paternal meiosis with subsequent loss of the maternal chromosome. Clinically, these cases both demonstrate bony spurs, a previously unreported skeletal feature associated with paternal UPD 14. To our knowledge, this is the first report of calcaneal spurs in a genetic disorder and anterior bony spurs from the mandible have previously only been seen in frontometaphyseal dysplasia. These cases illustrate the heterogeneity both in the mechanism of formation of the UPD, as well as clinical heterogeneity, which may be explained in part by the mosaic trisomy for chromosome 14 in patient 2, in contrast to the pure paternal UPD 14 in patient 1. These cases highlight the utility of SNP array analysis to both diagnose UPD and to provide information about the mechanism by which these occur.

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Accomulation of Iron in Mitochondria in Friedreich Ataxia may damage the mtDNA in this patients. *S. Sanjarian[1], M. Shafa shariat panahi[2], Z. Nourmohammadi[1], M. Naseroleslami[1], M. Houshmand[2,3].* 1) Science & Research branch Islamic Azad University, tehran, Iran; 2) National Institute of Genetic Engineering and Biotechnology , Tehran,Iran; 3) Special Medical Center,tehran, Iran.

Introduction:Friedreich ataxia is an autosomal recessive neudegenerative disorder caused by decreased expression of protein frataxin. The expansion of polymorphic GAA triplet repeat in the first inton may be caused by decreased frataxin expression by interfering with transcription. Frataxin deficiency leads to excessive free radical production and dysfunction of chain complexes. FRDA characterized by gait and limb ataxia, lower limbs areflexia, loss of joint position, cardiomyopathy, scoliosis. material and methods:We studied the frequency and the length of (GAA)(n) repeat in 25 Iranian patients (16 females and 9 males) from unrelated families (control= 50).DNA from each patient was extracted and frequency and length of (GAA)(n) repeat was analyzed using a long-range polymerase chain reaction (PCR) test. Also we investigated impact of GAA size on neurological findings, age of onset and disease development. In order to identify polymorphic sites and genetic background, the sequence of two hyper variable regions (HVR-I and HVR-II) of mtDNA was obtained from FA patients harboring GAA tri nucleotide expansions. We have found 13 mutations (T8614C,T8598C,C8684T, A8701G, G8994A, A9024G, G8251A, A8563G,G8584A, A3505, T3335C and G3421A) in tRNALeu and ND1 and tRNALys and ATPase gene in 20 patients and 80 control by PCR analysis. In other study in order to determine the exact number of GAA repeats in FRDA gene we used Fragment analysis. fragment analysis was used to investigate genotype and phenotype correlation. For this reason expand long PCR was used for amplification. We believed that fragment analysis is more helpful for: a) Carrier detection (even most patients are consanguinity marriage) b) Genotype and phenotype analysis. Results:Homozygous GAA expansion was found in 21 (84%) of all cases. In four cases (16%), no expansion was observed, ruling out the diagnosis of Friedreich's ataxia. Our results showed that the rate of D-loop variations was higher in FA patients than control (P<0.05). Only A9024G was reported for the first time. Fragment analysis determine exact number of GAA repeats and the result confirm that there was a significant inverse correlation between the average of two expansion size and age of onset of symptoms. Discussion:1) Because of Iron accumulation in mitochondria, it may damage to mtDNA. 2) for genotype phenotype correlation fragment analysis is a best method.

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THE IMPORTANCE OF BIN-AURAL HEARNESS ON THE COGNITIVE DEVELOPMENT IN PEDIATRIC PATIENTS WITH UNILATERAL MICROTIC ATRESIA IN RELATION TO THE AUDITIVE REHABILITATION. *A.Diaz. Romero[1], Y. Peñaloza-Lopez[2], J.M. Aparicio-Rodriguez[3], S.I. Assia-Robles[4].* 1) Audiology, Hospital para el Niño Poblano; 2) Neurophisiology, Instituto Nacional de Rehabilitacion; 3) Genetics; 4) Pediatrics, Hospital para el niño Poblano.

Microtia also called microtia-anotia, is a congenital deformity where the pinna (outer ear) is very small and underdeveloped (microtia) or absent (anotia). It can be unilateral (one side only) or bilateral (affecting both sides). It occurs in 1 out of about 8,000-10,000 births. In unilateral microtia, the right ear is founded more often. It is known that microtia can have different phenotipical degrees; Grade I: A slightly small ear with identifiable structures and a small but present external ear canal. Grade II: A partial or hemi-ear with a closed off or stenotic external ear canal producing a conductive hearing loss. Grade III: Absence of the external ear with a small vestige structure and an absence of the external ear canal and ear drum conductive hearing loss. Grade IV: Absence of the total ear or anotia. Unilateral deafness was not generally considered a serious disability before the nineties; it was thought that the afected person was able to adjust to it. In general, there are very good advantages to gain from an intervention to enable hearing in the microtic ear, especially in bilateral microtia. Patients with untreated unilateral hearing loss are eight to ten times more likely not to hear. However, with unilateral deafness often require years of speech therapy. Aural atresia is commonly associated with microtia. Atresia occurs because patients with microtia may not have an external opening to the ear canal, though the cochlea and inner ear are usually present. The grade of microtia is in relation to the development of the middle ear. Microtia is found isolated, but may occur in conjunction with hemifacial microsomia, Goldenhar Syndrome or Treacher-Collins Syndrome. Microtia1,2 can cause difficulties with life. It is also occasionally associated with syndromes as mentioned before that can cause, kidney problems, and jaw problems, and more rarely, heart defects and vertebral deformities. It is important the highly experienced audiologist for a better evolution of thge patient. It must be difficult that many surgeons believe that ear canal reconstruction is unnecessary and over complicated and that very good hearing is possible with modern hearing aids which can be hidden under the skin. Microtia eitology has been associated to vascular deficiency that is in relation to sporadic cases rather than inherited. Several teratogens have been associated as Talidomida, retinoic acid, alcohol and diabetes.

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Spondylospinal thoracic dysostosis, a new case with the longest survival. *L. Becerra-Solano[1,2], D. Morales-Mata[2], E. Monroy-marquez[2], L. Chacon-Alcaraz[2], Y. Rufino-Serralde[2], J. García-Ortiz[1], M. Ramirez-Dueñas[1].* 1) Div Genetica, CIBO-IMSS, Guadalajara, Jalisco, Mexico; 2) CRIT Chihuahua, Fundación México.

In 1997, Johnson et al. described the Spondylospinal thoracic dysostosis (STD, OMIM 601809) in two related sibs with dwarfism, short thorax, and curved spine; fusion of the vertebrae and spinal process; multiple pterygium and arthrogryposis. They postulated an autosomal recessive mode of inheritance and considered this entity as a lethal condition due to stillbirth of one patient and early death of the second one. Clinical report. A female patient 15 years-old, was evaluated by dwarfism, severe dorsal lordosis, and multiple pterygium. She was product of the second uneventful pregnancy from healthy non consanguineous parents (mother 41 and father 39 years-old). She was delivered by cesarean section. At birth her weight was 2850 g (10-25 centile), no Apgar score was recorded but spontaneous cry was referred. Psychomotor development was notoriously delayed. Physical examination: Height 93 cm (-3 centile), weight 23.2 cm (-3 centile), OFC 54 cm (70 centile); dolichocephaly, bitemporal narrowing, high forehead, left facial hemi-hypoplasia, up-slant palpebral fissures, telecanthus, high nasal bridge and broad nasal base, anteverted nares, short columella, thick lips, short lingual frenulum, micrognathia; posteriorly rotated ears; short neck with hyperextension, short and wide thorax; external genitalia pubic hair Tanner 4 and hypoplastic labia majora; upper limbs with limitation of extension and pronosupination due to elbow pterygium, and partial cutaneous syndactyly; lower limbs in abduction with popliteal pterygium, both patella were not palpable, overlapping of second over first toes, and bilateral brachydactyly of 4th and 5th toes. X-ray examination and 3D reconstruction showed: advanced pneumatization of paranasal sinuses; short and wide thorax, crowded and gracile ribs; multiple fusions of vertebral bodies and spinal processes, hyperextension of cervical-dorsal column and lumbar hyperlordosis (both angle of approximately 90=BA); narrow hip, hypoplastic and vertically placed pelvic bones, wide pubic simphysis, and the presence of a hole on the central portion of iliac bones; also fusion of carpal and tarsal bones were appreciated. Discussion. STD is a rare MCA entity mainly characterized by vertebral defects, thoracic anomalies, and multiple pterygium. Here, we described a female patient of 15 years of age, whom probably would be considered as the eleventh case of this entity and interestingly the one with a longer survival period.

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Fibular Aplasia in Acro-Cardio-Facial Syndrome: expanding phenotypic spectrum in search for causative gene(s). *D. Niyazov[1], R. Monier[1], L. Finger[1], H. Ginsberg[1], A. Kenney[2], B. Canzoneri[3].* 1) Dept Pediatrics, Ochsner Clinic Foundation, New Orleans, LA; 2) Dept Radiology, Ochsner Clinic Foundation, New Orleans, LA; 3) Dept Maternal & Fetal Medicine, Ochsner Clinic Foundation, New Orleans, LA.

Acro-cardio-facial syndrome (ACFS) is a rare disorder which includes split-hand/foot malformation (SHFM), cleft lip/palate (CL/P), cardiac defects, dysmorphic features, genital anomalies and mental retardation. To our knowledge, only 6 cases have been published to date. We report a new patient with the characteristic features of ACFS, and bilateral fibular aplasia which has not been previously described. The male proband was born with unilateral CL/P, hypoplastic left heart and atrial septal defect, single umbilical artery and lower limb deformities. His karyotype on amniocytes was negative. In addition, he had sparse scalp hair, dysmorphic facies and ears, unilateral CL/P, hypoplastic nipples and genitalia with bilateral cryptorchidism. His multiple lower limb anomalies included an absent left hallux, SHFM of the right foot with absent right toes T3-4, severe bilateral limb shortening and equinovarus deformities. The patient died at 6 days of life from cardiac complications. Postmortem xray revealed the right anterior femural bowing, bilateral fibular aplasia, absence of the 3rd and 4th rays on the right and the 4th ray on the left. Total body MRI with 3D reconstruction revealed no anomalies in the brain or abdomen. His 105K oligo array was negative and the TP63 gene is currently being sequenced. Etiology of ACFS is currently unknown. To our knowledge, fibular aplasia (FA) as seen in our patient, has not been previously described in ACFS. FA or FA with ectrodactyly may be a part of the phenotypic spectrum of ACFS which can help identify more genes important in limb patterning. For instance, the TP63 gene has been implicated in ectrodactyly, ectodermal dysplasia and CL/P syndrome (EEC). The proband had SHFM, hypoplastic nipples and toenails, sparse scalp hair, and CL/P. Genital anomalies have also been reported in EEC. While cardiac anomalies are rare the TP63 mutation R298Q was reported in a patient with arrhythmogenic right ventricular cardiomyopathy and a possible role of the TP63 in the heart development was explored. This gene encodes 6 protein isoforms and is highly expressed in many organs including the heart. It may enhance the ability of cardiac tissue to resist mechanical stress by promoting the stable assembly of desmosomal adhesive complexes. In conclusion, discovery of additional anomalies in ACFS may aid in identification of causative gene or interaction of several genes variably affecting multiple organ systems.

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Association of Lactotransferrin Gene Polymorphisms with Risk of Aggressive Periodontitis. *C. Chou[1,2], F. Kuo[1,2], C. Brooks[3], C. Huang[1], J. Burmeister[3], H. Schenkein[3], S. Diehl[1,2].* 1) Health Informatics, UMDNJ-School of Health Related Professions, Newark, NJ; 2) Center for Pharmacogenomics and Complex Disease Research, UMDNJ-New Jersey Dental School, Newark, NJ; 3) Clinical Research Center for Periodontal Diseases, School of Dentistry, Virginia Commonwealth University, Richmond, VA.

Periodontitis, an inflammatory disorder of the periodontium caused by microorganisms, is one of the most common oral infectious diseases. Aggressive periodontitis (AgP) is further subdivided as generalized (GAgP) or localized (LAgP) and affects 2% of the African American population after puberty and may lead to premature tooth loss. Lactotransferrin (LTF) is a protein present in saliva with bactericidal function and can inhibit bacterial growth and attachment to epithelial cells. LTF can also inhibit cytokine production through interference with NF-kB activation, a primary cause of inflammation. Thus, it is reasonable to believe LTF gene may be involved with the development of periodontitis. This work aims to investigate the association between polymorphisms of the LTF gene and aggressive periodontitis. A total of 428 African American subjects were recruited from the Richmond, Virginia area. One hundred and eighteen subjects with GAgP, 112 with LAgP and 198 healthy controls were genotyped for four single nucleotide polymorphisms (SNPs) in the LTF gene by using TaqMan genotyping assay (Applied Biosystems). One SNP is located in the 5' region, and three are non-synonymous SNPs that alter the amino acid coded in the mature protein. The difference in the genotype frequencies between patients and controls was calculated under dominant, recessive and additive model by Fisher's exact test and the Armitage trend test. To control for potential confounding effects, gender, age and smoking status were included as covariates in logistic regression analyses. Statistical analyses were performed using SAS software. SNP rs1126478 that codes for a Lys/Arg polymorphism showed evidence of association with LAgP under dominant and additive models (p=0.019 and p=0.021, respectively). The Lys allele was more common in cases, indicating this variant may be associated with increased risk of this disease. No association was found between any of the evaluated LTF polymorphisms and GAgP. Further investigations including with functional studies of gene and proteins, are needed to support this conclusion. Supported by the Foundation of UMDNJ and NIDCR grants DE13102 and DE016057.

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ALX3 gene is not associated with the syndrome of midline facial defects, callosal agenesis, basal encephaloceles, and eye anomalies. *R.G. Quiezi, A. Richieri-Costa, S.P Barros, L.A. Ribeiro.* Genetics, Hospital for Rehabilitation of Craniofacial Anomalies/USP, Bauru, Brazil.

Frontonasal dysplasia (FND) is the hallmark of several syndromes involving the frontonasal process that includes: isolated frontonasal dysplasia, acrofrontofacionasal dysostosis 1, acrofrontofacionasal dysostosis 2, frontofacionasal dysplasia, oculoauriculofrontonasal syndrome, among other related conditions. One of the syndromes within this spectrum comprises of the syndrome of midline facial defects, callosal agenesis, basal encephaloceles, and eye anomalies. It has been described under different eponyms, most commonly the morning glory syndrome. The anomalies observed in the present condition are embryological related, occurring in the same critical period of time, most likely during the early induction of the forebrain, resulting in anomalies involving frontonasal process, midfacial structures as well as the primordium of the eyes. Several genes are known to exert effects during this period resulting in different craniofacial anomalies. Up to now there is no evidence of any particular gene involved in etiology of the reported condition within the FND spectrum. The chromosome 1 interval contained a strong candidate gene, ALX3; related to the aristaless gene in Drosophila. This gene, located in band 1p13.3, encodes the ALX homeobox 3 transcription factor, a member of the Paired class of homeodomain proteins. Previous studies of the murine ortholog, Alx3, had demonstrated strong expression in the frontonasal mesenchyme. Although the phenotype of Alx3_/_ mice was normal, a cleft face occurred when these homozygotes were additionally mutant for the paralogous gene Alx4. Mutations in ALX3 have been found in patients with frontorhiny phenotype. We amplified each of the four exons of ALX3 and subjected the products to DNA sequencing in 8 patients presenting midline facial defects, callosal agenesis, basal encephaloceles, and eye anomalies. No sequence variation was identified in this analysis. We conclude that ALX3 mutations are not associated with the reported condition within the FND spectrum. Grants: CNPq (307595/2008-0).

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Epistasis between Folate Genes contributes to the Genetic Risk for Non-syndromic Cleft of the Hard Palate. *M. Rubini[1], M. Ferrian[1], C. Baluardo[1], L. Marullo[1], D. Balestra[1], S. Bruni[1], E. Calzolari[1,2], A. Franchella[2].* 1) Dip. MSD Sez. Genetica Medica, Universita' di Ferrara, Ferrara, Italy; 2) Dip. Riproduxzione e Accresciment6o, Az. Ospedal. Sant'Anna, Ferrara, Italy.

During embryogenesis folate metabolism is a critical component for the development of orofacial structures, especially for those deriving from neural crests. Cleft palate only (CPO) and cleft lip with/without cleft palate (CL/P) are common congenital malformations with an overall birth prevalence of 1:700. Most of orofacial clefts are non-syndromic (NS-OFCs), and are considered disorders of multifactorial origin. Epidemiologic evidence indicate that folic acid (FA) supplementation during early pregnancy significantly reduces the risk for NS-OFCs, while association studies suggest that the risk for NS-OFC is influenced by polymorphisms in genes of folate metabolism and transport. It is generally accepted that altered metabolic balance of folate in the embryo, resulting from interaction between functional variants in folate genes and B vitamins bioavailability, could contribute to the risk for NS-OFCs. The genetic basis of folate unbalance underlying NS-OFC is likely to be the outcome of complex epistatic interactions, rather than be due to single gene variants. So far very little is known about the etiology of non-syndromic CPO (NS-CPO), but a number of evidence suggest that In order to track down the network of gene-gene interactions that could lead to folate unbalance and contribute to the risk for NS-CPO we have studied a wide panel of polymorphisms in genes of folate metabolism in a group of NS-CPO nuclear families of Italian origin (Italcleft study). Using Multifactorial Dimensionality Reduction (MDR) we identified a redundant interaction between variants in MTRR and MTHFS genes. This epistasis was specific for cases with cleft of the hard palate (CPH), and was associated with a significant increased risk. This evidence suggests that cleft of the hard palate and cleft of the soft palate should be considered conditions with different etiology, and that folate unbalance could be a specific pathogenetic component of cleft of the secondary palate. Our results, if confirmed in an independent study, would support the hypothesis that complex epistatic interactions between variants in folate genes contribute to the pathogenesis of non-syndromic cleft of the hard palate, and pave the way toward new preventive and diagnostic measures for this congenital malformation.

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Association of CARD8 with IBD in Koreans. *K. Song[1], H. Kim[1], M. Hong[1], S. Yang[2].* 1) Dept Biochem & Molec Biol, Univ Ulsan Col Medicine, Seoul, Korea; 2) Dept. Internal Medicine, Univ Ulsan Col Medicine, Seoul, Korea.

Caspase recruitment domain (CARD)-containing protein 8 (CARD8) is an attractive candidate risk gene for IBD because of its location within inflammatory bowel disease (IBD) locus and its role as a nuclear factor (NF) kappaB inhibitor. A recent report that NOD-2 mediated signaling is negatively regulated by CARD8 in intestinal epithelial cells supports its candidacy for IBD susceptibility gene. However, previous studies have yielded mixed results in the association of a CARD8 nonsynonymous SNP, rs2043211, which changes a cysteine residue to a stop codon at amino acid 10, with IBD in Caucasians. The aim of this study was to test the genetic association of the CARD8 nonsynonymous SNP rs2043211 with Korean ulcerative colitis(UC) and Crohn's disease(CD) patients. We tested rs2043211 of CARD8 in 659 UC, 632 CD, and 687 healthy controls. Rs2043211 showed 5.5 % difference in allele frequency between the UC and control groups, while the difference between the CD and control groups was 1.2%. Logistic regression analysis revealed that rs2043211 was associated with UC significantly. The odds ratio of the rare stop allele homozygote (TT) in comparison with the combined genotype of AA and AT was 1.54 (95% CI, 1.17 - 2.04, P = 0.002). Our data suggests that the CARD8 variants might play a role in the pathogenesis of IBD in the Korean population.

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Recurrent left atrial myxomas in female patients with Carney complex: a genetic cause of multiple strokes in young adults that can be prevented. *G. Briassoulis[1], MS. Popovic[2], P. Xekouki[1], N. Patronas[3], V. Vukomanovic[2], V. Kuburovic[2], M. Keil[1], CA. Stratakis[1].* 1) NICHD, SEGEN,National Institutes of Health, Bethesda, MD; 2) Mother and Child Healthcare Institute New Belgrade, Serbia; 3) Department of Radiology, Clinical Center, National Institutes of Health, Bethesda, MD, USA.

Background: Intracardiac myxomas in the autosomal dominant syndrome Carney complex (CNC) are significant causes of cardiovascular morbidity and mortality through embolic stroke and heart failure. The genetic, clinical and laboratory characteristics of CNC-related strokes from atrial myxomas have not been described before. The PRKAR1A gene is mutated in more than 60% of the cases of CNC. Methods and Results: We studied patients with strokes and cardiac myxomas that were hospitalized in our institution and elsewhere out of a total of more than 500 patients with CNC worldwide: a total of 7 patients with 16 recurrent atrial myxomas and more than 14 episodes of strokes were indentified. Neurological deficits were reported; in one patient, an aneurysm developed at the site of a previous stroke. All patients were female, most of them had presented with Cushing syndrome and all had additional tumors or other CNC manifestations. Although there was a trend for patients being overweight and hypertensive no other abnormalities or risk factors were identified. A total of 5 patients (71%) had a PRKAR1A mutation. All detected mutations (c418_419delCA, c.340delG/p.Val113fsX15, c.353_365del13/p.Ile118fsX6, c.491_492delTG/p.Val164fsX4, c.177+1G>A) were located in exons 3-5 and introns 2-3, and all led to a non-sense PRKAR1A mRNA. Conclusions: Among CNC patients, recurrent atrial myxomas that lead to multiple strokes appear to primarily develop in female patients. Cushing syndrome may be a risk factor that increases the possibility of multiple strokes (Cushing syndrome is more frequent among females with CNC). Early identification of a female patient with CNC either through the application of the diagnostic criteria or through PRKAR1A sequencing (genetic testing) is of paramount importance for the early diagnosis of atrial myxomas and the prevention of strokes.

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Multisynostotic Osteodysgenesis (Antley-Bixler Syndrome). Three years follow-up in a new patient with a very severe form due the mutation c.870G>T (W290C) in the exon IIIa of FGFR2 gene. *M.L. Giovannucci Uzielli[1, 2], G. Scarselli[1], L. Di Medio[2], N. Dayan[2], D. Nannini[2], S. Guarducci[3], E. Gambineri[1].* 1) Dept Paediatrics, Univ Florence, Viale G. Pieraccini 24, 50139, Firenze, Italy; 2) Genetic Science, Piazza G. Savonarola 11, 50132 Firenze, Italy; 3) Meyer Children's Hospital, Viale G. Pieraccini 24, 50139 Firenze, Italy.

Antley-Bixler syndrome (ABS), first described in 1975 in a patient with craniosynostosis, midfacial hypoplasia, arachnodactyly, radiohumeral synostosis and femoral bowing, is a very rare condition. With now a little more than 50 published cases, ABS has evolved as a clinical entity over the past three decades. The clinical and RX phenotype has expanded to include many other characteristic abnormalities, and more or less severe phenotype, often responsible for early death. The proband was born at 34 weeks EGA from a 28year-old woman. Healthy non-consanguineous parents, and a normal brother. Amniocentesis revealed 46,XY normal male. Polyhydramnios from the second trimester, was not well investigated. Several examinations with ultrasounds only revealed bilateral fetal exophthalmos at 33 weeks gestation. Delivery was by C-section. Initial examination at birth, and postnatal clinical and RX studies revealed a severe abnormal phenotype: multiple, premature craniosynostosis, increased biparietal diameter, midfacial hypoplasia, depressed nasal bridge, bilateral choanal stenosis, and severe proptosis. The extremities had, and still have, multiple joint contractures, more severe in the elbow due to radiohumeral synostosis. Three, consecutive, craniotomies with craniofacial correction, were not able to control the progressive exophtalmos, and to improve the breathing: ocular globe was enucleated at age two years, and tracheotomy, performed few days after birth, is still essential. Intellectual and social performances are not assessable. Steroidogenesis and Sterol metabolism are normal. The external genitalia are normal. The mother didn't use Fluconazole during the pregnancy. By direct sequencing of the Fibroblast Growth Factor Receptor 2 (FGFR2) gene, we identified the mutation c.870G>T (W290C) in the exon IIIa.

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A familial case of Goltz-Gorlin syndrome. *L. Wong-Ley[1 2], S. Hernández-Córdova[1], E. Lechón-López[1], M. Messina-Robles[1 2], J. González-Zapata[1 2], M. Pérez-Nuño[2], L. Medina-Carrillo[1 2], A. Flores-García[2].* 1) Dept Gen, SSN-IMSS, Tepic, Mexico; 2) Dept Gen, UAN, Tepic, México.

In 1960 (1), Gorlin and Goltz gave a complete description of the Goltz-Gorlin syndrome (OMIM #305600). A systemic dysplasia of mesodermal and epidermal structures characterized by underdevelopment of the dermal connective tissue and various anomalies in the ocular and skeletal systems. (2). In 2007 mutations in the PORCN gene were found to be causative in Goltz-Gorlin syndrome (3), presents a quite variable estimated prevalence, which goes from 1 in 57.000 to 1 in 256.000 habitants (4). The present report is that of three women with the syndrome, a 29-year-old female, her 50-year-old mother and her daughter deceased to the birth. The three patients had similar clinical symptoms, phenotype characterized by microcephaly, protruding ears with thin pinnae, small orbits with overriding eyelids, microphthalmia, agenesis of the incisors, sensorineural and conductive hearing loss, upper extremities with bilateral syndactyly and camptodactyly, lower limbs with bilateral clinodactyly and ectrodactyly, lobster-clawing with equinovarus deformity. Linear areas of dermal hypoplasia, linear hypopigmentation with dermal hypoplasia, telangiectasias, dysplasia of the hair and nails, mental retardation and inguinal hernias. Examination of the cardiovascular and respiratory system was normal. All the haematological and biochemical studies carried out were normal. X-ray examination showed several skeletal malformations such as hypoplasia of the craniofacial skeleton, winged shoulder, pectus excavatum, defects of segmentation of the vertebrae, dorsal scoliosis and lumbar kyphosis. Chromosome analyses were normal females (46,XX). The genealogical analysis showed autosomal-dominant or sex linked dominant mode of inheritance. There were no other affected relatives. A diagnosis familial Goltz-Gorlin syndrome was made on the basis of clinical and imaging findings. The intention of this paper is to present three cases of Goltz-Gorlin syndrome, diagnosed in the dermatology, gynecology y genetics department of the Civil Hospital of Tepic, Nayarit, México. Bibliography. 1.Goltz,RW. et al. A Review of the Literature and Report of Two Cases. Arch.Dermatol.1970;101:1-11. 2.H. Tanaka, et al. The Goltz syndrome associated with giant cell tumour of bone A case report. International Orthopaedics 1990;14:179-181. 3.SM Maas, et al. Phenotype and genotype in 17 patients with Goltz-Gorlin Syndrome. J Med Genet 2009;46:716-720. 4.Pastorino L, et al. Hum Mutat. 2005;25:322-3.

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A Silver-Russell syndrome patient with 22q13.3 deletion and H19-DMR epimutation. *E. Nishi[1], S. Mizuno[1], K. Yamzawa[2], T. Ogata[2].* 1) Department of Pediatrics, Central Hospital, Aichi Human Service Center, Kasugai, Japan; 2) Department of Molecular Endocrinology, National Research Institute for Child Health and Development, Tokyo, Japan.

Introduction 22q13.3 deletion syndrome (MIM606232) is characterized by overgrowth, muscle hypotonia, severe speech retardation, and autism-like behavioral features, whereas Silver-Russell syndrome (SRS, MIM180860) is characterized by pre- and postnatal growth retardation, relative macrocephaly, muscle hypotonia, difficulty in suckling, and body asymmetry. Here we report a girl with SRS who has chromosome 22q deletion and H19-DMR epimutation. Patient This Japanese female patient was conceived naturally without any remarkable family history. In 34 weeks of gestation, asymmetrical IUGR was indicated. She was born at 37 weeks of gestation with a height of 40.5 cm (-3.8SD), a weight 1,342 g (-3.8SD), and a cephalic circumference 32.0 cm (-0.5SD). Relative macrocephaly, triangular face, short clinodactyly of the bilateral fifth fingers, muscle hypotonia, right hydronephrosis, and leg length discrepancy were observed at birth. Tube feeding was required during the neonatal period. At 2-year-and-4-month-old, her height is 77.5 cm (-3.0SD), weight 6.03 kg (-4.3SD), and cephalic circumference 45.8 cm (-1.0SD). Both cervical and sitting positions remain incomplete, and she crawls on her back. Severe speech delay has been noted, and severe psychomotor retardation is present. G-banding stain showed 46,XX,del(22)(q13.3q13.3). Subtelomere FISH analysis did not identify any chromosomal disturbances including 22q. A CGH array (Agilent, 244 k) test showed a decrease in the copy number of 22q13.2 to q13.33, suggesting that intra-arm deletion at approximately 7 Mb involving SHANK3. In addition, she was diagnosed with SRS based on the clinical symptoms. Methylation analysis revealed H19-DMR hypomethylation, whereas maternal uniparental disomy for chromosome 7 was not identified. Discussion To our knowledge, no case of SRS with 22q13.3 deletion has reported thus far. Although 22q13.3 deletion and H19-DMR epimutation appear to develop independently, a relationship between these two events remains to be elucidated. In our case, symptoms characteristic of SRS were marked at birth, and subsequently severe language development retardation characteristic of 22q13.3 deletion syndrome has become apparent since 2 years of age. In view of the fact that the site of deletion involves SHANK3 gene which is associated with autism spectrum disorders, further developmental follow-up is required.

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Craniotelencephalic Dysplasia. A rare and unusual form of Dysplasia of Frontal Bone. *G. Scarselli[1], L. Di Medio[2], E. Gambineri[1], N. Dayan[2], D. Nannini[2], M.L. Giovannucci Uzielli[1, 2].* 1) Dept Pediatrics, Univ Florence, Firenze, Firenze, Italy; 2) Genetic Science, Florence, Italy.

Craniotelencephalic Dysplasia (OMIM 218670) was firstly described in 1958 by Daum, LeBeau and Minuit in an infant with protrusion of the frontal bone, craniosynostosis, encephalocele, and mental retardation. A second patient with a similar appearance, but no encephalocele, was described in 1964 by Jabbour and Taybi, and the term craniotelencephalic dysplasia was suggested. In the literature we only found three other reports, by G.R. Hogan (1969), Holmes et al (1972), and Gorlin et al. (2001). All described patients were newborns. Here we report a new patient observed since the birth and periodically studied by a 17 years multidisciplinary follow-up. At birth, this patient, a female, showed protuberance of frontal bone, and premature closure of sagittal, coronal, and metopic sutures, corrected by multiple surgery operations. Staturo-ponderal and psychomotor development were quite normal. Of special interest are now the dental problems with progressive, abnormal, placement, shape, and size of teeth. The intellectual development is within the average, but the girl expresses, especially in the last two years, psychosocial discomforts, in spite of the very good results of multiple aesthetic surgery reconstructions. No genes were until now associated with this disorder. A suggestion to the mutational analysis of EFNB1 in our patient, originates from the observation of vertical splitting and striping of the nail of first toe: this clinical aspect is typically described in Craniofrontonasal dysplasia (CFNS), a well known genetic disorder caused in the majority of patients by mutations of EFNB1 gene. The mutational analysis of this gene, often deleted, as part of contiguous gene deletions is in progress. High resolution cytogenetic analysis of G-banded metaphases (550-750 bands) from peripheral blood lymphocytes revealed a normal 46,XX karyotype.

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Follow-up and Management of Young-Simpson Syndrome. *K. Enomoto[1], N. Furuya[1], M. Adachi[2], S. Mizuno[3], Y. Yamanouchi[4], M. Masuno[4], T. Kondoh[5], K. Kurosawa[1].* 1) Division of Medical Genetics, Kanagawa Children's Medical Center, Yokohama, Japan; 2) Division of Endocrinology and Metabolism, Kanagawa Children's Medical Center, Yokohama, Japan; 3) Department of Pediatrics, Aichi Prefectural Colony Central Hospital, Kasugai, Aichi, Japan; 4) Kawasaki University of Medical Welfare, Okayama, Japan; 5) Institute for The Handicapped, Mutsumi Home, Misakaeno-sono, Nagasaki, Japan.

Young-Simpson syndrome (YSS) is a rare congenital multi-system disorder, characterized by hypothyroidism, facial dysmorphic features including blepharophimosis, congenital heart defects, and mental retardation. Following the first report by Young and Simpson [1987], many cases with similar symptoms have been reported. Although there is a case with parental consanguinity [Bonthron et al., 1993], suggesting autosomal recessive (AR) inheritance, almost all the cases with YSS are sporadic. In addition, there are few distinct chromosomal aberrations associated with YSS. To date, the underlying genetic defect remains unresolved. The clinical classification or diagnostic criteria of this rare condition is also unsettled. To refine the long-term development and phenotype in YSS, we report clinical findings in 5 individuals (4 males and a female) presenting just typical features of YSS. They are ranging in age 2 months to 20 years. All of them present hypothyroidism requiring levothyroxine replacement and severe mental retardation, especially speech delay. All 4 male patients present bilateral cryptorchidism and micropenis. Ptosis and blepharophimosis were obvious in the neonatal period, so that parents were not able to confirm their babies' eyes open at all. Hypotonia and feeding difficulty in early infancy are common features. Skeletal complications including contracture, dislocation, and deformation of lower limbs are common to our cases. 18-year-old male patient has been requiring several operations for ankle joint deformations and femoral head osteonecrosis. As information about orthopedic complications and problems during the neonatal period of YSS is so limited, the long-time follow-up and the management are important for health and quality of life (QOL) in YSS. These observations support the idea that YSS is a distinctive entity and a recognizable disorder of newborn.

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Ectodermal Dysplasia, Vertebral Anomaly, Hirschsprung Disease, Growth and Mental Retardation: A Clinical Report of a Boy with BRESEK Syndrome. *S. Mizuno[1], M. Oshiro[2], M. Seishima[3], N. Okamoto[4], Y. Makita[5], N. Wakamatsu[1].* 1) Dept Pediatrics, Central Hosp, Aichi Human Service Ctr, Kasugai, Japan; 2) Dept Pediatrics, Ogaki Municipal Hosp, Ogaki, Japan; 3) Dept Dermatology, Ogaki Municipal Hosp, Ogaki, Japan; 4) Dept Clinical Genetics,Osaka Medical Ctr and Research Institute for Maternal and Child Health, Izumi, Japan; 5) Education Center, Asahikawa Medical College, Asahikawa, Japan.

We present the case of a boy with multiple anomalies, including Hirschsprung disease and specific features of the skin, vertebrae anomaly and mental/growth retardation. The patient's gestalt and combination of multiple malformation are rather similar to previously reported BRESEK syndrome. We report this congenital malformation complex as an established syndrome. The patient was born to healthy parents without consanguinity. The pregnancy was complicated with mild oligohydramnios. He was born at 38 weeks and weighed 1996g. He exhibited generalized alopecia with absence of scalp hair, eyebrows, and eye rashes. He had malformed large ears and an inferiorly curved penis and bifid scrotum. The testicles was not palpated. He experienced persistent constipation, and Hirschsprung disease was confirmed by rectal biopsy. Congenital scoliosis in this patient was attributable to an hemivertebrae. Brain imaging revealed dysmorphology of the gyrus in the fronatal and parietal lobes. Right kidney of the patient was small. The entire skin constantly desquamated and erythematous. Skin biopsy revealed a reduced number of hair follicles and units and excessive keratinization. The patient exhibited a moderate to severe delay in psychomotor development. Intractable epilepsy persited in his infancy. Genetic analysis: G-banded chromosome analysis and genome-wide subtelomere FISH analysis did not show any chromosomal rearrangements. Mutation analysis of *ZEB2*, causative gene for Mowat-Wilson Syndrome, was performed on genomic DNA from the patient and no mutation was detected. No copy number changes were found in the genome of the patient using array CGH analysis. Discussion: Our present case is assumed to be identical to the second case by Reish. Reish et al. used the acronym BRESEK /BRESHECK to denote the following findings in their patients: brain anomalies, retardation, ectodermal dysplasia, skeletal deformities, Hirschsprung disease, ear/eye anomalies, cleft palate/cryptorchidism, and kidney dysplasia/hypoplasia. Although this combination is specific, only 3 articles concerning this syndrome could be retrieved in PubMed search. Simultaneous occurrences of Hirschsprung disease, Vertebrae anomaly, Ectodermal dysplasia, Retardation of Growth and Mentality are the main features for this syndrome. The standard CGH-array analysis did not reveal any changes in the copy number within the genome of the patient. Further case reports will help in delineating this disorder.

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Associated malformations in cases with neural tube defects. *C. Stoll, B. Dott, Y. Alembik, M-P. Roth.* Faculte de Medecine, Strasbourg, France.

Infants with neural tube defects (NTDs) may have other associated congenital defects. The reported incidence and the types of associated malformations vary between different studies. The purpose of this investigation was to assess the prevalence of associated malformations in a geographically defined population. The prevalences at birth of associated malformations in infants with NTDs were collected between 1979 and 2008 on all infants born in the area covered by the registry of congenital anomalies of Northeastern France in 402,532 consecutive births. Of the 441 infants with NTDs born during this period, representing a prevalence at birth of 10.9 per 10,000, 20.4 % had associated malformations. The associated cases were divided into recognized syndromes, chromosomal: 11 cases (2.5%) and non chromosomal: 23 cases (5.2%), and non recognized syndromes, multiply malformed: 55 cases (12.5%). Associated malformations were more frequent in infants who had encephalocele (36.8 %) than in infants with anencephaly (11.5 %) or infants with spina bifida (23.8 %). Malformations in the face (oral clefts), in the musculoskeletal system, in the renal system, and in the cardiovascular system were the most common other anomalies. In conclusion the overall prevalence of malformations, which was one in five infants, emphasizes the need for a thorough investigation of infants with NTDs. A routine screening for other malformations especially facial clefts, musculoskeletal, renal and cardiac anomalies may need to be considered in infants with NTDs, and genetic counseling seems warranted in most of these complicated cases.

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Replication of genome wide association findings for Cleft Lip and Palate a role for variants in MAFB, VAX1 and PAX7 in Asian populations. *S. Suzuki[1,2,3], ML. Marazita[4,5], ME. Cooper[4], E. Dragan[1], A. Butali[1], M. Mansila[1], N. Nagato[2], Y. Suzuki[2], T. Niimi[2], M. Yamamoto[3], G. Ayanga[8], T. Erkhembaatar[8], H. Furukawa[2], K. Minami[2], H. Imura[2], K. Fujiwara[2], J. L'Heureux[1], K. Durda[1], AC. Lidral[1], JC. Murray[1,6,7].* 1) Dept of Pediatrics, Univ Iowa, Iowa City, IA; 2) Division of Research and Treatment for Oral and Maxillofacial Congenital Anomalies, School of Dentistry, Aichi-Gakuin University, 2-11 Suemori-Dori, Chikusa-Ku, Nagoya 464-8651, Japan; 3) Faculty of Psychological and Physical Science, Aichi-Gakuin University; 4) Center for Craniofacial and Dental Genetics, and Dept of Oral Biology, and Dept of Human Genetics, Univ of Pittsburgh, Pittsburgh, PA; 5) Dept of Psychiatry, Univ of Pittsburgh, Pittsburgh, PA; 6) Dept of Pediatric Dentistry, Epidemiology and Biology, univ of Iowa, Iowa City, IA; 7) College of Nursing, University of Iowa, Iowa City, IA; 8) Maternal and Children's Health Research Center Hospital, Ulaanbaatar, Mongolia.

Nonsyndromic cases of cleft lip with or without cleft palate (NSCL/P) and cleft palate only (NSCPO) are among the most common human birth defects caused by both genetic and environmental factors and/or by their cross talks. Recent genome-wide association studies discovered multiple loci such as MAFB, VAX1 and PAX7 associated with NSCL/P. In order to replicate the association of these genes in other populations, we used independent case-parents samples from Mongolia (204 case-parents trios) and Japan (98 case-parents trios). We genotyped single nucleotide polymorphisms (SNPs) in or near VAX1, MAFB and PAX7 using TaqMan assays which were performed used in GWAS study by Beaty, T. et al. (2010). Transmission disequilibrium tests were performed using the Family Based Association Test and PLINK programs. In Mongolian trios, potential candidate gene makers showed significant association (VAX1, rs7078160, p=5.32E-05, and MAFB, rs13041247, p=0.00108). In Japanese trios, one marker showed significant association (VAX1, rs 7078160, p=0.00511). This study suggests that VAX1 and MAFB are either directly or indirectly involved in the etiology of NSCL/P in Asian populations. This replication study further supports the role of common causative genes among different populations.

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An array-based approach to detect copy number changes in *SHOX* and other stature-associated genes in children with idiopathic short stature. *J. Keefe[1], P. Crotwell[2], S. Kirmani[1], G. Velagaleti[3], A. Lteif[1], L. Davis-Keppen[2], A. Karmazin[2], E. Thorland[1].* 1) Mayo Clinic, Rochester, MN; 2) Sanford Clinic USD Genetics Laboratory, Sanford Children's Hospital, Sioux Falls, SD; 3) Pathology Department, University of Texas Health Sciences Center, San Antonio, TX.

Idiopathic short stature (ISS) is defined as apparently isolated short stature with no other obvious clinical signs. Short stature is a developmental, multifactorial condition with a strong genetic background involving several genes. One such gene is the short-stature homeobox containing gene (*SHOX*) which maps to the pseudoautosomal region on chromosome Xp. The true frequency of *SHOX* gene mutations, including deletions of the coding region and deletions of upstream and downstream coding elements, in ISS is not known. It was our aim to utilize array comparative genomic hybridization (aCGH) technology to create a 'short stature' chip targeting the *SHOX* gene and other pertinent stature-related genes to gain a better understanding of the frequency of copy number changes contributing to short stature in ISS patients. An Agilent custom 8x60k array was designed to interrogate the *SHOX* gene and its regulatory regions, the Xp/Yp pseudoautosomal region, and 108 other genes implicated in short stature with high-density probe coverage. Following IRB approval and informed consent, the blood samples of 200 ISS patients are being collected. These patient samples are being tested using the custom aCGH chip and relevant copy number changes will be analyzed. Currently, there is no consensus on which subset of patients with ISS should be offered genetic testing. This study will allow us to determine the frequency of copy number changes in patients with ISS and help determine an appropriate testing algorithm for the evaluation of these patients.

801/T

Building up a genetic approach of nonsyndromic orofacial clefts in Alagoas, Brazil. *M.I.B. Fontes[1], V.L. Gil-da-Silva-Lopes[2], K.M. Santos[1], J.I. Vieira-Filho[1], F.S. Anjos[1], I.L. Monlleo[1,3], Alagoas' Cleft Team.* 1) Departament of Pediatrics, State University of Alagoas, Maceio, Alagoas, Brazil; 2) Departament of Medical Genetics, University of Campinas, Campinas, Sao Paulo, Brazil; 3) Clinical Genetics Unit, University Hospital Federal University of Alagoas, Maceio, Alagoas, Brazil.

There are regional disparities in public healthcare for patients with orofacial clefts in Brazil. This situation is much worse in poorest states such as Alagoas. There is just one clinical genetics unit and clinical geneticist for 3 million of inhabitants in this region and none specialized centre for orofacial cleft care. This study profiles nonsyndromic orofacial clefts (NSOC) of patients from this area. From September to December 2009, 53 families with NSOC were preliminarily seen by clinical geneticists. Data were collected through a pre-tested clinical protocol. Fisher Test was used for statistics with p-value<0.05. Age ranged from 0-31 years, sexual ratio was 1:1. Cleft lip with or without palate amounted to 84.9% while cleft palate, 15.1%. Rate of parental consanguinity and familial recurrence were 7.5% and 28.3%, respectively. Mother's age at birth ranged from 14-42 years. Level of instruction of 75% of mothers ranged from illiterate to primary school. In 15% of the families proband was a single child. In the remaining cases, patient was the first child and sibling was significantly smaller. Results suggest that the birth of a child with NSOC might have played a rule in reproductive decisions of these families although they had never benefited from genetic evaluation and counseling. Except for sexual ratio, results on clinical and genetic characteristics corroborate the literature. These data would be useful for the planning of genetic care for patients with orofacial clefts in Alagoas. Financial Support: FAPEAL, FAPESP.

802/T

Hypophosphatemic rickets, two unrelated case reports and review of the literature. *G.H. Galicia Hernandez[1], V.M. Anguiano-Alvarez[1], I.J. Garcia-Gonzalez[1], J.F. Michel Monrroy[4], J. Fonseca Cárdenas[4], R. Minerva Martínez[5], N.O. Davalos[1,2,3].* 1) Biologia Molecular, Instituto de Genética Humana Dr. Enrique Corona, Guadalajara, Guadalajara, Mexico; 2) Doctorado de Genética Humana, CUCS, Universidad de Guadalajara; 3) Servicio de Genética, HRVGF ISSSTE Zapopan, Jalisco; 4) Ortopedia y traumatología, HRVGF ISSSTE Zapopan, Jalisco; 5) endocrinología,HRVGF ISSSTE Zapopan, Jalisco.

INTRODUCTION: Hypophospatemic rickets (HR) is characterized by osseus hypomineralization, secondary to the increased expression of phosphatonins , especially FGF23, which acts in the sodium and phosphate cotransporters of the proximal convoluted tubule, inducing phosphaturia. HR exhibits AD, AR or X-linked inheritance patterns. OBJECTIVE: To present two compatible HR case reports, its osseous complications and adverse medical events. Case report: We present two unrelated familial cases, HR compatible. Proposita 1: A 7.6-year-old girl with disproportional short stature, genu varum, gait difficulties, asymmetric thorax; actually presenting normocalcemia, normophosphatemia, phosphaturia, calcitriol deficiency and secondary hyperparathyroidism. Proposita 2: 15-year-old girl with surgical antecedents, the first one in order to correct bilateral tibia deformity at age 3, and genu varum correction surgery at age 5; actually presenting genu varum recidivation, normocalcemia, hypophosphatemia, and phosphaturia. CONCLUSIONS: Due to functional complications in HR, early diagnosis and treatment is necessary, based in growth curves as well as serum values of phosphorus, calcitriol, alkaline phosphatase, and others, in order to offer an optimal medical care and prevent secondary osseous deformities.

803/T

Patients with heterozygous deletion of *NRXN1*α present with variable phenotypes from mental retardation, developmental delay, seizures, cardiac conduction defects to apparently normal phenotype. *K. Hovanes[1], J. Kim[1], M. Gorre[1], N. Shur[2], C. Harini[2], S. Gunn[1], D. Niyazov[3].* 1) Dept Gen, CMDX CombiMatrix Diagnostics, Irvine, CA; 2) Rhode Island Hospital, Providence, RI; 3) Pediatrics, Ochsner Clinic Foundation, New Orleans, LA.

We present *NRXN1* gene deletions in two unrelated patients with developmental delay and in four members of another family with variable phenotypes. Our first patient, a 5-year-old male, was evaluated for global developmental delay and seizures. The onset of his seizures was at the age of 13 months with frequency of 20-40 seizures per day. His evaluation at 5 years of age revealed mixed receptive and expressive language delays with obsessive compulsive behavior and had repetitive and hand flapping activities. Our second patient was a 3-year-old male with language delay and autistic features. These two patients were evaluated by array comparative genomic hybridization (aCGH) which revealed an approximately 255 Kb deletion of *NRXN1* (*Neurexin 1*) gene on chromosome 2p16.3. Parental analyses for these patients are pending. Two brothers, age 23 and 20, from the third family were also found to have a similar deletion in *NRXN1*. The older brother had motor and language delays. He also had advanced second degree atrioventricular (AV) block. He has profound mental retardation due to anoxic brain injury that he suffered during a prolonged cardiac arrest. His younger brother had normal development but required speech therapy at age 5 and was diagnosed with ADHD. Their 16-year-old sister had normal development but required special education classes until the 5th grade due to learning disability and currently struggles with dyslexia. The younger brother and sister also have second degree AV block. Both brothers were tested by aCGH and found to have a *NRXN1* gene deletion similar to the one found in the two patients mentioned above. FISH analysis revealed the same deletion in the mother and the sister. However, the mother, who is a licensed professional nurse, denied any history of developmental delay or learning disabilities. The deletion seen in these three families encompasses the promoter and part of the coding sequence of the neurexin1α without affecting the neurexin1β transcript. *Neurexin 1* has been a candidate gene for schizophrenia and autism spectrum disorder. Recently, Zahir et. al. reported a *de novo* heterozygous deletion of 320 kb affecting neurexin1α in a patient with vertebral, cognitive and behavioral abnormalities. Here we report three additional families with similar deletions affecting the neurexin1α transcript suggesting that haploinsufficiency of this transcript presents with significant variable phenotype and incomplete penetrance.

804/T

AN EXERCISE IN DIAGNOSING PROTEUS SYNDROME. *R.M. Minillo, C. Mcheletti, M.A.P. Ramos, A.B.A. Perez, V.F.A. Meloni, M.C.P. Cernach, F.T. Lima, D. Brunoni.* Universidade Federal de São Paulo (UNIFESP), São Paulo, Brazil.

INTRODUCTION: Proteus syndrome (PS), (OMIM %176920), is a rare genetic disease with unknown etiology and highly variable expression. No intraexonic mutations were identified, indicating that neither PTEN nor GPC3 was likely to have a major role in the etiology of the disease (Thiffault et al., 2004). The diagnosis criteria must be applied carefully in order to its confirmation, classify patients at risk and allow molecular and evolution evaluation. OBJECTIVE: Analyze the difficulty and complexity in confirming the diagnosis of PS as well as its differential diagnosis. MATERIALS AND METHODS: Retrospective analysis by chart review of 30 patients of Centro de Genética Médica of Universidade Federal de São Paulo with initial diagnostic hypothesis of PS, trying to apply the diagnosis criteria properly and check the complementary exams ordered during the investigation. RESULTS: PS was confirmed in 6 patients (20%;). Six patients (20%;) presented only macrodactyly and 7 patients (23,3%), hemihypertrophy (isolated, with hemangiomas or macrodactyly), still without final diagnosis, being that 10 were younger than 1 year old and needed periodic revaluations because of its progressive course. The diagnosis of PS was discarded in 11 patients (36,7%), (1 with encephalocraniocutaneous lipomatosis, 2 with Klippel-Trennaunay syndrome, 1 with macrolipodistrophy, 2 with lipomatosis, 1 with linfangiomatosis, 1 with hemifacial hemihypertrophy, 1 with Mafucci syndrome, 1 with vascular malformation and 1 with epibulbar dermoid). The exams of each patient were tabbed and their contribution to diagnosis was analyzed. CONCLUSION: PS is often associated with missed diagnosis and applying the diagnosis criteria is not always easy. It is important to highlight the correct investigation and the importance of periodic revaluations of oligosymptomatic patients so that the proper differential diagnosis is made, which requires a real diagnosis exercise in many instances.

805/T

MOMO Syndrome with holoprosencephaly and cryptorchidism: Further dilineation of the new obesity syndrome. *S. Sharda, I. Panigrahi, R. Marwaha.* Pediatrics, Advanced Pediatric Centre,PGIMER, Chandigarh, India.

There are multiple genetic disorders with known or unknown etiology grouped under obesity syndromes. Inspite of having multisystem involvement and often having a characteristic presentation, the understanding of the genetic causes in majority of these syndromes are still lacking. The common obesity syndromes being Bardet-Biedl syndrome Prader-Willi syndrome, Alstrom syndrome ,Cohen syndrome, Albright's hereditary osteodystrophy ,Carpenter syndrome, Rubinstein-Taybi syndrome, Fragile X syndrome and Börjeson-Forssman-Lehman syndrome. The list is ever increasing with new syndromes being added on. A recent addition is MOMO syndrome, with about five such cases being reported in the literature. Expanding the spectrum of clinical features, we report the first case of MOMO syndrome from India. The hitherto undescribed association with the lobar variant of holoprosencepahaly and cryptorchidism and the rarity of the syndrome prompted us to document this case. Key words: Obesity syndrome, MOMO, cryptorchidism holoprosencephaly.

806/T

Genetic risk factors for neonatal hyperbilirubinemia in North Indian population. *P.K. Tiwari[1], A. Kumar[1,3], A. Bhutada[1], R. Agarwal[1], R. Raman[2,3].* 1) Department of Pediatrics, IMS, Banaras Hindu University, Varanasi, Uttar Pradesh, India; 2) Department of Zoology, Faculty of Science, Banaras Hindu University, Varanasi, Uttar Pradesh, India; 3) Centre for Genetic Disorders, Faculty of Science, Banaras Hindu University, Varanasi, Uttar Pradesh, India.

Introduction: Jaundice is a common problem in newborn babies in first few days of life. In severe, untreated cases, it leads to the risk of bilirubin encephalopathy or kernicterus. The cause of neonatal hyperbilirubinemia (NNH) is multifactorial. There is a wide variation in the prevalence of NNH in different ethnic groups, suggesting the influence of genetic factors. Genetic factors may influence bilirubin metabolism by increasing bilirubin production and/or diminished bilirubin conjugation. It has been established that variants of bilirubin metabolism controlling genes, viz, Uridinediphosphate glucuronosyl transferase1A1 (UGT1A1), Solute carrier organic anion transporter family, member 1B1 (SLCO1B1) and Glucose -6-phosphate dehydrogenase (G-6-PD) may predispose to NNH. Objective: To understand the contribution of genetic variants of UGT1A1, SLCO1B1and G-6-PD genes to the etiology of NNH. Methods: A prospective case-control study was conducted, which included 247 newborns as cases (total serum bilirubin ≥15 mg/dl) and 278 newborns as controls (total serum bilirubin < 15 mg/dl). Polymorphisms of above mentioned genes were genotyped by PCR-RFLP, SSCP and DNA sequencing. Statistical analysis has been done by using SPSS, version 16.0. Results and conclusions: In our study NNH was significantly associated with certain polymorphisms of UGT1A1 gene such as G211A variant (OR= 3.03, 95%CI=1.06-8.63), enhancer region polymorphism -G3279T (OR=2.5, 95%CI=1.5-4.3) and TATA box polymorphism (TA)6®(TA)7 of promoter region (OR=1.5,95%CI=1.06-2.13).We did not observe any association between NNH and polymorphisms of CAT box, C686A, C1091T and T1456G. Similarly polymorphisms in coding region of SLCO1B1 gene did not appear to contribute to NNH as the occurrence of variants was comparable between the cases and controls. For G-6-PD gene Mediterranean mutation (C563T) was found in only 2 female (heterozygous) neonates with hyperbilirubinemia and 1 male (hemizygous) control neonate, suggesting infrequent occurrence of this mutation in this region of India. It is concluded that variants of UGT1A1 gene contribute significantly to NNH in Indian population.

807/T

The identification of SNPs associated with facial morphological traits in a genome-wide association study. *L. Paternoster[1,2], A.M Toma[3], C. Wilson[3], A.I. Zhurov[3], J. Kemp[2], G. Davey Smith[1,2], B. St Pourcain[2], G. McMahon[2], P. Deloukas[4], S.M. Ring[2], N. Timpson[1,2], S. Richmond[3], D.M. Evans[1,2].* 1) MRC CAiTE, University of Bristol, Bristol, United Kingdom; 2) Social Medicine, University of Bristol, Bristol, United Kingdom; 3) Department of Applied Clinical Research & Public Health, Cardiff University, Cardiff, United Kingdom; 4) Wellcome Trust Sanger Institute, Cambridge, CB10 1SA, United Kingdom.

Craniofacial morphology is highly heritable (with the easier to measure traits showing higher heritability) but very little is known about which individual genes are involved. We have carried out the first genome-wide association study (GWAS) of facial morphology to identify single nucleotide polymorphisms (SNPs) for facial characteristics.

Three-dimensional facial images of 2185 15 year-olds with genome-wide data from the Avon Longitudinal Study of Parents and Children (ALSPAC) were obtained using two high resolution Konica/Minolta laser scanners. The images were aligned and 21 key facial landmarks identified. The 3-D coordinates of these landmarks were used to derive linear distances and angular measurements. In addition, the lip morphology was categorized according to size, shape and features. We carried out genome-wide association analyses on these measures using imputed genome-wide data in MACH2QTL.

We have several exciting associations that meet $p<5x10^{-8}$, including SNPs near FAM44B, ISX, SIX2 and AQP4. The most promising of these was in a region on chromosome 2 that showed association with the nasion-mid-encanthion distance (the distance from the nasal root to the midpoint of the line made by the inner eye fissures). The top SNP in this region was rs7559271 ($p=2x10^{-10}$), which is in the PAX3 gene. This gene is known to play a role in Waardenburg syndrome and craniofacial-deafness-hand syndrome, both of which have distinct facial clinical features. We will now genotype this SNP (and others which reached $p<5x10^{-8}$) in a further 2500 ALSPAC 15 year-olds with facial data and DNA extracted and attempt to replicate these findings (results of this will be presented).

808/T

Novel association to the proprotein convertase *PCSK7* alongside *HFE* and *TMPRSS6* gene loci revealed by analysing soluble transferrin receptor (sTfR) levels. *J.S. Ried[2,17], K. Oexle[1,17], A. Hicks[3,17], T. Tanaka[4], C. Hayward[5], M. Bruegel[6], M. Gögele[3], P. Lichtner[7], B. Müller-Myhsok[8], A. Döring[2], T. Illig[2], C. Schwienbacher[3,9], C. Minelli[3], I. Pichler[3], G.M. Fiedler[6], J. Thiery[6], I. Rudan[10,11], L. Ferrucci[4], S. Bandinelli[12], P.P. Pramstaller[3,13,14], H.-E. Wichmann[2,15], C. Gieger[2], J. Winkelmann[1,16], T. Meitinger[1,7].* 1) Institute of Human Genetics, MRI, Technische Universität München, Munich, Germany; 2) Institute of Epidemiology, Helmholtz Zentrum München, Neuherberg, Germany; 3) Institute of Genetic Medicine, European Academy Bozen/Bolzano (EURAC), Bolzano, Italy, affiliated institute of the University of Lübeck, Lübeck, Germany; 4) NIA at Harbor Hospital, Baltimore, MD, USA; 5) MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine, Edinburgh, United Kingdom; 6) Institute of Laboratory Medicine, Universitätsklinikum Leipzig, Leipzig, Germany; 7) Institute of Human Genetics, Helmholtz Zentrum München, Neuherberg, Germany; 8) Max Planck Institute of Psychiatry, Munich, Germany; 9) Department of Experimental and Diagnostic Medicine, University of Ferrara, Ferrara, Italy; 10) Croatian Centre for Global Health, University of Split Medical School, Split, Croatia; 11) Centre for Population Health Sciences, The University of Edinburgh Medical School, Edinburgh, United Kingdom; 12) Geriatric Unit, Azienda Sanitaria Firenze (ASF), Florence, Italy; 13) Department of Neurology, General Central Hospital, Bolzano, Italy; 14) Department of Neurology, University of Lübeck, Lübeck, Germany; 15) Institute of Medical Informatics, Biometry and Epidemiology, Ludwig-Maximilians-Universität and Klinikum Großhadern, Munich, Germany; 16) Department of Neurology, MRI, Technische Universität München, Munich, Germany; 17) these authors contributed equally to the paper.

Serum iron parameters indicate the availability of and the demand for iron. This is the first meta-analysis of genome-wide association studies (GWAs) on soluble transferrin receptor (sTfR). This parameter is regarded as a more reliable index of iron deficiency in anemia, compared to other serum iron parameter such as ferritin which may be disproportionately influenced by inflammation or neoplasia. The meta-analysis of five GWAs on sTfR and ferritin serum levels revealed a novel association to the *PCSK7* locus on chromosome 11. Additionally, two genes, *TMPRSS6* and *HFE*, which are known to be associated with iron parameters such as free iron, ferritin or transferrin, have also been found to be associated with sTfR. The association with the *PCSK7* locus was the most significant (rs236918, $p = 1.1 \times 10^{-27}$) suggesting that proprotein convertase 7, the gene product of *PCSK7*, is involved in sTfR generation and/or iron homeostasis.

809/T

Replication study of the recently identified rheumatoid arthritis susceptible polymorphisms: a case-control study. *T. Suzuki[1,2], K. Ikari[1], Y. Toyama[2], A. Taniguchi[1], H. Yamanaka[1], S. Momohara[1].* 1) Inst Rheumatology, Tokyo Women's Med Univ, Tokyo, Japan; 2) Dept of Orthopedic Surgery, Keio Univ, Tokyo, Japan.

Background. Recently a large-scaled meta-analysis of genome-wide association studies (GWAS) and replication studies identified novel rheumatoid arthritis (RA) risk loci in European descent populations; SPRED2 (rs934734), ANKRD55 (rs6859219), C5orf30 (rs26232) (1). The aim of this study was to validate the association between these reported loci and susceptibility to RA in a Japanese population.

Methods. The present study is part of a RA cohort project with an enrollment of nearly 5000 patients established by the Institute of Rheumatology, Tokyo Women's Medical University (IORRA). All the RA patients fulfilled the American College of Rheumatology 1987 revised criteria for RA. DNA samples of 1504 RA patients were obtained from IORRA study and 752 population-based control DNA samples were obtained from the DNA collection of Health Science Research Resources Bank (Osaka, Japan), which was entrusted by Pharma SNP consortium (Tokyo, Japan). Tested SNPs on the genes were selected based on the published studies. Genotyping was performed using the TaqMan assay according to the manufacture's instructions (Applied Biosystems, Japan). The Chi-square test was used for the case-control study.

Results. We failed to genotype rs6859219 using the TaqMan assay. No significant differences were observed in allele frequencies of the two SNPs between cases and controls (rs934734 [P=0.86, OR=1.01, 95%CI=0.84-1.16], rs26232 [P=0.50, OR=1.05, 95%CI=0.83-1.10]. Our data could not support the evidence of association between these SNPs and RA.

Conclusion. Although strong association was found in European descents, these reported susceptibility genes analyzed here are not associated with RA susceptibility in a Japanese population. The result suggests that the SNPs may be associated with RA susceptibility in a specific ethnic group.

Reference.

1. Genome-wide association study meta-analysis identifies seven new rheumatoid arthritis risk loci. Nat Genet. (42):508-14.

810/T

Double methylation disorder: Beckwith-Wiedemann and Angelman syndromes in two patients. *L. Randolph[1], D. Wong[1], J. Waterson[2], J. Schoof[3], J. Johnson[3].* 1) Div Med Gen, Childrens Hosp, Los Angeles, Los Angeles, CA; 2) Children's Hospital Oakland, Oakland, CA; 3) Medical Genetics, Shodair Hospital, Helena, MT.

The concept of a multiple methylation disorder was recently proposed in both IVF and other pregnancies, (J Med Genet 2006;43:902-7). Our lab (JJ) analyzes both SNRPN and LIT1 methylation and found two patients with abnormal imprinting at both loci, but with different mechanisms Pt1 is a 3-y.o. F referred to genetics for DD and protruding tongue. She was born to a 31-y.o. G3 P2-3 woman with type I DM and preeclampsia after a naturally conceived pregnancy. Gestation was 34 wks, BW 4020 g and HC 34.5 cm, LGA with a normal head size. She had facial asymmetry, macroglossia, hand flapping and easy laughter. Methylation testing for BWS showed mosaic hypomethylation of LIT1, with a methylation index of .20. Uniparental disomy (UPD) studies for chromosome 11 were normal, indicating that she likely has a mosaic imprinting defect of LIT1. msPCR for SNRPN showed an MI of .08, consistent with Angelman syndrome (AS), similar to Southern blot results showing a faint methylated allele. UPD studies for chromosome 15 were normal. FISH for SNRPN has not yet been done. Pt2 was a 2 m.o. female infant seen by genetics at 2 d. age for suspected BWS. She was born at 30 6/7 wks to a 26-y.o. G1. Omphalocele and polyhydramnios were diagnosed prenatally. BW was 2.46 kg (>97th), length 49 cm (>97%) HC 29 cm (50%). She had hemihyperplasia and a globular pancreas. She died at two months of age. LIT1 msPCR showed an MI of .22, , and UPD 11 studies showed an average paternal/maternal allele ratio of 3.9, consistent with approximately 59% of cells being isodisomic for paternal alleles. Pt1, with signs of both syndromes, has a presumed mosaic imprinting defect of LIT1 resulting in BWS, and an almost complete lack of methylation of SNRPN, consistent with AS and a possible imprinting defect. The coincidence of the two syndromes, produced possibly by imprinting defects is unusual. More unusual is the same syndrome combination in Pt2, but produced by a different mechanism, mosaic UPD for two different paternal chromosomes, indicating a possible predisposition to post-zygotic non-disjunction. We report these patients to raise awareness of multiple imprinting syndromes in the same patient, with multiple mechanisms potentially responsible. We suggest that patients with atypical clinical presentations of BWS and other maternal hypomethylation disorders be tested for other methylation disorders.

811/T

The heritability of the common foot disorders, hallux valgus and pes cavus, in elder men and women: The Framingham Study. *Y. HSU[1], J. Jordan[2], LA. Cupples[3], DP. Kiel[4], M. Hannan[4].* 1) Molecular & Integrative Physiological Sciences Program, Harvard School Public Health, Boston, MA; 2) University of North Carolina at Chapel Hill, Chapel Hill, NC; 3) Dept. Biostatistics, School of Public Health, Boston University, Boston, MA; 4) Hebrew SeniorLife, Boston, MA.

Foot disorders, including hallux valgus, pes planus, pes cavus, lesser toe deformities, are common among adults, affecting approximately 20-60% of Americans and are increasingly linked to chronic mobility limitations and disability. Although genetic predisposition to foot disorders has been suspected, we are aware of only one study that reported family history of patients with hallux valgus, and this study concluded that this foot deformity may be inherited through an autosomal dominant transmission. To date, no linkage, candidate gene association, GWAS or heritability studies have been performed. Utilizing the pedigree structure in the Framingham Study, we estimated overall and gender-specific heritability of hallux valgus and pes cavus (our most common and least common foot disorders) by a standard quantitative genetic variance-components model implemented in the Sequential Oligogenic Linkage Analysis Routines (SOLAR) package. Foot disorders were assessed using the validated Foot Assessment Clinical Tool that captures the main features of common foot disorders. Briefly, trained clinical examiners conducted a physical examination of a participant's feet, collected data on medical history, plantar pressures using a computerized mat, and administered risk assessment questionnaires. The structural foot disorders were indicated as present or absent based on an atlas of pictorial depictions of each foot disorder. For example, hallux valgus was defined as the angular deviation of the hallux with respect to the first metatarsal bone toward the lesser toes at 15° or more, and appears as a medial bony enlargement of the first metatarsal head. The prevalence of Hallux Valgus was 31% in these participants (675 Hallux Valgus cases with available pedigree information). The overall heritability (h2) was 0.39 for women and 0.38 for men. For individuals < 60 years of age, the heritability was 0.89. The prevalence of pes cavus was only 7% (154 cases with available pedigree information). The heritability was 0.68 for women and 0.20 for men. For individuals < 60 years of age, the heritability was 0.99 for women and 0.63 for men. In summary, we found that hallux valgus and pes cavus are highly heritable, especially for younger adults. Genome-wide association analyses on three independent studies (including the Framingham Study) are planned to identify potential genetic determinants for these common foot disorders.

812/T

A genome-wide scan for quantitative trait loci that regulate canine skull shape. *J.J. Schoenebeck[1], A.M. Byers[1], J.W. Fondon III[2], L. Lin[3], A.R. Boyko[3], C.D. Bustamante[4], R.K. Wayne[5], E.A. Ostrander[1].* 1) Cancer Genetics Branch, National Human Genome Research Institute, Bethesda, MD; 2) Department of Biology, University of Texas at Arlington, Arlington, TX; 3) College of Veterinary Medicine and Department of Biological Statistics and Computational Biology, Cornell University, Ithaca, NY; 4) Department of Genetics, Stanford School of Medicine, Stanford, CA; 5) Department of Ecology and Evolutionary Biology, University of California, Los Angeles, CA.

An understanding of how gene products interact during development could lead to better diagnosis and treatment for individuals with craniosynostoses. However, the compendium of molecular machinery that dictates cranioskeletal morphology remains poorly defined. Dogs display tremendous intraspecific cranioskeletal variation, making them an appealing model for the identification of genetic regulators of skull shape. Using a microscribe digitizer, we collected coordinate data from 53 landmarks on 192 skulls of dogs and wild canids and analyzed skull shape variation using MorphoJ software (Klingenberg lab). Our data reveals three principal components (PCs) that cumulatively explain 68% of symmetrical variance seen across 61 dog breeds. The first PC explains snout length, a feature that differs prominently among brachycephalic (short-snouted) and dolichocephalic (long-snouted) breeds of dogs. Genome-wide scans using both breed averages for PC1 and binary status (case/control) reveal that regions on chromosomes 1, 5, and 26 are highly associated with snout length (best uncorrected quantitative associations: $P = 4.99 \times 10^{-43}$, 3.00×10^{-49}, and 1.65×10^{-46} respectively). PC2 explains variation, predominantly in the neurocranium and width of the snout, that distinguishes brachycephalic and molosser breeds from toy breeds. Quantitative associations using PC2 reveal a single, highly significant association on the X chromosome (best uncorrected SNP: $P = 2.28 \times 10^{-67}$). Together, our data begin to reveal how multiple genetic loci converge to generate the tremendous skull shape variation seen in domestic dogs. With the eventual identification of causal variants, we hope to relate our findings to human symmetric craniosynostoses.

813/T

A wide range of ophthalmologic anomalies are part of the broad holoprosencephaly spectrum. *D.E. Pineda Alvarez[1], B.D. Solomon[1], J.Z. Balog[1], D.W. Hadley[1], B.P. Brooks[2], E. Roessler[1], M. Muenke[1].* 1) Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD; 2) National Eye Institute, National Institutes of Health, Bethesda, MD.

Holoprosencephaly (HPE) is the most common disorder of the developing forebrain in humans, characterized by failure of cleavage of the cerebral hemispheres; additionally, it is accompanied by a spectrum of midline craniofacial anomalies such as cyclopia at the most severe end of the spectrum to mild hypotelorism, single central incisor, and flat nasal bridge, at the milder end. Besides obvious ocular anomalies easily apparent on physical exam, other eye findings have not been described formally within the HPE spectrum. One central cause of HPE is functional alterations of the Sonic Hedgehog signaling pathway. SHH is a well known morphogene that regulates several processes during organogenesis, including the forebrain, limbs, and eyes. For instance, SHH inhibits PAX6 in the midline in order to maintain the development of the optic cups in the lateral planes, and also regulates PAX2, an important molecule for normal eye development. Loss of PAX2 function causes cobolomatas and retinal anomalies in animal models. Thus, alterations in the SHH pathway are expected to cause alterations of the normal development of the eye. From our large cohort of over 1000 patients with HPE, we have evaluated ocular changes in patients carrying mutations or deletions in SHH (n = 203 patients), SIX3 (n = 141 patients), TGIF (n = 56), and ZIC2 (n=42). While cyclopia and extreme hypotelorism are relatively common in patients with severe HPE, other subtle findings were noted in patients with microform or mild HPE. Coloboma was reported in 4% (18/449) of our patients, with mutations representing all studied genes, 44.5% of patients with colobomas had no brain anomalies. Congenital ptosis was found in 1.3% (6/449) of the patients, most of whom had only subtle microforms of HPE (5/6). Except for a mother with microform HPE due to a ZIC2 mutation, only patients with SHH mutations had ptosis. Nine of 10 individuals (including patients with mutations in SHH, SIX3, TGIF, and ZIC2) with identified mutations in HPE-associated genes who were evaluated by an NIH ophthalmologic geneticist familiar with the condition were found to have subtle ocular anomalies. These anomalies include microcornea, microophthalmia, thinning of the cornea, subtle defects of the pigmentation of the retina, optic nerve cupping, and ocular position anomalies were noted. The results suggest that some subtle ocular anomalies could be used to predict HPE-associated mutations in otherwise unaffected individuals.

814/T

Crossed segment hemihyperplasia and constitutional epigenotypes. *M.I. Van Allen[1,2], G. Hulait[1,2], T. Oh[1,2], M.S. Peñaherrera[1], W.P. Robinson[1].* 1) Dept Med Gen, Univ British Columbia, Vancouver, BC, Canada; 2) Provincial Medical Genetics Programme B.C. Children's and Women's Health Centre Vancouver, B.C. Canada.

Crossed segment hemihyperplasia is an unusual finding in idiopathic hemihyperplasia (IH) and in Beckwith Wiedemann syndrome (BWS). Buek et al (2008) reported on the epigenotypes of 73 IH and 110 BWS patients, only one case (1/183) had crossed segment hemihyperplasia, suggesting that it is an infrequent finding. IH and BWS result from various genomic changes in molecular alterations of 11p15, including paternal uniparental disomy (UPD) and alterations of methylation of two imprinting centers at 11p15:ICR1(*H19*) and ICR2 (KCNQ10T1). We present two cases with crossed segment hemihyperplasia found to have constitutional mosaicism of epigenetic events. These rare cases shed light on the timing of mechanisms of genomic imprinting inducing parental-specific gene expression. ***Case (1)*** presented at 10 weeks old with crossed segment body asymmetry, Lt tongue hyperplasia, an ear tag and pit, and BWt 4220 gm. He had Lt face > Rt, Lt side tongue >> Rt, and limb segment measurements Rt upper arm > Lt, Rt forearm < Lt, Rt hand length < Lt. His leg length measurements were consistently Rt > Lt. The soft tissue circumferences were consistently Rt > Lt for all limb segments. There was no organomegaly. Molecular study results were consistent with mosaic paternal isodisomy for 11p14.3-pter in ~25-30% of cells and hypomethylation of ICR2. ***Case (2)*** presented at 7 months old with Rt tongue and face hyperplasia, occipital flamus nevus, and BWt 4128 gm. Limb segment lengths were variable with Lt forearm > Rt, Lt palm > Rt, Rt thigh > Lt, Rt lower leg > Lt. The circumferences varied with the Lt upper arm > Rt, Lt forearm < Rt, Lt thigh < Rt, Lt calf > Rt. There was no organomegaly. Molecular studies identified borderline low methylation at *H19/IGF2* ICR1 consistent with mosaicism for an imprinting mutation or mosaic UPD11p15.5 below our detection limits. Our cases have molecular changes typical of other cases of IH and BWS with unilateral hemihyperplasia, consistent with the same underlying mechanism, rather than another cause, for crossed segment hemihyperplasia. Epigenetic mechanisms play major gene-regulatory roles in development, differentiation and disease. The finding of crossed segment hemihyperplasia suggests that epigenetic events occurred prior to determination of Rt/Lt sidedness, most likely during secondary reprogramming and establishment of parental imprints in the pre- and post-implantation blastocyst.

815/T

Association analysis of the Class-II Division-2 (CIID2) malocclusion with *RUNX2*, *RUNX3*, *PAX9*, *MSX1* and *AXIN2*. *L.A. Morford[1, 2], T.J. Coles[2, 3], M.D. Wall[4], M.W. Morrison[4], D.W. Fardo[5], K.S. Kula[4], J.K. Hartsfield Jr.[2, 3, 6].* 1) Dept Oral Health Practice, Univ Kentucky College of Dentistry, Lexington, KY; 2) Hereditary Genomics Laboratory, Center for Oral Health Research, Univ Kentucky College of Dentistry, Lexington, KY; 3) Dept Oral Health Science, Division of Orthodontics, Univ Kentucky College of Dentistry, Lexington, KY; 4) Dept of Orthodontics and Oral Facial Genetics, Indiana Univ School of Dentistry, Indianapolis, IN; 5) Dept of Biostatistics, Univ Kentucky, College of Public Health, Lexington, KY; 6) Dept of Medical and Molecular Genetics, Indiana Univ School of Medicine, Indianapolis, IN.

Objectives: Development of the dental/skeletal Class II Division 2 (CIID2) malocclusion is a heritable trait. Multiple tooth anomalies, including hypodontia of maxillary incisors and/or third molars, coincide with the CIID2 phenotype at a higher frequency than observed with other dental/skeletal malocclusions. This study sought to determine whether five genes that influence incisor and third molar development, namely *RUNX2*, *RUNX3*, *PAX9*, *MSX1* and *AXIN2*, could influence the development of CIID2 in the presence or absence of hypodontia. Our null-hypothesis states there is no association of SNPs near or within these candidate genes and CIID2 (in the presence or absence of hypodontia). **Methods:** Both Indiana University and The University of Kentucky IRBs approved this study. Ninety-four Caucasian CIID2 subjects (31 with hypodontia) were compared to eighty-nine Caucasian non-CIID2 control subjects without hypodontia. Clinical exam, photographs, models, radiographs and saliva were collected and analyzed for all subjects. SNPs within or near the *RUNX2* (rs1406846, rs6930053), *RUNX3* (rs6672420), *PAX9* (rs8004560, rs1955734), *MSX1* (rs12532, rs3821949) and *AXIN2* (rs7591, rs2240308) genes were genotyped utilizing either Applied Biosystems Taqman ® or Roche SimpleProbe ® technology. A chi-square analysis was used to evaluate the potential association of CIID2 (in the presence and absence of hypodontia) and each SNP genotype. **Results:** All SNP alleles were in Hardy-Weinberg equilibrium. Assuming a co-dominant mode of inheritance, a marginal association was identified between CIID2 (in the presence and absence of hypodontia) and *RUNX2* rs6930053 ($p=0.09$) when compared to non-CIID2 controls. No association of rs6930053 was identified for CIID2 subjects with hypodontia of any permanent tooth or with hypodontia of one or more non-third molars, when compared to CIID2 subjects without hypodontia. **Conclusions:** The data suggest that *RUNX2* rs6930053 (or genetic loci in linkage-disequilibrium with *RUNX2*) plays a role in CIID2 development but not in the coincidental hypodontia. Studies are ongoing to expand the number of subjects being examined for SNPs from all five genes. **Supported by:** Indiana University Bixler Fund for Research in Genetics & Education, and the University of Kentucky E. Preston Hicks Endowed Chair.

816/T

Role of interleukin-28B polymorphisms in the treatment of Taiwanese patients with hepatitis C virus genotype 1 infection. *M.S. Wu[1], C.F. Haung[3], M.L. Yu[3], S.H. Juo[1,2].* 1) Department of Medical Genetics, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; 2) Department of Medical Research, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; 3) Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan.

Purpose: Interleukin 28B (IL28B) encodes interferon-λ3 on chromosome 19 and had been referred to be involved in the suppression of hepatitis C virus (HCV) replication. Several genome-wide studies had showed that single nucleotide polymorphisms (SNPs) at IL28B were related to the treatment response in HCV genotype 1 (HCV-1) patients. Our goal was to confirm whether SNPs at IL28B are associated with HCV-1 infection treatment response in a Taiwanese population. **Methods:** We enrolled a total of 473 HCV-1 Taiwanese treatment-naïve patients who received pegylated interferon plus ribavirin combination therapy in Kaohsiung Medical University Hospital (KMUH). Five tagging SNPs were selected. Genotyping was determined by using the Taqman 5 nuclease assay (Applied Biosystems). **Results:** Among the five candidate SNPs (rs8105790, rs8099917, rs4803219, rs8109886, and rs10853728) at the IL-28B, rs8099917, rs4803219 and rs8105790 were noted to be in very strong linkage disequilibrium with each other ($r2=0.94$-0.96). Sex, age, body weight, baseline HCV viral loads were adjusted in the statistical analysis. SNPs rs8105790 TT genotype, rs8099917 TT genotype, rs4803219 GG genotype, rs8109886 CC genotype and rs10853728 CC genotype were associated with a rapid virological response of Hepatitis C virus infection. (RVR, seronegativity of HCV RNA at treatment week 4, $p=0.00007$, $p=0.00008$, $p=0.00003$, $p=0.0002$, and $p=0.0247$ respectively) and also associated with an sustained virologic response of Hepatitis C virus infection. (SVR, seronegativity of HCV RNA throughout 24 weeks of post-treatment follow-up, $p=0.00001$, $p=0.000098$, 0.000005,0.0001,and 0.0044 respectively) **Conclusion:** SNPs at IL28 were associated with treatment responses in HCV-1 patients in Taiwanese.

817/T

Complex I deficiency in Persian multiple sclerosis patients. *S. Ardalan Khales[1], S. Ardalan Khales[1], M. Houshmand[2].* 1) Medical Genetic Laboratory of Special Medical Center, Tehran, Iran; 2) National Institute for Genetic Engineering and Biotechnology, Tehran, Iran.

Complex I deficiency in Persian multiple sclerosis patients S. Ardalan Khales1, S. Ardalan Khales1, M. Houshmand2 1)Medical Genetic Laboratory of Special Medical Center, Tehran, Iran 2)National Institute for Genetic Engineering and Biotechnology, Tehran, Iran Multiple sclerosis (MS) is an immunological inflammatory disease of the central nervous system. Until now, little attention has been paid to the contribution of mitochondrial respiratory chain enzyme activities to MS. We showed a biochemical defect in complex I activity may be involved in pathogenesis of MS and also point mutation occurred in mtDNA might be involved in pathogenesis of MS. So in this study we sequenced mitochondrial complex I subunit in MS patient (ND1-ND6). No pathogenic mutations were found in our patient's. We conclude that it may mitochondrial nuclear subunit of complex I gene play roll in MS patient. Keywords: Multiple sclerosis; Mitochondrial complex I; Mitochondrial DNA.

818/T

The association of VEGF C-634G polymorphism with diabetic retinopathy: a meta-analysis. *H. Chen[1,2], W. Chen[1], C. Pang[1], M. Zhang[1,2].* 1) Joint Shantou International Eye Center, STU/CUHK, Shantou, GUangdong, China; 2) Department of Ophthalmology and Visual Sciences, the Chinese University of Hong Kong, Hong Kong, China.

Purpose: To investigate the association of *vascular endothelial growth factor (VEGF)* gene polymorphism with diabetic retinopathy (DR). Methods: A meta-analysis was performed. Literatures reporting the association of *VEGF* C-634G polymorphism with DR was searched in PubMed and EMBASE. Allele and genotype frequencies of *VEGF* C-634G polymorphism were extracted. The statistical analysis was performed using Reviewer Manager comparing the allelic distributions between (1) any DR and control; (2) proliferative diabetic retinopathy (PDR) and diabetes without retinopathy (DC); (3) non proliferative diabetic retinopathy (NPDR) and DC; (4) PDR and NPDR. Results: Totally 9 reported studies were retrieved. There was statistical significant difference in the distributions of *VEGF* C-634G polymorphism between any DR and DC (OR = 1.13, 95% CI 1.01-1.26, P=0.04). The G allele is more frequent in NPDR than in DC (OR=1.61, 95% CI 1.23-2.10, P=0.0005). However, it is less frequent in PDR than in NPDR (OR= 0.76, 95% CI=0.57-1.01, P=0.06). There is no different of the G allele frequency in PDR and DC (OR=1.02, 95% CI 0.87-1.18, p=0.85). Conclusion: The *VEGF* C-634G polymorphism is more frequency in NPDR patients than in PDR and diabetes without retinopathy patients.

819/T

Neurocristopathies in DiGeorge Syndrome. *A. Myers[1], V. Schroeder[2], M. Dasouki[3].* 1) Department of Pediatrics, University of Nebraska-Creighton, Omaha, NE; 2) Department of Pediatrics, University of Kansas Medical Center, Kansas City, KS; 3) Departments of Pediatrics and Internal Medicine, University of Kansas Medical Center, Kansas City, KS.

DiGeorge Syndrome is a well-established multiple congenital anomalies syndrome usually caused by a 3-5Mb deletion within the 22q11.2 chromosomal region. Other phenotypes caused by this microdeletion include Velo-Cardial-Facial, Conotruncal Anomaly Face Syndrome, and Opitz G/BBB Syndrome. Deletions at 22q11.2 have been associated with craniofacial abnormalities, congenital heart disease, palatal abnormalities, hypoparathyroidism, and developmental delays. Labyrinthine malformation has been described only in one individual with DiGeorge syndrome. TBX1 (a gene within the 22q11.2 microdeletion region) is required for mouse inner ear morphogenesis and proper neural crest migration. In addition, Hirschprung disease has been reported in association with DiGeorge syndrome in a few patients, suggesting a locus for Hirschprung disease within the 22q11.2 region. Typically, Hirschprung disease is recognized as a polygenic and isolated neurocristopathy. However, it is frequently associated with congenital central hypoventilation syndrome (CCHS) caused by PHOX2B gene mutations. Recently, the PHOX2B gene was linked to Hirschprung disease in Chinese patients. Patient one (AO) presented with speech and developmental delays, PDA, and bilateral labyrinthine abnormalities. She was diagnosed with DiGeorge syndrome based on the presence of a 2.81 Mb deletion in the 22q11.2 region found on aCGH analysis. Patient two (LN) had congenital heart disease and polyhydramnios diagnosed prenatally. After birth, he was diagnosed with Tetralogy of Fallot, Hirschprung disease, and congenital central hypoventilation syndrome. His blood karyotype was normal while a deletion in 22q11.2 was found by FISH analysis. Sequencing of his PHOX2B gene showed an abnormally expanded polyalanine repeat. Both patients were diagnosed with DiGeorge syndrome and neurocristopathies. Given these clinical presentations and the known role of TBX1 in mouse inner ear development, the labyrinthine malformation appears to be explained by the 22q11.2 deletion. On the other hand, the PHOX2B mutation found in the second patient separates Hirschprung disease and CCHS from DiGeorge syndrome. While neurocristopathies cause a myriad of anomalies in patients with DiGeorge syndrome, the exact molecular basis of their pathogenesis needs to be delineated.

820/T

The Psychiatric and Neurocognitive Manifestations in 1q21.1 Deletion Syndrome. *E. Chow[1,2,3], A. Ho[1], T. Aidan[3], D. Skidmore[3], C. Morel[4].* 1) Clinical Genetics Service, Centre for Addiction & Mental Health, Toronto, Ontario, Canada; 2) Department of Psychiatry, University of Toronto, Toronto, Ontario, Canada; 3) IWK Health Centre, Halifax, Nova Scotia, Canada; 4) Fred A. Litwin Family Centre in Genetic Medicine, University Health Network / Mount Sinai Hospital, University of Toronto, Toronto, ON, Canada.

Background: The 1q21.1 Deletion Syndrome (1q21.1DS) is a recently identified microdeletion syndrome with a variable phenotype. It has an estimated prevalence of ~0.4% in the general population. Cognitive and psychiatric manifestations have been reported in this syndrome but few details are available. Method: We have ascertained two unrelated families with 1q21.1DS. A total of 7 individuals in these two families (5 adults and 2 children) have been identified with 1q21.1DS on microarray testing. We systematically assessed them for psychiatric and neurocognitive phenotypes. Results: High rates of psychiatric conditions were identified in the 7 affected individuals. Every affected individual, except the youngest who is only 6 years old, has suffered from at least one psychiatric disorder: 4 subjects were treated for attention deficit hyperactivity disorder, 4 suffered from a major depressive disorder, 2 had alcohol abuse, and one suffered from psychotic disorder not otherwise specified. No autism or autism spectrum disorder was found. Intellect ranged from normal to moderate intellectual disability. Median full-scale IQ was 65, median verbal IQ was 71, and median performance IQ was 74. Verbal IQ was lower than performance IQ in all subjects tested. One subject was diagnosed as having dyslexia in school and another subject had a Verbal-Performance IQ difference of 21 points in favor of performance IQ on testing. Verbal comprehension and reading skills were particular weaknesses, while perceptual organization, abstraction and mental flexibility were relative strengths. Conclusions: A high rate of psychiatric conditions is associated with 1q21.1DS. 1q21.1DS may also be associated with weaknesses in verbal abilities and a relatively lower verbal IQ. Early assessment and intervention for neuropsychiatric manifestations are recommended for individuals with 1q21.1DS.

821/T

Lipomatous cortical dysplasia with lipoma of the corpus callosum associated with an Xp deletion. *R. Regan[1], L. Baker[2], J. Conroy[1], V. Donoghue[2], M. Mullarkey[3], N. Shah[1], N. Murphy[2], S. Ennis[1], S.A. Lynch[3].* 1) School of Medicine and Medical Science, Univ College Dublin, Dublin 4, Ireland; 2) Temple Street Children's Hospital, Dublin 1, Ireland; 3) National Centre for Medical Genetics, Our Lady's Children's Hospital, Dublin 12, Ireland.

We report a 4 year old girl with a de novo 13.7Mb deletion on Xp who has lipomatous cortical dysplasia with lipoma of the corpus callosum. Antenatal ultrasound scans had initially revealed ventriculomegaly and a fetal magnetic resonance imaging (MRI) scan revealed a soft tissue mass in the region of the corpus callosum. Post-natal MRI confirmed the diagnosis of a lipomatous cortical dysplasia with agenesis of the corpus callosum. Karyotype analysis showed a deletion on the short arm of chromosome X. Chromosomal replication studies using a thymidine analogue (BrdU) and methylation analysis of DNA at the androgen insensitivity locus showed non-random X inactivation. Fine mapping studies carried out with the Illumina 1M SNP array confirmed the deletion spanned Xp22.31 to Xp22.12. Cytogenetic abnormalities have not previously been described with this malformation, although this may be attributed to selection bias. At four years of age, the girl remains clinically well with no evidence of seizure activity or headache. She has normal growth.

822/T

Phenotypic manifestations of copy number variation in chromosome 16p13.11. *S.C Sreenath Nagamani[1], A. Erez[1], P. Bader[2], S.L Lalani[1], D.A Scott[1], F. Scaglia[1], S.E. Plon[3], C.H. Tsai[4], T. Reimschisel[5], E. Roeder[6], A.D. Malphrus[7], P.A. Eng[1], P.M. Hixson[1], S.L. Kang[1], P. Stankiewicz[1], A. Patel[1], S.W Cheung[1].* 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA; 2) Parkview Hospitals, Fort Wayne, IN, USA; 3) Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA; 4) Division of Clinical Genetics, Children's Hospital, Aurora, CO, USA; 5) Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, USA; 6) Department of Pediatrics, University of Texas Health Science Center, San Antonio, TX, USA; 7) Division of Pediatric Neurology, Baylor College of Medicine, Houston, TX.

The widespread clinical utilization of array comparative genome hybridization, has led to the unraveling of many new copy number variations (CNV). While some of these CNVs are clearly pathogenic, the phenotypic consequences of others, such as those in 16p13.11 remain unclear. Whereas deletions of 16p13.11 have been associated with multiple congenital anomalies, the relevance of duplications of the region is still being debated. We report detailed clinical and molecular characterization of ten patients with duplication and four patients with deletion of 16p13.11. We found that patients with duplication of the region have varied clinical features including behavioral abnormalities, cognitive impairment, congenital heart defects and skeletal manifestations such as hypermobility, craniosynostosis and polydactyly. These features were incompletely penetrant. Patients with deletion of the region presented with microcephaly, developmental delay and behavioral abnormalities as previously described. One patient with deletion presented with Wilms' tumor. The CNVs were of varying sizes and were likely mediated by non-allelic homologous recombination between low copy repeats. Our findings expand the repertoire of clinical features observed in patients with CNV in 16p13.11 and strengthen the hypothesis that this is a dosage sensitive region with clinical relevance.

823/T

A novel familial 11p15.4 microduplication associated with developmental delay and hypercalcemia, likely secondary to an increased copy number of the OVHC2 gene involved in calcium ion binding. *N. Shur[1], D. Abuelo[1], S. Gunn[2], N. Golova[1], C. Harini[1], J. Quintos[1], E. Sofos[3], K. Hovanes[2].* 1) Rhode Island Hospital, Providence, RI; 2) Combimatrix Diagnostics, Irvine, CA; 3) Alpert Medical School at Brown University, Providence, RI.

We evaluated a seven-year-old boy with intellectual disability and seizures in genetics clinic. He was the six pound product of an uncomplicated term pregnancy born via repeat C-section to a 30 year-old mother and 24-year-old father. He appeared healthy until three months of age, when he was hospitalized for pneumonia. Soon after, developmental delays were noted. He walked at age 2, said his first words at around 3, and spoke in sentences at age six. Recently, he was hospitalized at a psychiatric institution for aggressive behavior. Family history was positive for significant medical problems in his father and siblings. The father and two younger brothers (age 4 and 6) all had developmental delay and familial hypocalciuric hypercalcemia (discovered through screening because of that diagnosis in the paternal grandfather). The 6-year-old also had an atrial septal defect, a seizure disorder, hernias, and kidney stones. An 8-year-old brother who did not have hypercalcemia had mild learning problems. Our patient's mother had learning disabilities and seizures. On physical examination, his weight and height were greater than the 95th percentile, and his head circumference was 52.5 cm (90th percentile). He was large for age and had a round face. Dysmorphic features included high arched eyebrows with synophorus, large posteriorally rotated ears, a long smooth philtrum, an overbite, and high palate. Calcium level was normal. Oligonucleotide array comparative genomic hybridization (aCGH) revealed a single copy number gain of approximately 2.3 Mb in the 11p15.4 region. His mother's aCGH result was negative for this duplication. The father and 8-year-old brother have not yet been tested. His six-year-old brother carried the same 11p15.4 duplication. The duplicated region seen in these two brother's genome spans 31 genes (6,904,229-9,243,447) and includes the ovochymase 2 (OVCH2) gene involved in calcium binding, which could explain the hypercalcemia in some family members with the duplication. A literature review showed only one other case of a patient with an overlapping, albeit larger, duplication. He had pharmacoresistant epilepsy, profound intellectual disability, and dysmorphisms. Our case represents a novel familial microduplication syndrome of chromosome 11p15.4 with variable expression of features that include developmental delay, dysmorphic features, and hypercalcemia.

824/T

Developing neurocognitive profiles of patients with recently reported CGH abnormalities. *G.C. Gowans, P.L. Brock, K.E. Jackson, S.D. Tomchek, L.L. Sears, E.R. Markham, A.M. Allard, Y.S Senturias, R.H. McChane, T.L. Erwin, K.M. Goodin, A. Asamoah, J.H. Hersh, P.G. Williams.* WCEC, Pediatrics, Univ Louisville, Louisville, KY.

Behavioral and developmental phenotypes are well recognized for a variety of genetic conditions, e.g. Down syndrome, Fragile X syndrome, Williams syndrome, Turner syndrome and 22q11.2 deletion syndrome. Comparative genomic hybridization (CGH) analysis with increasing resolution has resulted in detection of much smaller chromosome duplications and deletions. Physical and medical manifestations of some of these newly described CGH anomalies are beginning to emerge. We describe cognitive and behavioral assessments of selected CGH anomalies that we are developing through a multidisciplinary approach to patient evaluation that include not only geneticists and genetic counselors, but developmental pediatricians, child psychologists, child psychiatrists, speech therapists, occupational therapists and physical therapists. Our research at the Weisskopf Child Evaluation Center is currently focused on patients with the chromosome 1q21.1 deletion and duplication syndromes. However, we plan to similarly assess other conditions beginning with the chromosome 22q11.2 duplication syndrome.

825/T

Growth in Chilean infants with chromosome 22q11 microdeletion syndrome. *G.M. Repetto, M.L. Guzman, A.R. Puga, J.A. Justiniano.* Human Genetics, Clin Alemana- Univ Desarrollo, Santiago, Chile.

Chromosome 22q11.2 deletion syndrome (del22q11) is the most common known microdeletion syndrome, with an estimated frequency of 1/4000 to 1/6000. Though most patients share a deletion of similar size and location, the phenotypic variability among them is substantial. Growth failure is frequently described in patients with del22q11, and it is unclear whether this is an intrinsic manifestation of the syndrome or it is secondary to other medical problems, such as the presence of congenital heart disease (CHD) or endocrine deficiencies. We evaluated growth during infancy (newborn to 3 years of age), measured as weight and height, in a group of Chilean patients with del22q11. Data was collected through review of medical records, and compared with World Health Organization (WHO) growth curves, routinely used in the country for growth assessment in the pediatric population. In addition, we compared the findings between patients with and without CHD. Longitudinal measurements during infancy were obtained from 139 patients. Weight for age was below the 10th percentile of WHO curves in 86 of them (62%), and height for age was below the 10th percentile in 82 patients (59%). Only 2 patients had height/age above the 90th percentile, and the majority of remaining patients had measurements between the 10th and the 50th percentiles. Fifty two patients (37.4%) had CHD, all of them surgically repaired. We found no evidence of statistically significant differences in height or weight for age when comparing patients with or without CHD (p value of 0.07, Fisher's exact test). None of the patients had hypothyroidism or evidence of growth hormone deficiency. Although potentially biased due to its retrospective nature and lack of standarized measurements, our results show that the majority of Chilean patients with del22q11 in this study have growth in height and weights below the 10th percentile, at least during the first 3 years of life. Additionally, there was no evidence of association between growth pattern and presence of CHD or hormone deficiencies, suggesting that the findings may be due to direct effects of the deletion. Funded by Fondecyt-Chile grant #1100131.

826/T

Deletion of *MAP2K2*: A possible novel mechanism for RASopathies? *M. Nowaczyk[1], S. Zeesman[1], K.A. Rauen[2].* 1) Dept Pathology & Pediatrics, McMaster Univ, Hamilton, ON, Canada; 2) 3Dept. of Pediatrics, Division of Medical Genetics, University of California, San Francisco, San Francisco, CA.

The Ras/mitogen-activated protein kinase (MAPK) pathway is essential to embryonic development. Germline mutations of genes encoding components of this pathway have been described in a group of conditions referred to as RASopathies (which include, among others, Noonan (NS), and cardiofaciocutaneous (CFC) syndromes). RASopathies are characterized by craniofacial and cardiac defects, ectodermal anomalies, variable degrees of cognitive defects and of growth disorders. MEK2 (encoded by MAP2K2) is activated by Raf and in turn activates ERK1/2, the final effectors of the Ras/MAPK pathway. Activating mutations of MEK2 have been reported in patients with CFC which comprises NS-like facies, moderate-severe neurocognitive impairment, hyperkeratotic skin lesions, and sparse, curly hair and absent eyebrows, and heart defects. We report an 11 year old boy with a 1.5 Mb deletion in 19p13.3, which includes MAP2K2, who has clinical features suggestive of dysregulation of the Ras/MAPK pathway. He has macrocephaly, bicuspid aortic and dysplastic mitral valve, cardiac rhythm abnormalities, severe eczema with hyperkeratosis and nail dysplasia combined with severe developmental delay. His facial features include a triangular face with tall forehead, down slanting palpebral fissures, thick nasal tip, underdeveloped cheekbones, tall and prognathic chin, thin upper and thick lower vermilion border; his ears are small with abnormal antihelices, and posteriorly rotated. He has a history of severe GE reflux with recurrent aspiration pneumonias which required Nissen funduplication and G-tube feeding. He requires nighttime CPAP for obstructive sleep apnea. He has lymphopenia of unknown etiology. He has advanced bone age. BAC-based chromosomal microarray detected deletion of clone RP11-454N6 at 19p13.3. Haploinsufficiency of other Ras/MAPK components resulting in phenotypic effects reminiscent of RASopathies have been reported recently. Individuals with deletions including ERK2 present with craniofacial and cardiac anomalies, microcephaly, and developmental deficits; deletions ERK3 are associated with autism, and facial and cardiac anomalies. Deletion of MAP2K2 may produce similar effects during development and organogenesis. Our patient's phenotype may be a result of a dysregulation of the Ras/MAPK pathway caused by haploinsufficiency of MAP2K2. This is the first report of deletion of MAP2K2 associated with a phenotype suggestive of a RASopathy.

827/T

22q11.2 deletion syndrome: Three unexpected diagnoses through Single Nucleotide Polymorphisms (SNP) microarray in a four month period. *M. Ronningen Johnson[1], C. Dinsmore[1], A. Bytyci Telegrafi[1], D. AS Batista[2], M. Gunay-Aygun[1], R.D. Cohn[1], A. Hamosh[1].* 1) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Pathology Department, Johns Hopkins University, Baltimore, MD.

22q11.2 deletion syndrome is a common genetic condition with a current prevalence of 1/2000-1/4000 births. With the advent of SNP microarray technology, diagnoses of subtle and non-classic presentations are being made in individuals whose clinical features are not consistent with the clinical description of this condition. From 12/2009 to 4/2010 we evaluated three individuals with unanticipated diagnoses of 22q11.2 deletion syndrome. The first patient is a 12 year-old female who presented to the genetics clinic with a history of autism and hyperflexibility. A bifid uvula and hypertelorism were observed. An echocardiogram showed an aortic root measurement on the high end of normal (2.70 cm, z-score = 1.9) and was otherwise unremarkable. TGFBR1/2 sequencing and a SNP array were pursued. TGFBR1/2 was negative. The SNP array showed an interstitial deletion at 22q11.21 (2.68-2.73 Mb). The second diagnosis was made in a two day old male in the newborn nursery with prenatally diagnosed right multi-cystic dysplastic kidney, left hydronephrosis and hydroureter. Karyotype and SNP array were sent by the newborn nursery and a genetics consult was requested for questionable dysmorphic features. An echocardiogram showed only a small patent foramen ovale. Examination revealed no dysmorphic features. The karyotype was 46,XY and the SNP array showed a 2.6 Mb deletion of 22q11.2 deletion. The third case involved a genetics consult for a 2 month-old male on the GI service due to poor feeding and dysmorphic features. An echocardiogram showed a moderate atrial septal defect, and the examination revealed petite facial features, mild micrognathia, overfolded helices and an intact but highly arched palate. The karyotype was 46,XY and the SNP array diagnosed 22q11.2 deletion syndrome (2.58 Mb). These patients illustrate the broad phenotypic variability of 22q11.2 deletion syndrome and represent cases that would have been undiagnosed or diagnosed later without the utilization of SNP microarray technology. While 22q11.2 deletion syndrome is a common and well described genetic condition, these cases show that clinical examination alone may not identify all affected individuals. We anticipate that SNP array technology will result in an increasing number of diagnoses, spectrum expansion, and that prevalence estimates may rise.

828/T

Two identical cases of dup(16)(p13.3->p13.11); a new microduplication syndrome? *R. Pasion[1], B. Rush[1], S.S. Brooks[3], C. Botti[3], M. Holt[4], J. Tepperberg[1], S. Schwartz[1], R.D. Burnside[1], I. Gadi[1], V. Jaswaney[1], H. Risheg[2], E. Keitges[2], P. Papenhausen[1].* 1) Laboratory Corporation of America RTP, NC; 2) Laboratory Corporation of America Seattle, WA; 3) Department of Pediatrics, UMCNJ-Robert Wood Johnson Medical School, New Brunswick, NJ; 4) Blythe Family Health Clinic, Blythe, CA.

We present two patients referred for whole genome SNP microarray (Affymetrix 6.0). Both individuals have an identical 3.377 Mb interstitial duplication of 16p13.3->p13.11 (11,946,847-15,323,999). Patient 1 is a three year old male who was born at 25 weeks gestation, weighing 745 grams. Neonatally, he was in the NICU for 86 days due to respiratory distress, apnea, bradycardia, anemia and jaundice. Currently, he is non-dysmorphic, has global developmental delays, tip toe walks with an abnormal gait, and has significant language delays with only 2 words. He has slightly autistic behavior, but has not had a formal evaluation for autism. Patient 2 is a 12 year old female who was born at 32 weeks gestation, weighing 2353 grams, and was hospitalized neonatally for three weeks. She is non-dysmorphic, with cognitive and social delays. She also has a history of significant language delay, with no sentences until 7 years of age. While neither patient is dysmorphic, both demonstrate prematurity, significant speech delays, global developmental delays, and mild behavioral issues. The 3.377 Mb interval contains 8 OMIM annotated genes and numerous refseq genes. No previously reported cases of this duplication have been published. However, the duplicated interval encompasses the distal portion of the recently described 1.5Mb 16p13.1 microdeletion/microduplication, which is associated with a predisposition to autism, mental retardation, behavioral problems and speech delays. The deletion is more clearly implicated to be causal whereas significance of the duplication is unclear. Two candidate genes, NTAN1 and NDE1, have been hypothesized as contributing to the phenotype of the 1.5 Mb duplication. The NTAN1 candidate gene is included in the both the 3.377 Mb and 1.5 Mb duplications. Shared phenotype between the two microduplications includes speech delays and absence of dysmorphic features. Behavioral problems in individuals with the 1.5 Mb duplication appear to be more severe than the larger duplication, perhaps related to the duplication of NDE1 in the smaller duplication. The identical duplication in both patients and a similar phenotype further implicates duplication of the NTAN1 candidate gene in contributing to developmental and speech delays and the 3.377 Mb duplication may define a new duplication ASD related syndrome region.

829/T

Microdeletions and microduplications of 2q23.1 involving the *MBD5* gene. *E.A. Repnikova[1], A. Bailes[1], A. McKinney[1], A. Brock[1], C. Deeg[1], L. Erdman[1], J. Gordon[1], C. Weber[1], D. Bartholomew[1,3], K. Manickam[1,3], D. Lamb Thrush[1,3], S. Reshmi[1,2], C. Astbury[1,2], R.E. Pyatt[1,2], J. Gastier-Foster[1,2,3].* 1) Laboratory Medicine, Nationwide Children's Hospital, Columbus, OH; 2) Department of Pathology, Ohio State University, Columbus, OH; 3) Department of Pediatrics, Ohio State University, Columbus, OH.

Haploinsufficiency of the *MBD5* gene (methyl-CpG domain binding protein) is believed to be the primary cause of the features associated with the 2q23.1 microdeletion syndrome, which include microcephaly, intellectual disabilities, severe speech impairment, and seizures. The *MBD5* gene encodes several alternatively-spliced transcripts that are expressed in the cerebellum, muscle, heart, pancreas, and kidney. In all previously reported cases, deletions ranged in size from 200 kb to more than 4 Mb (which contained multiple genes including all the *MBD5* isoforms). Duplication of the 2q23.1 region is extremely rare (only four cases have been reported) with sizes ranging from 4-5 Mb and most are visible by standard cytogenetic analysis. In this study, we report the molecular and clinical characterization of two patients with alterations in the *MBD5* gene detected by oligonucleotide array comparative genomic hybridization. The first patient presented with developmental and speech delay. We identified a *de novo* 240 kb deletion which contained only the 5'UTR of the long *MBD5* transcript but did not disrupt shorter isoforms. A previous report described two siblings with 400 kb deletions that also contained a portion of only the *MBD5* long isoform 5'UTR. Similarly, these patients had developmental and speech delay and stereotypic behavior. However, all the previously reported deletion cases, including our case, involving only the long *MBD5* transcript lacked other features characteristic of the 2q23.1 microdeletion syndrome. Our second patient presented with a mild Angelman-like phenotype including mild developmental delay and seizures. She also exhibited motor tics and skin picking behavior. In this patient, we detected an approximately 600 kb duplication in chromosome 2q23.1 which included the majority of the *MBD5*, as well as *EPC2* (a member of the polycomb protein family, involved in heterochromatin formation), a portion of *KIF5C* (a neuron-specific kinesis family member) and *MIR1978*. To our knowledge, submicroscopic duplications at 2q23.1 have not been previously reported. These cases suggest that the *MBD5* gene may play an important role in pathogenesis involving deletions and duplications of the 2q23.1 region.

830/T

Chromosome 1q21.2 microdeletion in a child with phacomatosis pigmentovascularis type IIIb. *J. Stone[1], W.R. Heymann[2], S.M. Manders[2], R.E. Schnur[1].* 1) Genetics/Pediatrics, Cooper University Hospital/Robert Wood Johnson Medical School, Camden, NJ; 2) Dermatology/Internal Medicine, Cooper University Hospital/Robert Wood Johnson Medical School, Camden, NJ.

Phacomatosis pigmentovascularis (PPV) refers to a group of disorders with congenital nevoid pigmentary and vascular skin lesions, +/- systemic anomalies. The underlying genetic etiology remains elusive, but PPV is thought to represent post-zygotic somatic mutation/mosaicism, given the segmental distribution of lesions, sporadic occurrence, and discordance in MZ twins (e.g., Moutray et al. 2010). PPV is classified based on the type of cutaneous abnormalities. Type IIIb PPV includes speckled lentigines/nevus spilus, vascular lesions and systemic anomalies. We report an 8 year-old boy with PPV type IIIb and a 77.6 kb chromosome 1q21.2 deletion detected by microarray analysis. He has mild learning disability and craniofacial variations (hypertelorism, high arched palate). Pigmented lesions include segmental lentigines on a background of increased pigment (nevus spilus) and pigmentary clumping of the irides. Several lesions cross the midline. Vascular lesions include portwine stains, conjunctival telangiectasias and a hemangioma of the right temporoparietal region. He also has left leg hemihyperplasia, pelvic tilt and scoliosis. MRI showed low-lying cerebellar tonsils at the level of the foramen magnum, a venous anomaly, hypoplastic frontal sinuses, and a posterior location of the conus medullaris without tethered cord. He also had hepatomegaly with a relatively small spleen. Parental karyotypes, performed previously, were normal. Mother does not have the 1q21.2 deletion by microarray analysis; paternal testing is pending. The microdeletion contains two OMIM genes, *ENSA* and *MCL1*. *MCL1*, a member of the *BCL2* gene family, may function as a cell growth regulator/anti-apoptotic agent. Could hemizygosity of *MCL1* contribute to the dysregulation of melanocytic and endothelial cell growth that comprises the PPV phenotype? Also, *ADAMTSL4* lies only ~4 kb centromeric to the deletion and is part of a gene family involved in angiogenesis, cell adhesion and CNS patterning. In summary, our patient has the first reported chromosome anomaly associated with PPV. Genes within chromosome 1q21.2 may underlie PPV. Overt chromosomal or gene mosaicism may not be the underlying mechanism as has been assumed, but localized factors during embryogenesis or a "second hit" mechanism may underlie the patchy distribution of PPV lesions. We suggest that other children with PPV be screened with microarray and sequencing of *MCL1* and *ADAMTSL4* as candidate PPV genes.

831/T

Interstitial deletion of the 6q16.3q21 chromosomal region: report on a new patient and review of the literature. *J. Puechberty[1,2], A. Schneider[2], M. Tournaire[2], M. Girard[2], C. Coubes[1], L. Pinson[1], E. Haquet[1], AM. Chaze[2], M. Vincent[1], G. Lefort[2], P. Sarda[1,2], D. Geneviève[1], P. Blanchet[1].* 1) Centre de Référence Anomalies du Développement et Syndromes Malformatifs, Département de Génétique Médicale, CHRU Arnaud de Villeneuve, Montpellier, France; 2) Laboratoire de Génétique Chromosomique, Département de Génétique Médicale, CHRU Arnaud de Villeneuve, Montpellier, France.

We report on an interstitial 6q16.3q21 deletion in an 8.5-year-old girl. The patient was referred to our Department of Medical Genetics because of psychomotor delay of undefined origin associated with developmental delay and dysmorphic features. The prenatal period was marked by moderate intrauterine growth retardation. Clinical examination found facial features (prominent forehead, high arched eyebrows, slight bilateral ptosis with downslanting palpebral fissures, slightly elongated face, macroglossia, cup-shaped ears), body and facial asymmetry, abnormal asymmetric skin pigmentation, ligament hyperlaxity, hypermetropia and strabismus. She presented with psychomotor delay (developmental delay, delayed walking, ataxia, delayed language, hypotonia), and echolalia. She had a friendly and amiable behavior. Skeletal X-ray study revealed delayed bone age (18 months at 5 year-old), 11 pairs of ribs, delayed ischio-pubic bone ossification, coxa valga, thin diaphyses. Psychomotor development was improved through appropriate medical care and supportive educational management. Initial blood chromosome studies using standard cytogenetic methods (RHG and GTG banding) at a 550 band level were interpreted as normal. Microarray analysis (SNP 6.0 Affymetrix) subsequently showed a 4.85 Mb interstitial deletion of the long arm of chromosome 6 (6q16.3q21). To date, less than one hundred 6q deletions have been reported and are subdivided in three cytogenetic groups (proximal, middle and terminal). Constitutional middle deletions of the long arm of chromosome 6 are rare chromosomal events with around 20 patients reported in the literature. We compare the phenotype observed in our patient to the phenotype observed in the patients with overlapping deletions reported in the literature and discuss candidate genes linked to the features described in patients with 6q16.3q21 deletion.

832/T

A family with microdeletion at 2q14 associated with IUGR, OFC at the 2nd%, cognitive impairment and hypercoagulability. *J. Richer, M. Lines, J. McGowan-Jordan, K. Boycott.* Med Gen, CHEO, Ottawa, Canada.

The proband was born following a pregnancy complicated by gestational diabetes on diet, maternal cigarette smoking (1 pack/day) to a mother on Novolox for a prior history of phlebitis. He was born at $37^{6/7}$ weeks gestational age. Birth weight was 2050g (<3rd%), length 47cm (10th%) and head circumference 30.8cm (<3rd%). At 5 months of age he had been admitted 4 times for failure to thrive. Dysmorphic features included: 2 posterior hair whorls, frontal upsweep, medial eyebrow flaring, mild telecanthus with bilateral epicanthal folds, depressed nasal bridge and long philtrum with thin upper vermillon . He re-presented at 3 years of age with significant development delay with impulsive and aggressive behaviors. Head circumference was 2nd%, weight 5th% and height 10-25th%. His mother required special education since grade 2 and had been unable to retain employment for 15 years. Her head circumference was 52 cm (2nd%) and she had a history of multiple thrombotic events for which she is now on warfarin. Three siblings had normal birth weights, head circumferences and early developmental milestones within normal limits, the fourth sibling had IUGR (birth weight at 36 weeks of 1786g, <3rd%), severe developmental delay with a head circumference at the 2nd%. At 3 years of age, microarray (Baylor, oligonucleotides V8.0) showed a maternally inherited 2 megabase deletion at 2q14.3. The deletion was also present in the affected brother and was absent in the 3 unaffected brothers. In the neonatal period, the proband had a normal brain MRI, echocardiogram and karyotype (500-550 bands). This region contains 2 genes of clinical importance: BIN1 and PROC. Haploinsufficiency of PROC causes protein C deficiency thereby explaining the hypercoagulability in this family. BIN1 is a highly conserved gene in mammalian and alternative splicing leads to the expression of 2 isoforms: amphiphysin 2, primarily expressed in muscle, and amphiphysin 2a (amph2a) which is mainly expressed in brain (particularly at nodes of Ranvier). Amph2a forms heterodimers with Amph1 forming a complex which is required in the endocytic recycling pathway and is evolutionarily conserved from worms to mammals. Knock-out mouse for amph1 show cognitive deficits and a higher rate to sudden death due to paroxysmal seizures. Our findings suggest that haploinsufiency of BIM1 may lead to dosage imbalance between amph2a and amph1 and explain our patients' cognitive deficits and microcephaly.

833/T

Interstitial deletion of 3q22-q25 in associated with multiple congenital abnormalities, growth retardation and intellectual disability. *E.C. Tan[1], E.C.P. Lim[1], B. Cham[2], L.K. Ching[1], I.S.L. Ng[2].* 1) KK Research Centre, KK Women's & Children's Hospital, Singapore; 2) Genetics Service, KK Women's & Children's Hospital, Singapore.

Interstitial deletions on the long arm of chromosome 3 are very rare, and most of the few recorded cases involved 3q12 - 3q21. More common abnormalities involving chromosome 3 are structural rearrangements, translocations and mutations in FOXL2 which have been associated with blepharophimosis, ptosis, and epicanthus inversus syndrome (BPES). In this report, we describe a boy with normal karyotype. He was born at term by normal delivery. He had low birthweight of below 2.5 kg. His length at birth was 45 cm and his occipito-frontal circumference was 30 cm. Clinical examination at 8 years of age showed severe growth retardation with height, weight and occipito-frontal circumference all below the 3rd centile. Dysmorphism in this patient include turricephaly, ptosis, bilateral microphthalmia, low-set ears, micrognathia, overlapping fingers and rocker bottom feet. Other abnormalities include cleft palate, moderate ventral septal defect, small patent ductus arteriosus, absent right kidney, and bilateral inguinal hernia. The non-BPES-associated abnormalities are probably the result of abnormalities in genes other than FOXL2. Array-CGH analysis revealed an interstitial deletion from 3q22.1 to 3q25. This deletion was confirmed by qRT-PCR. The chromosomal region deleted in this patient provides candidate genes for molecular genetic studies related to the phenotypic presentations.

834/T

The 12q14 microdeletion syndrome : description of a new case without osteopoikilosis. *L. Telvi[1], L. Boutaud[1], O. Brichet[2], A. Coussement[1], JM. Dupont[3], G. Viot[4], C. Bellesme[2].* 1) Cytogenetics Laboratory, Hosp St Vincent de Paul, Paris, France; 2) Department of Pediatric Endocrinology, Hosp St Vincent de Paul, Paris, France; 3) Cytogenetics Laboratory, Hosp Cochin, Paris, France; 4) Genetics Department, Hospital Cochin, Paris, France.

Recently, the microdeletion of 12q14 region has been reported as a new chromosomal syndrome (Merten,2007). The phenotype of these patients was defined with mental retardation, short stature, dysmorphia and osteopoikilosis. We report a nine-years-old boy with mental retardation, dysmorphic features, unusual hyperactivity and short stature. The genital examination showed cryptorchidia and small penis. At nine-years-old he had no speech and showed an unusual hyperactivity. The patient had a phenotypically normal brother. The karyotype with RHG banding of the patient was found normal. By using Cytochip Bac Arrays v20 Bluegnome (Amplitech) we identified a microdeletion at 12q14. The RP11-366L20 and RP11-109N1 probes was used with FISH analysis and confirm the deletion. The size of the deletion was 5.8 Mb (linear position 60,290,737-66,163,925). The karyotype of the parents was found normal with absence of 12q14 deletion after FISH analysis. Few cases was described to date with 12q14 microdeletion (Merten & al, 2007; Mari & al, 2009; Buysse & al, 2009). The patient described did not showed osteopoikilosis and/or skin manifestions caracteristic of Buschke-Ollendorff syndrome after squeletal X-rays, ultrasound examination and RMI. The LEMD3 gene of our patient was found deleted. However, LEMD3 gene was described as implicated with ostepoikilosis and/or skin manifestations caracteristic of Buschke-Ollendorff syndrome. The relationship of LEMD3 deletion with the absence of osteopoikilosis in this case is discussed.

835/T

A patient with Williams-Beuren syndrome and multicystic kidney disease with deletions at 7q11.23 and 17q12. *L. Cohen[1], J. Samanich[1], Q. Pan[2], A. Ludtke[3], L. Mehta[3], R. Marion[1].* 1) Williams Syndrome Center, Division of Genetics, Department of Pediatrics, Children's Hospital at Montefiore, Bronx, NY; 2) Dept. of Pathology, Montefiore Medical Center, Bronx, NY; 3) Dept. of Genetics & Genomic Sciences, Mount Sinai School of Medicine, NY, NY.

The Williams-Beuren syndrome (WBS) is a complex genomic disorder entailing distinctive facial dysmorphisms, cardiovascular abnormalities (predominantly supravalvular aortic stenosis [SVAS]), intellectual disabilities, unusual behavioral features, and a specific cognitive profile with considerable variability. Additional symptoms include endocrine abnormalities, renal anomalies and connective tissue weakness. We report a monozygotic twin patient with WBS who presented with multicystic kidneys in the newborn period, and, in addition to the typical WBS deletion at 7q11.23 (detected by FISH), was found to have a de novo 1.7 Mb deletion in the 17q12 region on microarray comparative genomic hybridization (array CGH). Prenatal history was significant for the patient's co-twin being diagnosed with bilateral multicystic dysplastic kidneys and anhydramnios. A selective termination of the co-twin was performed at 23 weeks gestation. In the prenatal period, the patient herself had intrauterine growth retardation and single umbilical artery. At birth, she was symmetrically small (<3%). Facial features were suggestive of WBS and FISH revealed the typical deletion at 7q11.2. Echocardiogram showed mild bilateral peripheral pulmonic stenosis with no significant SVAS. Renal sonograms showed small kidneys with bilateral tiny cysts. Neonatal course was otherwise uncomplicated. While renal anomalies are reported in WBS, the severe renal involvement in the patient's twin and her own presentation are unusual. Hence, array CGH using a custom designed Agilent 44K oligonucleotide microarray with 50kb resolution in targeted regions was performed. In addition to the 1.3Mb deletion on 7q11.2, a second deletion at 17q12 was detected. Karyotype and array CGH on parents were normal. Review of literature shows that deletion of chromosome 17q12, encompassing the HNF1beta gene, is associated with cystic renal disease and is the first recurrent genomic deletion associated with maturity onset diabetes of the young (MODY5). In addition, two cases of Mayer-Rokitansky-Kuster-Hauser syndrome with identical deletions on chromosome 17q12, including TCF2 and LHX1 genes, have also been described. It is likely that the additional 17q12 deletion has played a role in modifying the phenotype in our patient. This case highlights the importance of using array CGH in the clinical setting to uncover the etiology of atypical findings in individuals with known microdeletion syndromes.

836/T

Microdeletions within the "Imprinting region 2" on 11p15 identify a variant of the Beckwith-Wiedemann syndrome at risk for cardiac arrythmia. *G. Neri[1], F. Gurrieri[1], M. Zollino[1], G. Marangi[1], D. Orteschi[1], R. Pietrobono[1], M.G. Pomponi[1], A. Camporeale[2], F. Bellocci[2].* 1) Inst Di Gen Medica, Univ Cattolica, Rome, Italy; 2) Istituto Di Cardiologia, Univ Cattolica, Rome, Italy.

Beckwith-Wiedemann syndrome (BWS) is a relatively common genetic overgrowth condition, characterized by clinical variability and genetic heterogeneity. Cardinal features include macroglossia, omphalocele, and macrosomia. Psychomotor retardation is uncommon and familial recurrence rare. BWS is caused by genetic or epigenetic alterations of two imprinting domains (ICR1 and ICR2) on 11p15, ICR2 being involved in most cases. Hypermethylation in ICR1 is associated with BWS, whereas hypomethylation of the same region is associated with Silver-Russel syndrome (SRS), a condition characterized by growth delay. Abnormalities of ICR2 include loss of methylation of KvLQT1-AS or point mutations of CDKN1C, both occurring on the maternal allele. We here report on two unrelated females , aged 16 and 19 years respectively, both carrying a 11p15 microdeletion involving ICR2. The deletion spanned about 900 kb in the first case, including CDKN1C, KCNQ1 and additional 15 flanking genes. The size of the deletion in the second patient was 200 kb, encompassing the entire KCNQ1OT1 antisense transcript (AS), and exons 11-16 of KCNQ1. Both patients presented with an atypical BWS phenotype, including mild mental delay in the first patient [Zollino et al, J Med Genet, 2009], a long QT syndrome in the second and overgrowth and facial dysmorphisms in both. The long QT syndrome was responsible for a cluster of life-threatening ventricular fibrillation episodes, needing an urgent implant of intra-cardiac defibrillator. In this patient loss-of-function mutation of KCNQ1 is likely responsible for the long QT trait, although the severity of clinical presentation may be due to additional mutations either in the remaining KCNQ1 allele or in other genes known to cause long QT. Interestingly, the ICR2 deletion in patient 2 was inherited from the mother, who carries the same alteration on the paternal allele. The mother has minor physical anomalies, short stature and long QT. The first patient was re-called and checked for a long QT, which she did not have. Nonetheless, patients in which ICR2 is deleted should be monitored for long QT syndrome. Both patients were counselled that a 50% recurrence risk of BWS expected in the offspring.

837/T

Immune deficit in a cohort of 90 patients with 22q11 deletion. *M. PEREZ, P. SARDA, E. HAQUET, A. SCHNEIDER, G. LEFORT, D. GENEVIEVE.* Medical Genetics, Arnaud de Villeneuve Hospital, Montpellier, France.

The 22q11 deletion causes the most common microdeletion syndrome. During a period of five years, the Montpellier Reference Center for Developmental Abnormalities and Malformative Syndromes has coordinated the multidisciplinary management of 90 patients aged from a few months to 45. Ear-nose-throat (ENT) infections are particularly frequent during the first years of life for children. ENT infections in children have multiple origins, associating inner and middle ear abnormalities, pharyngeal and velar abnormalities and " transitory" immune deficit. CD4 lymphocytes and CD8 lymphocytes deficits probably play a role in these infections in the young child. To investigate the immunologic constitution of patients we characterized the T-cell deficiency in the follow-up of patients with 22q11 deletion. CD4 levels (number/mm3 and percentage) normalize for the majority of patients before the age of 8. CD8 lymphocytes levels (number/mm3 and percentage) remain low until the age of 15, as opposed to what is reported in the literature. In most patients, the immune deficit improves with time, therefore CD4 and CD8 lymphocytes levels do not need to be regularly measured once normalized, unless a clinical problem appears. Autoimmune disorders are more frequent in the group of patients with 22q11 deletion (10%). Many patients who were properly vaccinated do not achieve immunity for certain vaccinations. With advancing age it is thus important to check residual antibody levels after certain vaccinations (polio, tetanus, diphtheria, hepatitis, mumps for men and rubella for women).

838/T

Paternal duplication 15q11-q13 results in sleep problems, anxiety and abnormal EEG patterns. *N. Urraca[1], J. Cleary[2], E. Pivnick[3], K. McVicar[3], V. Brewer[3], R. Thibert[4], L.T. Reiter[1,3].* 1) Department of Neurology, UTHSC, Memphis, TN; 2) Department of Speech and Language Pathology, University of Memphis, Memphis, TN; 3) Department of Pediatrics, UTHSC, Memphis, TN; 4) Department of Neurology, Mass General Hospital, Boston, MA.

The widespread use of clinical microarrays has substantially increased the detection rate of 15q11-q13 interstitial duplication cases. In most cases the duplication is de novo, however there are few familial cases. Although the majority of duplications in this region are maternal in origin, some cases of paternally transmitted 15q duplication have been reported with a presumably normal phenotype since unaffected mothers have transmitted paternally derived duplication chromosomes to their affected children. A few paternal cases have been reported with speech delay and behavior problems. Here we report for the first time a father with a paternal duplication who transmitted to both his son and daughter. Both children and the father had macrocephaly (>97%), an asymmetric face due to unilateral relaxed nasal labial fold, and mild unilateral eyelid ptosis. They also shared the following features: downslanting of palpebral fissures, prominent infra-orbital creases, upturned, short nose, mildly elongated philtrum, thin upper lip, round, moon-like face and mild orofacial hypotonia. The son had radiological signs of Chiari I malformation. In addition he also had a pineal gland cyst. The daughter had a voracious appetite since infancy. ADOS/ADI-R testing indicated that the son meets the criteria for autism, however his sister does not. Both children had normal awake EEG's with excessive bi-frontally predominant 18-22Hz beta activity throughout the recording, which may be characteristic of int dup 15. The father and both children have sleep problems and showed signs of anxiety. Pediatric polysomnogram showed abnormal sleep architecture in both children. Molecular analysis indicated a class II duplication in all three individuals of paternal origin. The majority of int dup 15 cases detected are maternal in origin; this is most likely due to the coincidence of autism with maternal duplications. Although clinically neither sibling clearly fit an autism diagnosis, the male proband did meet the minimal criteria for autism on the ADOS. Further evaluation must be conducted on this family and others with paternal 15q duplication syndrome, including a special emphasis on sleep evaluation, evidence of anxiety, EEG features and detailed physical description. These investigations may help to establish as well as distinguish the unique characteristics of paternal 15q duplications from the more common maternal 15q duplications that cause autism.

839/T

Refinement of the interstitial microdeletion in chromosome 6q25 associated with mental retardation. *A.B. Ekici[1], J. Hoyer[1], C. Petsch[1], I. Göhring[1], A. Rauch[1,2], A. Reis[1].* 1) FAU Erlangen-Nuremberg, Institute of Human Genetics, Erlangen, Germany; 2) University Zurich, Institute of Medical Genetics, Zurich, Switzerland.

Interstitial deletions of chromosome 6q are very rare findings. Deletions range from 0.3 Mb to several megabases spanning multiple chromosomal bands. Some associated clinical features are commonly seen including mental retardation, hypotonia and facial dysmorphism, while others like microcephaly, brain malformation, micrognathia, hearing loss, hernia and heart malformation are only seen in some cases. The high clinical variability is explained by the large divergence in the overlap and gene coverage of the deletions. We report on a 28 month old infant with normal female karyotype, mental retardation (MR), facial dysmorphism, strabismus alternans, plagiocephaly, hyperopy and body measurements on 10th centile. After exclusion of known aberrations with various commercial MLPA-kits, Angelman Syndrome with methylation specific PCR and Rett Syndrome (MECP2 point mutations) we conducted a genome-wide analysis of copy number variation (CNV). We performed molecular karyotyping in the patient and both parents with high density Affymetrix Genome-Wide Human SNP 6.0 microarrays. In addition to a paternally inherited 121 kb duplication on chromosome 12 we detected a de novo deletion of 2.3 Mb on chromosome 6q25. This deletion encompasses only 5 known genes and lies within the previously reported deletion region. We hypothesize that haploinsufficiency for a gene within the 2.3 Mb deleted region may impair normal development. In order to identifying the phenocritical gene we are screening these five positional candidates for point mutations in 144 patients with moderate to severe MR from our MRNET-cohort. To date we found a sequence alteration in one of the genes, highly expressed in brain. One patient of our screening cohort showed an exonic 11 bp deletion leading to a frameshift resulting in a premature stop codon, which would shorten the protein by 58 amino acids. Sequencing of both parents to prove de novo origin of the mutation is ongoing as well as mutation analysis in further patients and controls. This study is part of the German Mental Retardation Network (www.german-mrnet.de).

840/T

Intragenic deletion of the *AUTS2* gene in patients with mental retardation, autistic behaviour and dysmorphic features. A new microdeletion syndrome. *E.A. Sistermans[1], E. Voorhoeve[1], G. Beunders[1], B. van der Zwaag[2], H.Y. Kroes[2], S. Vergult[3], G. Mortier[4], B. Menten[3], S. Gana[5], J.J. Saris[6], M. van der Mespel[1], D. Linders[1], A. Nieuwint[1], J.M. van de Kamp[1], S. Groffen[1], M.M. Weiss[1].* 1) Dept Clinical Genetics, VU University Medical Center, Amsterdam, The Netherlands; 2) Dept Medical Genetics, University Medical Center Utrecht, The Netherlands; 3) Center for Medical Genetics, Ghent, Belgium; 4) Dept Medical Genetics, Antwerp University, Edegem, Belgium; 5) Genetica Medica, Pavia, Italy; 6) Dept Clinical Genetics, Erasmus MC, Rotterdam, The Netherlands.

Disruption of the *AUTS2* gene on 7q11.2 has been described in five patients with a translocation with one of the breakpoints in 7q11.2 by Kalscheuer et al. (2007) and Sultana et al. (2002). These findings suggested that rearrangements of *AUTS2* cause mental retardation and/or autism. We here present three patients with (partial) deletions of *AUTS2* detected by array CGH (Agilent 180k oligo-array), and an additional patient with a translocation disrupting *AUTS2*. One patient lacks the 3' end of the gene, which is likely to result in inactivation of *AUTS2* only. This patient has mental retardation, and shares several distinct dysmorphic features described in the translocation patients, confirming that aberrations of the *AUTS2* gene are causative for MR and/or autism. This new microdeletion syndrome is unique in that it is more often caused by translocations than by "pure" microdeletions. Furthermore, the gene is located close to the Williams-Beuren syndrome critical region on 7q11.23, and deleted in rare patients with larger non-standard deletions (including one of our patients). It is very likely that deletion of *AUTS2* will add to the phenotype in these patients. Functional studies are currently performed to assess the biological role of AUTS2, which is highly expressed in fetal and adult brain.

841/T

Parathyroid gland dysfunction in a cohort of 90 patients with 22q11 deletion. *P. Sarda, M.J. Perez, G. Lefort, E. Haquet, A. Schneider, D. Genevieve.* Dept Med Gen, Arnaud de Villeneuve Hosp, Montpellier, herault, France.

The 22q11 deletion causes the most common microdeletion syndrome. During a period of five years, the Montpellier Reference Center for Developmental Abnormalities and Malformative Syndromes has coordinated the multidisciplinary management of 90 patients aged from a few months to 45. 35% of the subjects had hypocalcemia, half in the neonatal period and for the other patients hypocalcemia was diagnosed after the first year of life. 6% of the patients developed hypocalcemia after puberty or at adult age. The diagnosis of late-onset hypocalcemia in 15% of the patients implies the organization of a regular calcemia monitoring at all age. In the population with normal calcemia, the level of calcium showed a progressive decrease with age (y=-0.0026x + 2.38). Ionized calcium was always low (1.15 +/- 0.1 mmol/l , normal between 1.20 and 1.35), which was probably responsible for the cramps the patients presented. PTH levels in blood are low but seem to be of little interest for the follow up of these patients (PTH for patients with normal calcemia : 17.3 +/- 8.3 pg/ml versus 14.9 +/- 8.3 pg/ml for patients with hypocalcemia). The results we observed concerning the risk of late-onset hypocalcemia suggest that the monitoring of calcemia should not be neglected. The frequency and the method of follow-up still need to be defined as well as the therapeutic regimen and its management. We suggest a check-up every 2 years for patients with 22q11 deletion. However this raises the problem of patient accessibility and participation.

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Functional and cellular characterization of human Retinoic Acid Induced 1 (RAI1) mutations associated with Smith-Magenis Syndrome. *P. Carmona-Mora[1], C.A. Encina[2], C.P. Canales[1], J. Molina[2], L. Cao[1], J.I. Young[1, 2, 3], K. Walz[1, 2].* 1) Dr. John T. Macdonald Foundation Department of Human Genetics, John P. Hussman Institute for Human Genomics, University of Miami Miller School of Medicine Miami, Florida, USA. 1501 NW 10th Avenue Miami, FL 33136; 2) Centro de Estudios Científicos, CECS, Valdivia, Chile. Arturo Prat #514, Valdivia, Chile; 3) Centro de Ingeniería de la Innovación (CIN), CECS, Valdivia, Chile. Arturo Prat #514, Valdivia, Chile.

Smith-Magenis Syndrome is a contiguous gene syndrome with a birth prevalence estimated at 1/25,000. The clinical phenotype includes craniofacial abnormalities, brachydactyly, self injurious behavior, sleep abnormalities and mental retardation. The dosage sensitive gene causative of SMS has been identified: the Retinoic Acid Induced 1 (RAI1). Little is known about the function of human RAI1. We generated the full-length cDNA of the wild type protein and four mutated forms: RAI1-HA Q1562R, RAI1-HA S1808N, RAI1-HA 2687delC and RAI1-HA 3103delC, three of them previously associated with SMS clinical phenotype. Molecular weight, subcellular localization and transcription factor activity of the wild type and mutant forms were studied by western blot, immunofluorescence and luciferase assays respectively. The wild type protein and the two missense mutations presented a higher molecular weight than expected, localize to the nucleus and activate transcription of reporter gene. The frameshift mutations generated a truncated polypeptide with transcription factor activity but abnormal subcellular localization. Besides, we have generated two different C-terminal halves of the RAI1 protein (1038aa-end and 1229aa-end). Both were able to localize into the nucleus but have no transactivation activity. With this work, we demonstrate that the human wild type protein has transcription factor activity, and is able to localize to the nucleus. Moreover, it is possible to define a transcription factor domain within the first 1034 aa and that the nuclear localization signals reside in the second half the protein. Our results indicate that transcription factor activity and subcellular localization signals reside in two separate domains of the protein and both are essential for the correct functionality of RAI1. The pathogenic outcome of some of the mutated forms can be explained by the dissociation of these two domains.

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Branchio-oculo-facial syndrome: Inner and middle ear malformations. *M. Carter[1], S. Blaser[1], W. Meschino[2], B. Papsin[1], W. Reardon[3], R. Klatt[1], R. Babul-Hirji[1], J. Milunsky[4], D. Chitayat[1].* 1) Hospital for Sick Children, Toronto, ON, Canada; 2) North York General Hospital, Toronto, ON, Canada; 3) Our Lady's Hospital for Sick Children, Crumlin, Dublin, Ireland; 4) Center for Human Genetics, Boston University School of Medicine, Boston, Massachusetts, USA.

Branchio-oculo-facial syndrome is an autosomal dominant disorder of the first and second pharyngeal arches. The main features of this syndrome are cutaneous defects in the cervical, infra-auricular and/or supra-auricular region; ocular anomalies; and characteristic craniofacial features including dolichocephaly, malformed pinnae, broad nasal tip, pseudocleft, up-slanting palpebral fissures, and cleft lip with or without cleft palate. Other common findings are conductive hearing loss, prematurely gray hair, ectopic dermal thymus, and scalp cysts. Recently, the gene responsible for BOFS was identified as TFAP2A (Milunsky, 2008). The gene is expressed in cranial neural crest cells and their derivatives including the mesenchyme of the medial and lateral nasal prominences and the maxillary prominence. We report middle and inner ear abnormalities in three individuals with mutation-proven BOFS. Patient 1 is a 13-year old boy with bilateral cleft lip and palate, malformed pinnae, congenital conductive hearing impairment, and bilateral cervical skin defects. Temporal bone (TB)-CT showed deficient long process of the right incus, bony atresia plate covering left oval window, and mild prominence of the left vestibular aqueduct. Patient 2 is 10-year old boy with moderate bilateral conductive hearing impairment, nasolacrimal duct stenosis, myopia, and skin defects superior to the malformed pinnae bilaterally. TB-CT showed mildly prominent vestibular aqueduct on the left and small bone islands. Patient 3 is an 11 year old girl previously reported as an infant by Raveh et al. (2000). She has mixed conductive and sensorineural hearing impairment, with narrow external auditory canals, cochlear dysplasia, small horizontally-oriented semicircular canals and small bone islands. She has right-sided facial nerve palsy and CT revealed an accessory facial nerve canal with an ectopic origin on the right side. Hearing impairment in individuals with BOFS is common and variable. The majority of described individuals have conductive hearing impairment due to malformed ossicles and/or external canal stenosis/atresia. A sensorineural component to the hearing impairment is increasingly being reported. Sophisticated computed tomography of the temporal bone has revealed inner ear malformations in three reports of patients with BOFS (Raveh 2000, Tekin 2009, and Stoetzl 2009). Our study further delineates the variety of inner and middle ear abnormalities associated with BOFS.

844/F

The low-frequency hearing loss: a mexican family. *E. Hernandez Gomez[1], L. Hernandez Gomez[2], S. Juarez Garcia[3], D. Gomez Torres[4].* 1) Biology, Facultad de Estudios Superiores Iztacala, Mexico; 2) Dept Audiologia Instituto Nacional de Rehabilitacion Mexico; 3) Dept Neuropsicologia Instituto Nacional de Rehabilitacion Mexico; 4) Instituto Nacional de Rehabilitacion Mexico.

The low-frequency hearing loss is genetically heterogeneous, caused by mutation of WFS1 gene located in 5q31. It has been observed in parent families. The hearing loss usually begins in the first decade of life, being non-syndromic and postlingual. Which may progress to profound hearing loss. Audiometric studies show an apical initial, probably a result of endolymphatic hydrops caused by alterations in the stria vascular sclerosis secondary to labyrinthine. Present the case of a Mexican family where the father of four male children have bilateral hearing loss for low frequencies, detected between 11 and 16 years old, There are no previous medical history, hearing loss, exposure to noisy environment or use ototoxic in 5 patients. Physical examination is normal in five patients. The audiometric study on five patients were observed for low frequency hearing loss. Speech audiometry in the five cases was sensory. Tympanometry was observed in two curves A of Jerger and 3 with curves As stapedial reflex absent bilaterally in all patients. Caloric tests in 1 patient is reported with areflexia, 1 with hyporeflexia and 3 patients are normal. Computed tomography normal in all patients. Corroborate the clinical cases with hereditary hearing loss for low frequencies and are similar to the results presented in other studies, only pending the molecular studies give us a definitive diagnosis.

845/F

Congenital deafness diagnosed by custom molecular microarray (ARRAY CGC): A new rapid and cost-effective approach. *P. Rendeiro[1], L. Lameiras[1], A. Lopes[1], L. Dias[1], L. Nunes[2], J. Saraiva[3], A. Fortuna[4], M. Cunha[5], M.H. Carreiro[6], M. Rosa[7], J. Sá[1,3], S. Almeida[1,2], A. Palmeiro[1], P. Tavares[1].* 1) Cytogenetics, CGC Genetics (www.cgcgenetics.com), Porto, Portugal; 2) Centro Hospitalar de Lisboa Central, EPE, Lisboa, Portugal; 3) Hospital Pediátrico de Coimbra EPE, Coimbra, Portugal; 4) Instituto Nacional de Saúde Dr. Ricardo Jorge, IP, Porto, Portugal; 5) Centro Hospitalar Trás-os-Montes Alto Douro EPE, Vila Real, Portugal; 6) Hospital Professor Doutor Fernando Fonseca EPE, Amadora, Portugal; 7) Hospital Garcia de Orta EPE, Almada, Portugal.

Introduction: Congenital hearing loss/deafness is the most common birth defect and the most prevalent sensorineural disorder in developed countries but currently only a minority of genes is included in genetic diagnostics. Genetic factors are considered to cause more than 50% of the cases of congenital deafness in children. Genetic deafness can be inherited, as an autosomal dominant, autosomal recessive, or X-linked recessive trait, as well as by mitochondrial inheritance. Over 400 genetic syndromes that include deafness have been described. Moreover, in some syndromes deafness may appear as the first symptom, while other pathological manifestations may have a later onset during development. Molecular testing is a vital asset to complement the differential diagnosis between nonsyndromic and syndromic hearing loss. Method: Using a custom microarray panel (Arrays CGC - Patent Pending) that contains 312 point mutations, identified in 31 main genes involved on congenital deafness it is possible to identify the molecular basis of the most common forms, both syndromic and nonsyndromic. Results: The samples analyzed were obtained from an already scrutinized population, so the most common genetic alterations were already excluded. We analyzed 110 cases and in 24 we detected mutations or sequence variants on CDH23, GJB2, GJB3, MYO1A, MYO7A, OTOF, SLC26A4 and WFS1 genes. Turnaround time was one week after DNA extraction. Conclusion: The usual diagnostic approach only analyses few genes. With this approach we can drastically increase the number of genes/mutations analyzed maintaining accuracy but reducing turnaround time. This approach greatly enhances genetic diagnostics, allowing early decision-making process in patient management as well as new epidemiologic data.

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The Oculocutaneous Albinism Natural History Study at the National Institutes of Health. *D.R. Simeonov[1], B.P. Brooks[2], C.C. Brewer[3], W.M. Zein[2], C.K. Zalewski[3], T.D. Ngugyen[4], M. Huizing[1], W.A. Gahl[1], D.R. Adams[1].* 1) NHGRI, National Institutes of Health, Bethesda, MD; 2) NEI, National Institutes of Health, Bethesda, MD; 3) NIDCD, National Institutes of Health, Bethesda, MD; 4) Clinical Center, National Institutes of Health, Bethesda, MD.

Introduction: Autosomal recessive oculocutaneous albinism (OCA) causes low vision, hair, skin and eye hypopigmentation, and skin ultraviolet-light sensitivity in 1:18,000 newborns. Approximately 85% of OCA is caused by defects in one of four known genes: *TYR*, *OCA2*, *TYRP1* and *SLC45A2*, leaving a substantial minority of OCA cases without molecular confirmation. The pathogenic potential of many individual variants of the known genes has yet to be quantified. Key aspects of the natural history and cellular biology of OCA also remain to be elucidated.

Methods: The NIH OCA Natural History Study is actively recruiting individuals and families with OCA to participate in a 3-5 day evaluation at the NIH Clinical Center in Bethesda, Maryland. Participants are clinically evaluated at the NIH, focusing on comprehensive evaluation of the visual system with an attempt to uncover clinically relevant endpoints, and evaluation of hearing, auditory processing abilities, and low-vision adaptation. Functional assays are being developed to assess the pathogenic potential of individual mutations in known genes.

Results: Forty individuals have been recruited to date. Demographic and preliminary molecular data are presented along with examples of functional assessment of DNA variants.

Conclusions: The careful collection of clinical data, genetic data and cell biological data for persons with albinism has the potential to yield new albinism-related genes, new biological insights and novel therapeutic targets. Medical providers are encouraged to provide information about the study to their patients with albinism.

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In Silico and Experimental Analysis of a Novel Intronic *LMNA* DNA Variation Implicated in Limb Girdle Muscular Dystrophy Type 1B and Dilated Cardiomyopathy. *B.W. Darbro[1], E.M. Cox[1], A.A. Stence[1], B.M. Cabuay[3], K.D. Mathews[2], P.L. Nagy[1,4], S.A. Moore[1].* 1) Department of Pathology, University of Iowa, Carver College of Medicine, Iowa City, IA; 2) Departments of Pediatrics and Neurology, University of Iowa, Carver College of Medicine, Iowa City, IA; 3) Department of Internal Medicine, University of Iowa, Carver College of Medicine, Iowa City, IA; 4) Department of Pathology, Columbia University Medical Center, New York City, NY.

Mutations in the *LMNA* gene encoding lamins A and C result in a wide variety of dominant disorders known as laminopathies that include the striated muscle phenotypes limb-girdle muscular dystrophy type 1B (LGMD1B) and dilated cardiomyopathy (DCM). We identified a four-generation family with a novel sequence variant in *LMNA* (c.639+1 G>A) and clinical features of both LGMD1B and DCM. The variably expressed familial phenotype includes increased creatine kinase levels, muscle weakness, and, in some family members, severe cardiomyopathy leading to heart transplant or death. Muscle biopsies in adult members of the second generation revealed mild dystrophic pathology. Cultured fibroblasts from affected family members had increased nuclear blebbing, a feature common to a variety of laminopathies. The *LMNA* sequence variant segregated with affected family members and is present in three currently asymptomatic members of the third generation. To confirm that the novel sequence variation affects proper pre-mRNA splicing, a thorough in silico analysis of the mutation using several online computational tools was performed and predicted improper splicing that would lead to the formation of a premature stop codon (r.635_639del, p.Ser212ArgfsX4). This prediction was confirmed through the use of mutation specific RT-PCR studies in cultured fibroblasts. These data indicate that this novel *LMNA* change is pathogenic and consequently responsible for the LGMD1B and DCM phenotypes seen in this family. Clinical experience with this family affirms that patients with autosomal dominant LGMD should be screened for cardiac abnormalities and mutations in *LMNA*. Furthermore, when novel DNA variants are discovered by gene sequencing, proper in silico analysis can provide valuable clues to mechanisms of pathogenicity and guide confirmatory testing.

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Delineation of novel phenotypes and the identification of new disease genes from United Arab Emirates. *L. Al-Gazali[1], N.A. Akawi[2], S. Ben-Salem[2], B.R. Ali[2].* 1) Dept Pediatrics, Faculty of Medicine and Health Sciences, UAE University, Al Ain, Abu Dhabi, United Arab Emirates; 2) Dept Pathology, Faculty of Medicine and Health Sciences, UAE University, Al Ain, Abu Dhabi, United Arab Emirates.

The local inhabitants of the United Arab Emirates (UAE) are ethnically diverse with ancestries from the south and north of the Arabian Peninsula, Persia, Baluchistan and East Africa. In addition, the majority of the current 5 million inhabitants are expatriates from the Asian subcontinent, Middle Eastern, African and European countries. Despite this admixture of populations, intermarriages between the groups are still rare, with consanguineous marriages within the UAE local and other Arab populations still the norm, leading to the formation of population isolates and indeed the appearance of recessive conditions. Like other regional populations, the UAE has high frequencies of blood disorders including beta and alpha thalassemias, sickle cell disease and glucose-6-phosphate dehydrogenase (G6PD) deficiency. In addition, some other genetic disorders are relatively common in the UAE including cystic fibrosis, deafness, Joubert syndrome and Meckel syndrome. Furthermore, numerous rare congenital malformations and metabolic disorders caused by defects in recessive genes have also been reported. Clinical characterization of phenotypes of patients with recessive disorders from the UAE led to the delineation of rare and new conditions. In addition, molecular studies led to the discovery of a significant number of disease genes. In this paper, we will present the novel phenotypes observed in the UAE and we list the conditions and disease genes identified. Examples include new disorders like BNAR caused by mutations in FREM1 gene, CHIME-like syndrome caused by mutations in SRD5A3 gene, AR cutis laxa type IIB caused by mutations in PYCR1 gene and well established disorders like Hennekam syndrome caused by mutations in CCBE1 gene, Joubert syndrome caused by mutations in INPP5E gene and Crisponi syndrome caused by mutations in CRLF1 gene.

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The D538H mutation in ENPP1 is associated with generalized arterial calcification of infancy and pseudoxanthoma elasticum. *G. Baujat[1], S. Hadj-Rabia[2], M. Le Merrer[1], B. Bader-Meunier[3], N. Boddaert[4], C. Gay[5], G. Guest[6], K. Lambot[4], A. Linglart[7], L. Martin[8], F. Tazarourte[9], Y. Nitschke[10], F. Rutsch[10].* 1) Department of Genetics, Necker Hospital, AP-HP, Paris, France; 2) Department of Dermatology, Necker Hospital, AP-HP, Paris, France; 3) Department of Pediatric Immunology and Rheumatology, Necker Hospital, AP-HP, Paris, France; 4) Department of Pediatric Radiology, Necker Hospital, AP-HP, Paris, France; 5) Department of Pediatrics, Saint Etienne University, Saint Etienne, France; 6) Nephrology Unit, Necker Hospital, AP-HP, Paris, France; 7) Department of Pediatric Endocrinology, Saint Vincent de Paul, AP-HP, Paris, France; 8) Department of Dermatology, Angers University Hospital, Angers, France; 9) Pediatrics Unit, CH, Montreuil, France; 10) Department of Pediatrics, Muenster University Children's Hospital, Muenster, Germany.

Generalized arterial calcification of infancy (GACI) is an autosomal recessive disorder characterized by diffuse vascular and periarticular soft tissue calcifications, frequently leading to death in early infancy. A few patients present with a milder phenotype and hypophosphatemic rickets. This disorder is due to mutations in ENPP1 in most cases. Pseudoxanthoma elasticum (PXE) is another autosomal recessive multisystem disorder characterized by ectopic mineralization and fragmentation of elastic fibers of connective tissues, including skin, vascular walls, and the eye. Classic PXE results from mutations in the ABCC6 gene. We report a boy with a unique phenotypic overlap between PXE and GACI. He presented with neonatal periarticular and arterial calcifications, which completely resolved during childhood. However, ectopic calcification in both kidneys and eyes developed, associated with hypophosphatemic rickets. He lost his hearing due to stapedo-vestibular ankylosis and showed diffuse angiomatomateous skin lesions. The diagnosis of PXE was confirmed by histology and immunohistochemistry from a skin lesion at the age of 10 years. Sequence analysis of ABCC6 revealed no mutation, while the patient was demonstrated to carry the ENPP1 mutation p.D538H in exon 16 on both alleles. This particular overlapping phenotype between PXE and GACI appears to be associated with a better prognosis than classical GACI, with spontaneous resolution of vascular calcifications, but with severe hypophosphatemic rickets and hearing loss. Our case study confirms that GACI and PXE may have more similarities than previously thought. Since ABCC6 was recently discussed as the causative gene in a case of GACI without mutations in ENPP1 (Am J Med Genet 152A:118-123; 2010), we demonstrate here that, conversely, ENPP1 may be involved in some cases of PXE in the absence of ABCC6 mutations. This emphasizes that ENPP1 and ABCC6 are probably involved in the same molecular pathway.

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Case Report: Fraternal twins with Kniest dysplasia. *E. Carter[1], P. Brill[3], C. Raggio[1], J. Davis[1,2].* 1) Ctr Skeletal Dysplasias, Hosp Special Surgery, New York City, NY; 2) Div. Human Genetics, New York-Presbyterian Hospital, Weill Cornell Medical College, New York City, NY; 3) Pediatric Radiology, New York-Presbyterian Hospital, Weill Cornell Medical College, New York City, NY.

Kniest dysplasia is characterized by short trunk and limbs, kyphoscoliosis, midface hypoplasia, hearing loss, and severe myopia. Some individuals have cleft palate and/or club feet. Dominant mutation of *COL2a1* is causative. Severity varies, with severely-affected individuals dying from respiratory failure after birth and mildly-affected individuals having mild short stature, scoliosis and/or craniofacial findings. Adult height ranges from 106-145cm. We present 14-month-old dizygotic twins, conceived with assistance (IVF) using germ cells from both parents. The 36-yr-old mother (G1P2) is 5'3" tall with a history of polycystic ovarian syndrome. The 37-yr-old father is 6' tall. No consanguinity, no family history of skeletal dysplasia or metabolic bone disease. Prenatal history significant for bed rest from 23wks gestation, gestational diabetes, and blood pressure fluctuations. The twins were born at 34 wks gestation (10 days past EDD). Twin A (male) weighed 4lbs5oz and was 16.75" long at birth. He spent 3wks in the NICU with respiratory problems, a grade 1 germinal matrix hemorrhage and GE reflux. Developmental milestones are delayed. At 1yr height was in the 0.01 percentile. Radiographic evaluation of his pectus deformity showed abnormal vertebrae, leading to genetics referral and a full skeletal survey which we interpreted as Kniest dysplasia. Ophthalmology evaluation within normal limits. No hearing problems. He is normocephalic with prominent eyes, hypertelorism, anteverted nares with normal bridge, no cleft palate. Rhizomelic, no bowing of the limbs. Twin B (female) weighed 4lbs2oz and was 18" long at birth. She has had no medical problems or developmental delay. At 14mos a skeletal survey revealed similar findings to her brother's. Height at 14mos was 26" (0.09 percentile) and weight 20lbs14oz (26th percentile). Regular facial features, hypertelorism. Some bowing of her lower extremities, no pectus deformity. Radiographically, Kniest is distinguishable from other type II collagenopathies by coronal clefts of the vertebrae and dumbbell-shaped femora. Dysplastic epiphyses and metaphyses. Platyspondyly, some wedging and anterior beaking. Shortened tubular bones with narrowed joint spaces. In summary, this is a set of dizygotic twins with Kniest dysplasia, first identified at 14mos of age when twin A underwent radiographs for evaluation of a pectus deformity. Molecular genetic analysis of *COL2a1* is pending. Paternal mosaicism is possible.

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Fractures in children with neurofibromatosis type 1. *J. George-Abraham[1], E. Schorry[1], S. Allen[2], M. Rieley[1], H. Kalkwarf[1], L. Martin[1], D. Viskochil[2], D. Stevenson[2].* 1) Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 2) University of Utah School of Medicine, Salt Lake City, UT.

Neurofibromatosis type 1 (NF1) is an autosomal dominant genetic disorder affecting many different body systems including the musculoskeletal system with bony abnormalities occurring in up to 1/3 of patients. There is a growing body of evidence supporting the idea of an underlying bony dysplasia predisposing individuals with NF1 to orthopedic complications, which include dystrophic scoliosis, tibial dysplasia, and bone cysts. Several studies have documented osteopenia in both children and adults with NF1, with bone mineral densities (BMD) about 1 standard deviation below the mean compared to control groups. However, the significance of this degree of osteopenia in relationship to fracture incidence is not well elucidated, particularly in children. We undertook a retrospective study to determine incidence and types of fracture in children with NF1, ages 5-20 years using a standardized questionnaire. Patients with known tibial pseudoarthrosis were excluded from the analysis. We surveyed 98 individuals with NF1 from two multidisciplinary NF centers and compared to 74 controls without NF1 of similar ages and gender as the NF1 individuals. Data collected included numbers and types of fractures, dietary calcium intake, and physical activity levels. The fracture incidence for the NF1 group was 29.6% versus 20.3% in the control group. Further, NF1 individuals with fractures had a higher incidence of multiple fracture sites and occurrences (44.4% and 36%, respectively) than the controls with fractures (21.4% and 14.3%, respectively). NF1 individuals with fractures also exhibited a significantly lower age at fracture compared to control individuals with fractures ($p = 0.01$). Our data suggest that fracture incidence is higher in young NF1 individuals in comparison to healthy controls. We are currently evaluating additional factors (dietary, exercise, BMD) which could contribute to fractures in NF1 children.

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Mutations in FKBP10 cause Bruck syndrome type I and Osteogenesis Imperfecta type III. *B.P. Kelley[1,2], F. Malfait[3], L. Bonafe[4], D. Baldridge[1], S. Symoens[3], A. Willaert[3], N. Elcioglu[5], L. Van Malderghem[6], C. Verellen-Dumoulin[7], Y. Gillerot[7], D. Napierala[1], D. Krakow[8], P. Beighton[9], A. Superti-Furga[4], A. De Paepe[3], B. Lee[1,2].* 1) Human and Molecular Genetics, Baylor College of Medicine, Houston, TX; 2) Howard Hughes Medical Institute, Chevy Chase, MD; 3) Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium; 4) Division of Molecular Pediatrics, Centre Hospitalier Universitaire Vaudois, 1011 Lausanne, Switzerland; 5) Department of Pediatric Genetics, Marmara University Medical Faculty, Istanbul, Turkey; 6) Centre de Genetique Humaine, Universite de Liege, Liege, Belgium; 7) Center for Human Genetics, Cliniques Universitaires St Luc and University of Louvain Medical School, Brussels, Belgium; 8) Medical Genetics Institute, Cedars-Sinai Medical Center, David Geffen School of Medicine at UCLA, Las Angelas, CA; 9) Division of Human Genetics, University of Capetown, Observatory, 7925 South Africa.

Osteogenesis imperfecta (OI) is a heritable disorder of connective tissue characterized by bone fragility and alteration in synthesis and post-translational modification of type I collagen. Autosomal dominant OI is caused by mutations in the genes (COL1A1 or COL1A2) encoding the chains of type I collagen. Bruck syndrome is a recessive disorder featuring congenital contractures in addition to bone fragility; Bruck syndrome type 2 is caused by mutations in PLOD2 encoding collagen lysyl hydroxylase, while Bruck Syndrome type 1 has been mapped to 17q12 but the gene has remained elusive so far. Recently, the molecular spectrum of OI has been expanded with the description of the mechanistic basis of a unique post-translational modification of type I collagen, i.e., 3-prolyl-hydroxylation. Three proteins, cartilage-associated protein (CRTAP), prolyl-3-hydroxylase-1 (P3H1, encoded by the LEPRE1 gene), and the prolyl cis-trans isomerase Cyclophilin-B (PPIB) form a complex that is required for fibrillar collagen 3-prolyl-hydroxylation and mutations in each gene have been shown to cause recessive forms of OI. Recently, an additional putative collagen chaperone complex, composed of FKBP10 (also known as FKBP65) and SERPINH1 (also known as HSP47), has also been shown to be mutated in recessive OI. Here, we describe five families with OI-like bone fragility in association with congenital contractures who all had FKBP10 mutations. Given the previous mapping of Bruck syndrome type 1 to the chromosomal region containing FKBP10, we conclude that FKBP10 mutations are the cause of Bruck syndrome type 1.

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FATCO Syndrome: A Case Report. *M.G. Lopez-Cardona[1,2], A. Del Toro-Valero[1], D. Carmona-Navarro[1], J. Fonseca[2].* 1) Dpto Biologia Molecular y Genomica, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Jalisco, Mexico; 2) Hospital Regional "Dr. Valentin Gomez Farias", ISSSTE, Zapopan, Jalisco, Mexico.

Introduction: FATCO syndrome also known as aplasia of the fibula, tibial campomelia and oligosyndactyly syndrome, has an autosomal dominant pattern of inheritance, although isolated cases have been reported. It is characterized by absence or hypoplasia of the fibula, shortening of the tibia and oligosyndactyly of feet and hands. Objective: To present a case report of a patient with FATCO syndrome without upper limb involvement. Case Report: 33 year old female product of the first pregnancy, apparently healthy nonconsanguinous parents. A maternal cousin has oligosyndactyly. The patient has been operated 56 times to lengthen both lower limbs. The physical examination revealed disproportioned short stature, normocephalous, no facial dysmorphies, normal ches, central obesity, lumbar hyperlodoris, normal genitals. Upper limbs with bilateral brachydactyly of 5th finger, normal palmar creases and cubitus valgus. Lower limbs with bilateral hypoplasia of tibiofibular segment. Right foot with absence of 4th and 5th toes and cutaneous syndactyly. Left foot with cutaneous syndactyly between all the toes and fusion of the 2nd and 3rd toes and brachydactyly of the 5th toe. Finger and toe nails are normal. Conclussions: The diagnosis of FATCO syndrome should be carefuly analyzed because it could be confused with other syndromes characterized by absence of the fibula, such as Fuhrman, Du Pan and Brachydactyly-ectrodactyly with fibular aplasia or hypoplasia syndromes. This patient shows the inferior limb characteristics of FATCO syndrome described by previous authors, however there is no upper limb affectation.

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Geleophysic dysplasia is a clinically and genetically heterogeneous disease with aberrant autophagic function. *P. Piccolo[1], P. Campeau[2], R. Polishchuk[1], J. Hicks[3], J. Tolmie[4], G. Andria[5], G. Parenti[1,5], V. Sabatino[1], B. Lee[2,6], C. Bacino[2], N. Brunetti-Pierri[1,5].* 1) Telethon Institute of Genetics and Medicine, Naples, Italy; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA; 3) Department of Pathology, Baylor College of Medicine, Houston, TX, USA; 4) Institute of Medical Genetics, Yorkhill Hospital, Glasgow, UK; 5) Department of Pediatrics, Federico II University, Naples, Italy; 6) Howard Hughes Medical Institute, Houston, TX, USA.

Geleophysic dysplasia (OMIM 231050) is an autosomal recessive disorder characterized by short-limb dwarfism, brachydactyly, and a 'happy-looking' facial appearance. It is frequently associated with cardiac valvular disease although the incidence and natural history of the heart complications remain unclear. Mutations in the *ADAMTSL2* gene resulting in dysregulation of TGF-β signaling have been recently recognized to be responsible for this condition. We screened for *ADAMTSL2* mutations seventeen cases of geleophysic dysplasia diagnosed on the basis of clinical and radiological findings. Pathogenic mutations were found in three of the seventeen cases, suggesting that additional and yet unknown gene(s) are involved in the pathogenesis of the disease. Ultrastructural analysis of skin fibroblasts of affected patients, either *ADAMTSL2* mutation positive or negative, showed the presence of cytoplasmatic lysosome-like inclusions. Biochemical analyses of fibroblasts failed to reveal the composition of such inclusions and therefore, the nature of the accumulated material remains unclear. Intriguingly, fibroblasts from affected patients showed a significative increase of autophagic functions as shown by Western blot and immunofluorescence analyses of LC3-II. TGF-β signaling pathway and autophagy and their potential relationship may play an important role in the pathogenesis of the disease.

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Cornelia de Lange syndrome: report a new case. *S. Juarez Garcia[1], L. Hernandez Gomez[2], E. Hernandez Gomez[3], D. Gomez Torres[4].* 1) Dept Neuropsicologia Instituto Nacional de Rehabilitacion, Mexico; 2) Dept Audiologia Instituto Nacional de Rehabilitacion, Mexico; 3) Biologia, Facultad de Estudios Superiores Iztacala; 4) Instituto Nacional de Rehabilitacion, Mexico.

Cornelia de Lange syndrome can be caused by mutation in the NIPBL gene. A mild variant of Cornelia de Lange syndrome (CDLS3) has been related to mutation in the SMC3 gene, which encodes another component of the cohesin complex. The de Lange syndrome is recognized on the basis of characteristic facies (low anterior hairline, synophrys, anteverted nares, maxillary prognathism, long philtrum, 'carp' mouth) in association with prenatal and postnatal growth retardation, mental retardation and, in many cases, upper limb anomalies. Type I, or classic the patients have the characteristic facial and skeletal changes of the diagnostic, prenatal growth deficiency, moderate to profound psychomotor retardation, and major malformations which result in severe disability or death. Type II, or mild, the patients have similar facial and minor skeletal abnormalities to those seen in type I; however, these changes may develop with time or may be partially expressed. They have mild-to-borderline psychomotor retardation, less severe pre- and postnatal growth deficiency, and the absence of (or less severe) major malformations. Type III, or phenocopy, includes patients who have phenotypic manifestations that are causally related to chromosomal aneuploidies or teratogenic exposures. Small hands and feet, limb reduction anomalies, proximally placed thumbs, hirsutism, synophrys, low hairline, cutis marmorata, low birth weight, and growth retardation are more common in the de Lange syndrome Most cases are sporadic. We present a new case: female mexican child 10 years old is the three child of non consanguineous parents, pregnancy without adversities prenatal and postnatal. Delay in the psychomotor development and of the language. Low size and weight. She present hirsutismo, synophrys, nares anteverted, depressed nasal bridge, long philtrum, low implantation of hair, hipertelorism, epycanto, sunken brown eyes, long eyelashes, 4 metacarpiano its short, limb muscle hypotrophy . Language and learning disabilities. Mild mental retardation.

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Mandibulofacial Dysostosis with Microcephaly, Cleft Palate, and Anomalous Ears. *M.A. Lines[1], S. Douglas[2], M. Guion-Almeida[3], Y. Alanay[4], G. Utine[4], D. Wieczorek[5], D. Bohm[6], A. Grix[7], C. Nava[8], L. Huang[2], J. Kohlhase[5], G. Baujat[8], D. Bulman[2], K. Boycott[1].* 1) Genetics, Children's Hospital of Eastern Ontario, Ottawa, Canada; 2) Ottawa Health Research Institute, Ottawa, Canada; 3) Clinical Genetics, Hospital for Rehabilitation of Craniofacial Anomalies, University of Sao Paulo, Sao Paulo, Brazil; 4) Clinical Genetics Unit, Hacettepe University, Ankara, Turkey; 5) Institute für Humangenetik, Universitätsklinikum Essen, Essen, Germany; 6) Center for Human Genetics Freiburg, Freiburg, Germany; 7) Genetics, Permanente Medical Group, Sacramento, CA, United States; 8) Medical Genetics, Université Paris Descartes, Necker-Enfants Malades Hospital, Paris, France.

Mandibulofacial dysostosis (MFD) is an inborn error of craniofacial development affecting structures derived from the first and second branchial arches. Treacher Collins syndrome is the prototypical form of MFD; however, MFD is also a feature of several other syndromes of unknown genetic etiology. Two previously published reports describe seven patients with a novel MFD phenotype characterized by microcephaly, cleft palate, and anomalous ears (Guion-Almeida et al, Clin Dysmorphol 15(3):171-4; Wieczorek et al, Am J Med Genet A 149A(5):837-43); we believe these reports describe the same phenotype, and have provisionally termed this conditon 'MFD with microcephaly' (MFDM). Building upon these earlier reports, and incorporating five additional cases, we present an updated clinical description of the MFDM phenotype. Cardinal features of this condition are microcephaly, metopic craniosynostosis, midface hypoplasia, micrognathia, cleft palate, short stature, and developmental delay. A highly specific ear phenotype is also observed, characterized by small, low-set ears, preauricular tags, auditory canal stenosis, and hypoplastic lobe/tragus. Other, inconsistently observed anomalies include cardiac septal defects, polydactyly, choanal atresia, and epilepsy. We are presently determining the molecular basis of this condition. *TCOF1* mutations have been excluded in several probands, confirming that MFDM is genetically distinct from Treacher Collins syndrome. In one affected individual, a complex de novo rearrangement was delineated on chromosomal microarray; this individual has a microdeletion of an agenic region of 4p12, in conjunction with a deletion/duplication of 17q21.31. The latter region contains a number of appealing positional candidates for the causative gene underlying this condition.

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LRRK2 exons 31, 34, 35, 38 and 48 mutation analysis in a cohort of PD patients with a dominant pattern of inheritance from South Italy. *V. Scornaienchi[1], P. Tarantino[1], F.E. Rocca[1], E.V. De Marco[1], F. Annesi[1], D. Civitelli[1], V. Greco[1], G. Provenzano[1], A. Bacilieri[1], G. Nicoletti[1,2], A. Quattrone[2], G. Annesi[1].* 1) Institute of Neurological Sciences, National Research Council, Mangone, Cosenza, Italy; 2) Institute of Neurology, University of Magna Graecia, Catanzaro, Italy.

Mutations in the LRRK2 gene have been associated both in PD patients with a dominant pattern of inheritance and patients which appear to be sporadic because of reduced penetrance. Reported frequencies may be lower limits because only particular exons or mutations were screened in most studies. Mutations affecting residues p.G2019 (p.G2019S) in exon 41 and p.R1441 (p.R1441C, p.R1441G, p.R1441H) in the exon 31 are common confirmed disease-associated variations. The frequencies of these mutations are highly population dependent as well as the putative risk factor variations R1628P and G2385R. We hereby present the results of mutation screening of exons 31, 34, 35, 38, and 48 of LRRK2 in 69 autosomal dominat PD patients from South Italy. Diagnosis of PD was based on the UK Brain Bank criteria. All participants were recruited through the Institute of neurology at the "University of Magna Graecia" of Catanzaro and all of them gave informed consent. Mutation analysis of these five LRRK2 exons was carried out by PCR and sequencing. Our cohort was previously analyzed for alpha-synuclein gene mutations and LRRK2 exon 41 mutations (p.G2019S, p.I2012T and p.I2020T) and all patients resulted negative. Until now we screened all the 69 patients for LRRK2 exon 31, 35, 38 and 48. We identified the R1441C mutation (exon 31) in heterozygous state in a 56-year-old male with dominant PD. This patient descends from a PD family with a deceased affected mother and a deceased father with gait impairment. There was not other affected members in both maternal and paternal ascendants. In the other PD cases we found three described polymorphisms; IVS47-9 del(T) (58%) and A7155G (17%) in exon 48 and IVS35+23T®A in exon 35 (53%). We observed a R1441C mutation frequency of 1,5% (1/69) in our PD cohort from South Italy. This frequency is higher than other studies on italian sporadic PD patients have shown (0,01- 0,6%) thus confirming that the role of mutations in PD genes varies according to the origin of the studied population. Further, in order to well assess the R1441C mutation frequency in South Italian PD patients we will effect a mutation analysis of 500 PD sporadic cases.

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OTO-PALATO-DIGITAL SYNDROME TYPE 2. CLINICAL AND MOLECULAR CORRELATION. *R. Baez-Reyes[1,2,3], A. Hidalgo-Bravo[4], S. Kofman-Alfaro[4].* 1) Dept Gen, Inst Natl de Perinatologia,; 2) Dept Gen, Clinic of Specialities of Women, SEDENA ,; 3) Escuela de Ciencias Quimico-Biologicas, IPN,; 4) Dept Gen, General Hospital of Mexico, Mexico City, MEXICO.

The otopalatodigital type 2 syndrome (OPD2), it's part of a spectrum of four entities with phenotype related and considered alellics variants (OPD1, OPD2, frontometaphyseal dysplasia and Melnick-Needles syndrome). It has a model of inheritance X-linked, associating to mutations that generate in the majority gain in the function of the gene of the Filamin A (FLNA), located in Xq28, composed by 48 exons and that codes for the Filamin protein with molecular weight of 280 kD uniting to actin filaments. The patient is product of first pregnancy, his mother and father with 18 and 25 years old, not consanguineous. The mother went to the consultation of Prenatal Dignosis to the 23.3 weeks with an ultrasound that showed multiple defects. It's was realized an amniocentesis with result 46,XY. To the 34.2 weeks was born a male for caesarean operation, with many features to the clinical examination: skull with wide fontanel and sutures, prominent forehead, ocular hypertelorism, antimongoloid slant to palpebral fissures, flat nasal bridge, small mouth with central cleft palate, micrognathia, dysmorfics ears, short neck, extremities upper with flexed fingers and syndactyly fingers in bilateral form, the extremities lower with limitation to the extension of both knees and feet with absence of great toes and left cryptorquidism. X-ray of skull base with undermineralizaion of cranial vault, humerus, femur and tibia were curved and small fibula, hypoplastic and irregular metacarpals and absent halluces; Echocardiogram with minimum interventricular communication subaortic; renal ultrasound normal; magnetic resonance with loss of volume left corticosubcortical, ventriculomegaly, callous body and fossa brain hypoplastic; visual potentials with decrease in the vision; auditory potentials with conductive hearing loss, realizing the diagnosis of OPD2. We took a blood sample of the baby's and mother for the extraction of the DNA and by means of PCR the exon was amplified 3 of the gene FLNA using the primers: 5'-TCC AGA ATC TGT TCC AGA GCG-3'(forward) and 5'-GCT GTG AAG GTT GCT GTT CCT-3'(reverse). In the patient and in her mother were discovered an insertion of a single nucleotide identified (Adenine) in the codon 209 of the exon 3 of the gene FLNA, same that generates a landslide of reading mark seemingly without codon of premature unemployment. This mutation yet not reported into the literature. The genetic counseling was given to his parents.

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Interval Disease despite Routine Radiographic Screening in Vascular Ehlers-Danlos Syndrome. *M.A. Giovanni, M.F. Murray, J.P. Annes.* Division of Genetics, Brigham & Women's Hospital, Boston, MA.

Vascular Ehlers-Danlos syndrome (EDS) is an autosomal dominant connective tissue disorder characterized by vascular and visceral organ rupture with minor findings of thin translucent skin, easy bruising, acrogeria, and characteristic facial features. Vascular EDS is caused by a mutation in COL3A1 causing abnormalities in type III collagen. Here we present a 39 year old man who is monitored in an adult genetics clinic due to a familial mutation in COL3A1 (c.3473G>A, p.Gly1158Asp). The patient denied easy bruising, joint hypermobility, history of vascular, muscle, or tendon rupture; he denied any medication use. The patient reported a history of competitive athletics through his high school years with significant weight training into his 20's. Physical exam revealed a 5'7.7", normotensive, 187 pound Chinese-American man with pes planus and velvety skin; his Beighton score was 2. Notably absent was the presence of cigarette-paper scars, thin lips, narrow nose, high arched palate, or pectus deformity. The patient's family history included the death of his mother at age 53 secondary to intracranial hemorrhage. One maternal uncle died at age 36 of intracranial hemorrhage. A second maternal uncle brought the family to clinical attention with a celiac artery dissection at age 44, leading to COL3A1 testing. We instated our usual screening protocol including vascular imaging at an interval of every six months, alternating between CT angiography and MRA with annual clinical exams. Baseline imaging of the vascular tree was read as normal. Subsequent imaging studies showed normal vascular anatomy at each time point including November 2009. In February 2010, three months after his radiographic screening the patient presented to the emergency department at an outside institution with fever, cough, and left-sided abdominal pain. He was discharged with a diagnosis of community acquired pneumonia and possible renal infarct. The patient then followed up at our institution where CT angiography performed approximately one week later revealed left renal artery dissection with pseudoaneurysms and a left renal infarct. The patient was stented and successfully completed a three month period of anticoagulation. We present this case as a challenge in the medical management and screening of individuals with Vascular EDS. Due to the rapidly progressing nature of aneurysm and rupture, the utility of vascular imaging in preventing emergent events is questionable.

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Generalized arterial calcification of infancy (GACI) is associated with biallelic mutations in *ABCC6*. *Y. Nitschke[1], U. Botschen[1], N. Chassaing[2], C. Deshpande[3], S. Garber[4], R. Chikarmane[5], B. Steinmann[6], T. Shahinyan[7], L. Martorell[8], L. Martin[9], R. Terkeltaub[10], F. Rutsch[1].* 1) Department of General Pediatrics, Münster University Children's Hospital, Münster, Germany; 2) Department of Genetics, Toulouse University Hospital, Toulouse, France; 3) Department of Clinical Genetics, Guy's Hospital, London, UK; 4) Division of Neonatology, Department of Pediatrics, Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, PA, USA; 5) Institute of Medical Genetics, St. Peter's University Hospital, New Brunswick, New Jersey, USA; 6) Division of Metabolism and Molecular Pediatrics, University Children's Hospital, Zurich, Switzerland; 7) Arabkir Joint Medical Centre, Yerevan, Armenia; 8) Department of Genetics, Hospital Sant Joan de Déu, Barcelona, Spain; 9) Department of Dermatology, Angers University Hospital, Angers, France; 10) VA Medical Center, Department of Medicine, University of California San Diego, San Diego, California.

Generalized arterial calcification of infancy (GACI, MIM 208000) is a rare autosomal recessive disorder characterized by calcification of the media and myointimal proliferation leading to arterial stenoses frequently associated with death in early infancy. In 80% of GACI patients, the disease is caused by mutations in *ENPP1*, which encodes ecto-nucleotide pyrophosphatase/ phosphodiesterase 1 (NPP1). NPP1 is a generator of extracellular inorganic pyrophosphate, a physiologic inhibitor of hydroxyapatite deposition. The genetic cause of 20% of analysed GACI patients remains obscure. Most recently, mutations in *ABCC6* (ATP-binding cassette subfamily C number 6), the gene associated with pseudoxanthoma elasticum (PXE, MIM 264800), have been found in a male patient with PXE, whose younger brother died of generalized arterial calcification of infancy at the age of 15 months (Am J Med Genet 152A:118-123; 2010). Unfortunately, no DNA of the deceased younger brother was available. No *ENPP1* mutation was found in the living family members. We therefore hypothesized that GACI could be independent of *ENPP1*, but related to *ABCC6* mutations. To prove our hypothesis, we sequenced all 31 exons and their flanking splice sites of *ABCC6* in 18 GACI patients without mutations in the coding region of *ENPP1*. Excluding SNPs, we detected in 5 patients 6 different biallelic mutations in *ABCC6* known to cause PXE and the novel mutation c.450insC (p.A151fsX45). Two patients were homozygous for mutation c.3662G>C (p.R1221H) and c.3940C>T (p.R1314W) respectively. The other three patients were compound heterozygous carriers of at least two of the following mutations: c.2787+1G>T and 3736-1G>A affecting splicing, c.1552C>T (p.R518X), c.3105-3107delCTT (p.F1036del), c.3940C>T (p.R1314W) and c.450insC (p.A151fsX45). Our study emphasizes that GACI is not only associated with mutations in *ENPP1*, but also with mutations in *ABCC6*, recently described in PXE. We conclude that deficiencies of NPP1 and ABCC6 lead to alterations in the same pathogenetic pathway.

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Dysmorphic features, learning disability, immune abnormalities and predisposition to vasooclusive disease in two sisters: a new autosomal-recessive syndrome? *M. Velinov[1], N. Dolzhanskaya[1], P. Ramaswamy[2], L. Barinstein[2], R.M. Stuart[3], P. Kahn[4], N. Feldstein[3], R.E. Madrid[1].* 1) NYS Institute for Basic Research in Developmental Disabilities, Staten Island, NY; 2) Maimonides Infants & Children Hospital Brooklyn, NY; 3) Columbia University Medical Center, New York, NY; 4) New York University School of Medicine, New York, NY.

A 20 year-old female patient presented with mental retardation and a history of Moya moya disease with ischemic strokes at the age of 8. She had a learning disability prior to the stroke. After the stroke her cognitive impairment became more pronounced. This patient's parents were first cousins of Middle Eastern origin. The family history was significant for 6 close family relatives, all of whom suffered strokes in their 60s or 70s. The patient's 16 year-old sister had learning disability and chronic muscle pain in her legs. Her EKG showed evidence of mild intraventricular conduction delay with a QRS duration of 100 msecs with T wave changes suggestive of myocardial damage. By echocardiogram her ventricular systolic function was at the lower end of normal. The two sisters had a similar dysmorphic facial appearance including prominent philtrum, bulbous nose and severe acne. They both had increased subcutaneous tissue in their faces and slim bodies. Both sisters were found to have elevated levels of Rheumatoid factor, C-reactive protein, total IgM and erythrocyte sedimentation rate on repeat measurements. Comprehensive laboratory testing for rheumatoid/ autoimmune disorders was negative. Chromosome analysis and array CGH analysis were normal. Muscle biopsy was done on the younger sister and revealed normal morphology. Genome array expression analysis on peripheral native lymphocytes showed abnormal expression levels of a number of immune response-associated genes in both patients compared to healthy controls. Significant up-regulation was observed in the gene RNF125, a gene involved in T lymphocyte activation. Most significant down-regulation was observed in the gene IFIT1 that is involved in suppression of anti-viral responses. We propose that these two sisters suffer from a new autosomal-recessive syndrome. Carrier status for the reported condition may predispose to strokes as observed in family relatives.

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A Spectrum of Mutations found in Noonan Syndrome. *K.M.B. Vinette[1], S.M. Kirwin[1], E. Hopkins[2], K. Gripp[2], C. Jornlin[1], N. Manolakos[1], V. Funanage[1]*. 1) Molec Diagnostics Lab, Nemours/A I duPont Hosp, Wilmington, DE; 2) Medical Genetics, Nemours/duPont Hosp for Children, Wilmington, DE.

Objective: As a Molecular Diagnostics Laboratory in a pediatric setting, we encounter a wide variety of Noonan and LEOPARD syndrome associated mutations. These clinically related disorders are caused by a family of genes encoding the RAS/MAPK cell signaling pathway. We describe here several novel changes found in patients with a clinical diagnosis of Noonan syndrome referred for molecular testing. Methods: A 5-tiered sequencing approach with sequential testing for the genes associated with Noonan syndrome is performed in our laboratory. The typical order is as follows: PTPN11, SOS1, RAF1, KRAS and SHOC2. Results: A total of 65 probands and 21 family members were tested for mutations consistent with Noonan syndrome. 1) PTPN11, [n=47], 14 (30%) samples positive, showing 10 different mutations. Two patient results were found of great interest, as one patient carries two different mutations within exon 3 in cis (1 novel and 1 previously described); another patient carries a previously described mutation within exon 4 and a second change (deletion of at least exon 4) in trans. 14 family members were tested with 2 positives. 2) SOS1, [n=28] with 4 confirmed mutations (14%), 3 previously described and one novel mutation. 3) RAF1, [n=33] 5 patients positive (15%), and interestingly, no parent [n=3] was found to carry their child's mutation. 4) KRAS, [n=24] 2 patients were found to carry a mutation (8%); one determined to be de novo and the other mutation was previously described. 5) SHOC2, no mutations were detected in 5 patients tested. Conclusion: Molecular testing confirmed a suspected diagnosis of Noonan syndrome in 25 of 65 patients (38%), with mutations in PTPN11 in 14 of 25 mutation positive individuals (56%), SOS1 in 4/25 (16%), RAF1 5/25 (20%), and KRAS 2/25 (8%). This mutation distribution is consistent with previous data suggesting that about half of all cases are due to PTPN11 mutations, and that changes in KRAS are less common than those in RAF1 and SOS1. This mutation spectrum supports the use of our sequential testing strategy, as the percentage of positive results for each gene sequenced is higher than it would be if all genes were studied in parallel.

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Severe course of Alagille syndrome associated with a novel NOTCH2 mutation. *C. Zweier[1], G. Hammersen[2], R. Behrens[3], C. Gläser[4], C. Schmidt[4], A. Müller[5], H.E. Ulmer[5], A. Rauch[1,6]*. 1) Institute of Human Genetics, University Erlangen-Nuremberg, Erlangen, Germany; 2) Cnopf's Pediatric Hospital, Nuremberg, Germany; 3) Zentrum für Neugeborene, Kinder und Jugendliche, Südklinikum, Nuremberg, Germany; 4) Institute of Human Genetics and Medical Biology, Halle, Germany; 5) Department of Paediatric Cardiology, University Medical Centre, Heidelberg, Germany; 6) Institute of Medical Genetics, University of Zurich, Schwerzenbach-Zurich, Switzerland.

Alagille syndrome is a multisystemic disorder with hepatic bile duct paucity and cholestasis in association with other manifestations. In most of the cases haploinsufficiency of JAG1 can be identified as causative for the disease. Only recently also mutations in NOTCH2, the receptor of Jagged 1, have been shown to cause Alagille syndrome in two families. We report on the third mutation in NOTCH2 associated with an unusual severe course of Alagille syndrome with systemic arterial dysplasia. The male patient was born small for gestational age and the neonatal period was complicated by bilateral pneumothorax, renal insufficiency, persistent ductus arteriosus, arterial hypertension and cholestasis. No ophthalmologic or skeletal anomalies were found. At the age of 6 months the boy was dystrophic and showed jaundice and alopecia. In the further course he developed hyperparathyreoidism and recurrent crises with seizures, hypoxia and lactate acidosis. Extensive metabolic testing showed no abnormal results, liver biopsy showed moderate cholestasis and mild hypoplasia of bile ducts. At the age of 12 months the patient started to sit and spoke first words. Because of increasing hypertension of the pulmonic arteries intracardiac catheter examination was performed which revealed filiform endings of the peripheral vessels and furthermore hypoplasia and multiple stenosis of the aorta and large arteries. The patient deceased at the age of 15 months. Karyotyping with FISH-analysis at the JAG1-Locus, JAG1 sequencing and MLPA had revealed normal results. NOTCH2 sequencing showed the novel de novo splice site mutation c.6027G>A. This is the third mutation in NOTCH2, confirming its causative role in Alagille syndrome. If the severe vascular phenotype is an effect of the novel NOTCH2 mutation or an extreme manifestation within the Alagille spectrum regardless of the underlying gene remains unclear.

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HEMATOLOGIC DIAGNOSIS OF MUCOPOLISACCHARIDOSIS (MPSs)IN PEDIATRIC PATIENTS FROM THE DEPARTMENT OF GENETICS. TWO CASES REPORT, HOSPITAL PARA EL NIÑO POBLANO, MEXICO. *P. Sanchez-Meza[1], J.M. Aparicio-Rodriguez[2,4], M.L. Hurtado-Hernandez[3], I. Marroquin-Garcia[3]*. 1) Hematology; 2) Genetics; 3) Cytogenetics, Hospital para el Nino Poblano, Puebla; 4) Estomatology, Benemerita Universidad Autonoma de Puebla, Mexio.

INTRODUCTION. The mucopolysaccharidosis (MPSs) are rare genetic disorders in children and adults. They involve an abnormal storage of mucopolysaccharides, caused by the absence of a specific enzyme. Without the enzyme, the breakdown process of mucopolysaccharidosis is not completed. Development and the function of various organs of the body. The MPS diseases are part of a larger group of disorders known as Lysosomal Storage Disorders (LSDs). A deficiency in the activity of a specific protein (enzyme) is observed in our body cells. MATERIAL AND METHODS. At hematology, the type of MPSs was diagnosed as MPS type VI or Maroteaux Lamy syndrome taking in consideration; some lymphocytes has multiple blue intra lysosomal granulations, big vacuoles and different granulations size. The monocytes had more and bigger blue intra lysosomal granulations than normal. All mature neutrophils had also a huge quantity of blue intra lysosomal granulations that can not be different from those toxic cell granulations. However, in both patients from this study, blue granulations were observed into the esosinophils, such granulations had different size, shape and color that varied from gray to black. CONCLUSION. The role of lysosomes is to degrade final material made by these cells into simpler products in order to be used again. This process requires the sequential action of different enzymes. If one of these enzymes is present in insufficient amounts, the recycling process cannot proceed and the undegraded material remains stored within the lysosome and, therefore, the cell. leading to the emergence of hematological and clinical symptoms. It is important to mention that lysosomal granulation storage in both hematological cells (eosinophils y neutrophils) are patgnomonic characteristics in order to confirm with the clinical symptoms MPSs. Therefore we considered that hematological morphology became a very powerful tool in this metabolic diseases. Some of the MPSs are inherited in a autosomic recessive trait, as MPS II or Hunter Syndrome, the MPS diseases are caused by a recessive gene. Where both parents are considered as healthy carriers. However only the Hunter syndrome has a different form of inheritance as sex-linked where females may be carriers of the disease. The early genetic diagnosis is important since some of the MPSs have already enzymatic treatment and not only the clinical manifestations are stopped but the quality of life is improved.

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TRPV4 gene analysis in three Brazilian patients with skeletal dysplasias - A novel mutation in metatropic dysplasia. *G.L. Yamamoto[1], F.B. Piazzon[1], M.C. Moreira[1], L.A.N. Oliveira[2], C.A. Kim[1], A.C. Pereira[3], D.R. Bertola[1]*. 1) Unidade de Genética Médica, Instituto da Criança, Faculdade de Medicina da Universidade de São Paulo, São Paulo, São Paulo, Brazil; 2) Radiologia, Instituto da Criança, Faculdade de Medicina da Universidade de São Paulo, São Paulo, São Paulo, Brazil; 3) Laboratório de Biologia e Cardiologia Molecular, InCor, Faculdade de Medicina da Universidade de São Paulo, São Paulo, São Paulo, Brazil.

The TRPV4 gene, that codes a calcium-permeable ion channel, is associated with several previously considered distinct skeletal dysplasias - autosomal dominant brachyolmia, spondylometaphyseal dysplasia Kozlowski type, metatropic dysplasia, spondyloepiphyseal dysplasia Maroteaux type and parastremmatic dysplasia. Due to their rarity, few patients have been screened for mutations in this gene, but it seems that these disorders are allelic conditions. It is therefore important to evaluate additional patients, especially from different populations to confirm this specificity and to expand the mutational spectrum. We performed a bi-directional sequencing analysis of the TRPV4 gene in three patients, one presenting a clinical/radiological diagnosis of spondylometaphyseal dysplasia Kozlowski type and additionally a mild ventriculomegaly, and two with diagnosis of metatropic dysplasia, one with a mild form and the other severe with death due to respiratory compromise at 4 months of age. The results showed p.A594H, p.P799L and p.W733G mutations in the TRPV4 gene, respectively. The latter is a novel mutation which Polyphen analysis predicts damage to the protein. The other two mutations found in our patients were highly prevalent in the few cases described by different groups. None of the parents carried the specific mutation found in their child. These results corroborate the fact that spondylometaphyseal dysplasia Kozlowski type and metatropic dysplasia are caused by different mutations in the TRPV4 gene, all in heterozigosity. The presence of ventriculomegaly in a patient with spondylometaphyseal dysplasia Kozlowski type could be coincidental or this SNC abnormality could be part of this skeletal dysplasia. Further clinical description of other cases is necessary to confirm this association.

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Survival of a Male Mosaic for PORCN Mutation with Mild Focal Dermal Hypoplasia Phenotype. *K. Kosaki[1], H. Yoshihashi[2], H. Ohki[2], A. Ishiko[3].* 1) Dept Pediatrics, Keio Univ, Tokyo, Japan; 2) Dept Clin Genet, Tokyo Metropolitan Childrn's Med Ctr; 3) Dept Dermatol, Keio Univ, Tokyo, Japan.

We report a 46,XY boy with a mild FDH phenotype who had both wild-type and mutated copies of the PORCN gene and was, therefore, mosaic for the mutation. He had cutaneous syndactyly, hydronephrosis, and nail dystrophy. Small whitish depigmented spots, which were slightly depressed from the skin surface, were distributed linearly on the trunk and arms. Aside from these findings, streaks of brown-pigmented macules were seen on the dorsal aspect of the legs. Both the linear arrangement of the whitish spots and the streaks of pigmented macules followed the lines of Blaschko. The phenotype of the patient, who did not exhibit cribriform atrophy, telangiectasia or fat herniation, seemed to be much milder than that of typical female patients with FDH. Analysis of the genomic DNA extracted from the peripheral lymphocytes revealed a transition 129 (G to A9 within exon 1 of PORCN, which leads to a non-sense mutation W43X. The percentage of peripheral lymphocytes carrying a mutation was estimated to be 50% by the subcloning and sequencing of individual clones of the PCR product amplified across the mutation. This patient's case history provides further molecular evidence supporting the concept that "male FDH" does exist and that typical features such as telangiectasia and fat herniation are sometimes abs.

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A Case of Suspected Ankyloblepharon-ectodermal dysplasia-clefting syndrome(AEC) from Iran with possible autosomal recessive inheritance. *H. Pour-Jafari[1,2], R. Yadegarazari[1], B. Pourjafari[2], A. Yazdanfar[3], S. Irani[4], F. Talebzadeh[2], K. Hasrak[1].* 1) Molec Med & Gen, Sch Med, Hamadan Un, Hamadan, Iran; 2) Research Center for Molecular Medicine, Hamadan University of Medical Sciences, Hamadan, Iran; 3) Dermatology Dept., School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran; 4) Oral Pathology Dept., School of Dentistry, Hamadan University of Medical Sciences, Hamadan, Iran.

Ankyloblepharon-ectodermal dysplasia-clefting syndrome or Ankyloblepharon filiforme adenatum-ectodermal dysplasia-cleft palate syndrome (AEC) is one of at least 150 known types of ectodermal dysplasia. These disorders affect tissues that arise from the ectodermal germ layer, such as skin, hair, and nails. According to most references, it is an autosomal dominant disorder and caused by different mutations in TP73L (p63) gene. In the other hand some studies have showed an autosomal recessive mode of inheritance. We report a case with AEC syndrome and possible autosomal recessive inheritance from Iran. The patient was an 8-month-old girl, with ankyloblepharon filiforme adnatum, cleft palate and skin erosions at birth. She had parental consanguinity and presented many of major and minor criteria of the disease. Other disorders like CHAND syndrome, Rapp-Hodgkin syndrome, ectrodactyly-ectodermal dysplasia-clefting (EEC) syndrome and other congenital ectodermal dysplasia diseases can mimic AEC syndrome because of similar and overlapping clinical features. Those points were considered in our study, but her clinical features had no conformity and we rolled them out according to diagnostic criteria of the mentioned disease. However, more studies need to be done for revealing the type of molecular defect and definite pattern of inheritance.

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***POMC* gene mutation analysis and genotype-phenotype correlations in two patients with early-onset obesity.** *Y. Yang[1], M.S. Mendiratta[2], A. Balazs[2], A. Willis[1], L. Potocki[1], V.C. Sheffield[3], L.P. Karaviti[2], C.M. Eng[1].* 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Pediatrics, Baylor College of Medicine, Houston, TX; 3) Department of Pediatrics, University of Iowa, Iowa City, IA.

Pro-opiomelanocortin (POMC) is the precursor protein for several peptide hormones including adrenocorticotropic hormone (ACTH), melanocyte-stimulating hormones (MSH) α, β, and γ, and β-endorphin. The *POMC* gene, as well as four other genes in the leptin signal pathway, *LEP*, *LEPR*, *PCSK1*, *MC4R*, is associated with rare, monogenic forms of obesity. Additional phenotypes caused by bi-allelic *POMC* mutations include adrenal insufficiency due to lack of ACTH and red hair pigmentation due to MSH deficiency. The latter may be less obvious in patients of ethnic groups other than Caucasian.

We have recently developed clinical test for *POMC* gene sequencing and identified *POMC* mutations in two patients with early-onset obesity. The first patient is an 18-month Hispanic girl with a history of adrenal deficiency, obesity and developmental delay. The patient was found to be homozygous for a novel c.231C>A (p.Y77X) nonsense mutation in the *POMC* gene. Parental studies showed that both parents were heterozygous for the mutation and therefore confirmed the homozygous state of the mutation in the patient. This deleterious mutation introduces a premature stop codon at amino acid position 77 of the POMC protein and is predicted to result in absence of hormones encoded downstream of the mutation, namely γ-MSH, ACTH, α-MSH, β-MSH, and β-endorphin. The second patient is a 20-month Caucasian boy with morbid obesity, tall statue, rapid excessive weight gain and developmental delay. A heterozygous c.706C>G (p.R236G) missense mutation was identified in the *POMC* gene of this patient. A second mutation was not detected. The c.706C>G (p.R236G) mutation in the heterozygous state was previously reported in patients with obesity but not affected by adrenal insufficiency or red hair pigmentation. In vitro and populations studies indicated that the mutation may confer an inherited susceptibility to obesity by producing an aberrant β-MSH/β-endorphin fusion protein that competes with the nature product for MC4R binding and interferes with central melanocortin signaling.

In summary, we have identified a novel nonsense mutation and a previously characterized missense mutation in the *POMC* gene of two obese children. In addition to confirmation of diagnosis, the clinical molecular testing of *POMC* mutations may provide better understanding of genotype-phenotype correlations and enable better disease management and genetic counseling.

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A case of Congenital Hypomyelinating Neuropathy, a rare disorder and expanding the natural history. *A. Alfares[1], S. Melançon[1], N. Braverman[1], C. Hawkins[2].* 1) Department of Medical Genetics, McGill University Health Center, Montreal, QC, Canada; 2) Dept of Paediatric Laboratory Medicine, The Hospital for Sick Children, Toronto, ON, Canada.

Congenital hypomyelinating neuropathy (CHN) is characterized clinically by early onset of hypotonia, areflexia, distal muscle weakness, and very slow nerve conduction velocities (NCV). Only 8 cases have been previously reported. The neonatal forms of CHN feature severe decreased myelin sheath formation and are associated with defects in the Myelin Protein Zero (MPZ) or in the Early Growth Response 2 (EGR2) genes. Myelin protein-zero is the major structural protein of peripheral myelin, accounting for more than 50% of the protein present in the sheath of peripheral nerves. Expression of the MPZ gene is restricted to Schwann cells. Mutations in the MPZ gene cause distinct neurologic diseases, including CHN, Déjérine-Sottas syndrome (DSS), and CMT1B. The protein encoded by EGR2 is a transcription factor targeting genes critical for myelination in Schwann cells. Defects in EGR" are associated with Charcot-Marie-Tooth disease type 4E (CMT4E), CHN with early-onset slow NCV and a Déjérine-Sottas syndrome-like presentation. Respiratory dysfunction and cranial nerve abnormalities may also occur. We describe a new case of CHN in an infant with diffuse hypotonia and weakness, mild dysmorphic features and joints contractures. A nerve biopsy confirmed the diagnosis of CHN by showing diffusely thin myelin sheaths. Molecular analysis of MPZ, EGR2 and seven other genes associated with CHN or CMT failed to identify any pathological variants. This case provides additional insights into the natural history of this rare congenital disorder and suggests that (1) there are mutations in these genes not identifiable by routine gene sequencing, or that (2) since the sensitivity of mutation detection in these genes is low, perhaps defects in other genes involved in myelin synthesis pathways might cause this disorder. We will review these potential candidates.

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IL1RAPL1 Gene Deletion as a Cause of X-linked Mental Retardation. *J.A. Hellings, E.L. Youngs, M.G. Butler.* Departments of Psychiatry & Behavioral Sciences and Pediatrics, University of Kansas Medical Center, Kansas City, KS 66160.

Mental retardation affects approximately 2% of the population with males outnumbering females due to involvement of over 300 genes on the X chromosome. The most common form of X-linked mental retardation (XLMR) is fragile X syndrome. We report a family with an apparent XLMR pattern with both the proband and his mother having an Xp21.3 deletion detected with chromosomal microarray analysis involving the IL1RAPL1 gene. He was born full term, walked at 16 months and had first words at 8 months. A normal chromosome study was reported at 4 years of age. Our proband has one full brother with learning problems and a maternal half brother with 47,XYY syndrome and mental retardation. Learning problems were reported in two maternal uncles, one maternal aunt and three cousins. The proband's mother attended special education classes and dropped out of school in the eleventh grade. On physical examination at 15 years, 9 months of age, our proband was cooperative and had a normal height, weight and head circumference. He had prominent supraorbital ridges, an anterior tilt to his head, deep set eyes, a sloping forehead with a broad face, synophrys and anterior hair whorls, and a short prominent nose with a long wide philtrum. He presented with atypical autism, ADHD, self-injury, impulsive aggression and an IQ of 55 with limited expressive language. A chromosomal microarray study showed a 950 kb deletion (located at 29.13 to 30.08 Mb from pter) involving bands Xp21.3-p21.2. The interleukin 1 receptor accessory protein-like 1 (IL1RAPL1) gene located in this region was partially deleted and confirmed by FISH. The proband's mother had the same deletion. IL1RAPL1 is highly expressed in the postnatal brain, specifically hippocampus suggesting a specialized role in memory and learning abilities. This gene is coined mental retardation, X-linked 21; MRX21 (OMIM 300143) and illustrates the importance of interleukin signaling pathways and the immune system in cognitive function. Hence, our family is the third reported with involvement of this gene in causing XLMR and the first identified with chromosomal microarray studies. Our proband showed similar dysmorphic features noted in previously reported males including a broad face with sloping forehead, ptosis, synophrys, prominent suprorbital ridges and nasal root, long and wide philtrum and a small mouth. Additional reports are needed to further characterize whether syndromic features are related to disturbances of this gene.

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Ablepharon Macrostomia syndrome, a heterogeneous disorder: evidence for both autosomal dominant and recessive inheritance. *S. Kallish[1], SP. Bartlett[2,3], JA. Katowitz[4,5], DM. McDonald-McGinn[1,6], EH. Zackai[1,6].* 1) Division of Human Genetics, Children's Hospital of Philadelphia, Philadelphia, PA; 2) Division of Plastic Surgery, Children's Hospital of Philadelphia, Philadelphia, PA; 3) Department of Plastic Surgery, University of Pennsylvania School of Medicine, Philadelphia, PA; 4) Division of Ophthalmology, Children's Hospital of Philadelphia, Philadelphia, PA; 5) Division of Ophthalmology, University of Pennsylvania School of Medicine, Philadelphia, PA; 6) Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, PA.

Ablepharon Macrostomia syndrome (AMS) is a rare syndrome characterized by absent eyelids, fusion defects of the mouth, abnormal ears, ambiguous genitalia, skin findings, and speech delay. We present three patients with features of AMS.

Patient 1 was noted prenatally to have micrognathia, finger anomalies, and club feet. At birth she was found to have deficient skin of the eyelids and lips, macrostomia, absent nipples, and hypoplastic labia majora and minora. Examination of her extremities revealed 1st-2nd finger syndactyly. Her left hand had a small left thumb, which was syndactylous with the 2nd digit. Her feet had total syndactyly of digits 2 through 5 bilaterally, with partial syndactyly of the 1st digit. They were medially rotated bilaterally. Her development was normal at 7 months of age. Syndactyly of the toes has been reported once before in association with AMS, in two siblings. One had typical features of AMS and the other had overlapping features of AMS and Fraser syndrome (in which syndactyly is more common), suggesting autosomal recessive inheritance (Cavalcanti et al, Am J Med Genet 2007).

Patient 2 presented prenatally with an omphalocele. At birth she was also noted to have exophthalmos, absent eyelids, abnormal ears, and a large mouth with unfused labial commissures. On examination, she was found to have the hair abnormalities typical of AMS and hypoplastic labia of her genitals. Her development was normal at 2 months of age. Patient 3 is the father of patient 2. He also had an omphalocele. He had hypoplastic ears, minimal eyelid tissue with sparse eyebrows, and absent labial commissures of the mouth. His hands had single palmar creases with webbing of his fingers. The diagnosis of AMS in both father and daughter in this family suggests autosomal dominant inheritance. One other family with autosomal dominant inheritance of AMS has been reported (Ferraz et al, Am J Med Genet 2000).

Fewer than 20 patients with AMS have been described in the literature thus far. These patients represent further clarification of the features of AMS, including more severe foot anomalies than previously described. These cases also provide further evidence that AMS is a heterogeneous disorder, demonstrating both dominant and recessive inheritance.

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Axenfeld-Rieger, microcephaly and congenital hypothyroidism: A New Syndrome? *E. McPherson[1], C. Zaleski[1], I. Zador[1], H. Murali[1], A. Grajewski[2].* 1) Marshfield Clinic, Marshfield, WI; 2) University of Minnesota, Minneapolis, MN.

We report a family in which 8 individuals in 3 generations have had anterior chamber abnormalities, primarily Axenfeld-Rieger anomaly along with varying combinations of congenital hypothyroidism, microcephaly, developmental delay and minor limb anomalies. Although they have minor facial anomalies similar to other patients with Rieger syndrome (flat midface, thin upper lip), these individuals do not fit any of the known syndromes with Axenfeld-Rieger anomaly such as Rieger syndrome type 1 (with hypodontia and periumbilical abnormalities due to mutations in PITX2), Rieger syndrome type 2 (dental, hearing, and cardiac anomalies, hydrocephalus), Rieger syndrome type 3 (cardiac and sensorineural hearing loss due to mutations in FOXC1), Axenfeld-Rieger with leukoencephalopathy (due to mutations in COL4A1), or other syndromes with anterior chamber anomalies such as SHORT syndrome or Peters Plus. Furthermore, one affected child with bilateral congenital glaucoma due to Rieger anomaly, congenital hypothyroidism, microcephaly with OFC -3SD, and developmental delay tested negative for mutations in PITX2 and FOXC1. He also had a normal karyotype (46,XY), CGH microarray and extensive biochemical testing including lactate, pyruvate, very long chain fatty acids, amino acids, organic acids, carnitine, acylcarnitine profile, and carbohydrate deficient transferrin all with normal results. An MRI of his head showed only minimal volume loss. Expression of the condition varied among family members. One obligate heterozygote was said to be normal but never had a detailed examination. Anterior chamber abnormalities including Axenfeld-Rieger anomaly, glaucoma and/or retinal detachment were found in all 8 affected. 4 had congenital hypothyroidism. 5/6 with known head measurements were microcephalic and the 6th had an OFC just above the 3rd percentile. 4 had hearing loss. 4 had developmental delays not attributable to vision or hearing impairment. All who were closely examined had minor limb anomalies (including one with hypoplastic toes and one with severe metatarsus adductus). We believe this family has a new autosomal dominant syndrome with Axenfeld-Rieger anomaly, congenital hypothyroidism, microcephaly, developmental delay, hearing loss and minor limb anomalies.

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Mild Cornelia de Lange phenotype with single central incisor and insertional polydactyly. *J. Ranells, T. Ferlita, P. Newkirk.* Dept Pediatrics, Univ South Florida Col Med, Tampa, FL.

A 33 month old male was evaluated for unusual polydactyly with extra digit between the 4th and 5th fingers of the left hand, left choanal stenosis, left cryptorchidism and dysmorphic features. Physical exam showed height of 90 cm (25th centile), weight 12.1 kg (10th centile), OFC 47 cm (2nd centile), narrow forehead, epicanthal folds, long lashes, slight synophros, inner canthal distance 2.2 cm (10th centile), outer canthal distance 7.3 cm (30th centile), palpebral fissure length 2.3 cm (mean 2.5 cm), short broad nose with upturned tip, narrow philtrum with bowed upper lip, single upper central incisor, narrow palate with deep bilateral grooves, prominent ears, micrognathia, left mandibular spur, flattened umbilicus, left simian crease, hand and finger length <3rd centile, 5th finger clinodactyly with single flexion crease, minimal syndactyly of toes 2-3 and mild speech delay. Mild Cornelia de Lange syndrome (CdLS) was suspected. Brain MRI scan, echocardiogram, renal sonogram, 1.8 million SNP chromosome microarray and NIPBL analysis (research, courtesy of Dr. Krantz) were normal. Prenatally, reduced fetal activity and oligohydramnios were noted. Delivery was by cesarean section at 36 weeks for poor biophysical profile. Birth weight was 2520 gm, length 46 cm and OFC 34 cm. Radiographs showed distal left radius slightly smaller than right. He had severe GE reflux. Thickened bony cartilage was encountered during placement of a right lacrimal duct stent. At age 7 years, height is 103.6 cm (10th centile), weight 18.7 kg (50th centile), OFC 48.7 cm (2nd centile). He has lateral nasal prominences, mandibular spurs, short neck, glandular hypospadias, ulnar loops on all digits except left 2nd finger with radial loop, high pitched nasal voice and mild anxiety. He is sociable, has mild speech and fine motor difficulties. A brother was stillborn at term, birth weight 2.3 kg. He had single umbilical artery, bilateral bilobed lungs and heterotopic spleen within the tail of the pancreas. Karyotype was normal. CdLS is a complex developmental disorder with wide phenotypic variability. Mutations in NIPBL, SMC1A and SMC3, encoding proteins in the cohesin pathway, have been identified in 55-60% of affected individuals. Recognition of variant phenotypes may lead to identification of other genes involved in the pathogenesis of CdLS.

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Craniosynostosis: Thinking Beyond *FGFR* and *TWIST*! *E.H. Zackai[1], S.P. Bartlett[2], L.A. Whitaker[2], T. Shaikh[1], N.B. Spinner[3], D.M. McDonald-McGinn[1].* 1) Division of Human Genetics; 2) Division of Plastic Surgery; 3) Department of Pathology and Laboratory Medicine, The Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, PA.

Craniosynostosis may occur alone or in association with well-described syndromes due to mutations in *FGFR1*, *FGFR2*, and *FGFR3* or as a result of mutations/deletions/duplications in *TWIST*. Here we report 4 patients with craniosynostosis whose presentations were not consistent with a classic syndrome. Of these, 2 were found to have chromosomal aneuploidy suggesting the possibility of new associated loci and 2 with metopic synostosis and polysyndactyly were found to have GLI3 mutations suggesting a novel association. Patient 1, a 4-year-old male presented with pancraniosynostosis, hypertelorism, hearing loss, laryngotracheomalacia and developmental delay. Using array studies he was found to have a 4q27-q31.22 microdeletion. Of note, Del Valle Torrado reported a similarly affected patient with a cytogenetically visible deletion in 1982 thus suggesting a potential locus for craniosynostosis in this region which we may now have narrowed by use of the array. Patient 2, a 3-month-old female presented with unicoronal craniosynostosis, IUGR, and an umbilical hernia. She was found to have trisomy 9 mosaicism in 50% of her lymphocytes. Interestingly, coronal synostosis with trisomy 9 mosaicism has been reported previously (Diaz-Mares 1990; Kaminker 1985; Mantagos 1981; Tarani 1994). Patients 3 & 4 with metopic craniosynostosis and polysyndactyly were found to have mutations within the last third (Exon 14) and first third (Exon 6) of the GLI3 gene, respectively. Although the association of metopic craniosynostosis with GLI3 mutations has not been reported previously, two patients were described with metopic synostosis and polysyndactyly prior to mutational analysis (Hootnick 1972, Guzzetta 1996). Thus, these latter 4 patients likely fall within the GLI3 morphopathy spectrum and the list of findings associated with GLI3 mutations should now be broadened to include metopic craniosynostosis. In summary, these cases emphasize the importance of thorough evaluation of the patient and in the presence of findings outside the usual craniosynostosis syndromes suggest further workup for appropriate counseling and management.

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Holoprosencephaly, ectrodactylt, and bilateral cleft lip - palate syndrome and Xq microduplicationl A clue to understanding the genetic cause. *R. Kosaki[1], N. Okuno[2], C. Torii[2], K. Kosak[2].* 1) Dept Ped, Keio School of Med, Tokyo, Japan; 2) Dep Clin Genet & Mol Med, Natl Ctr Child Hlth & Dev, Tokyo, Japanc.

The unique combination of holoprosencephaly and ectrodactyly was first described by Hartsfield et al in 1984. Fifteen cases have been reported to date and the condition is likely to represent a single genetic entity (OMIM 300571, Hartsfield syndrome). The underlying genetic defect is yet to be unraveled. Mutation analyses of genes which cause holoprosencephaly including SHH, SIX3, ZIC2, an TGIF or genes which cause ectrodactyly including TP63 have been negative. Based on the observation that male patients have preferentially been affected, X-linked recessive mode of transmission has been suggested for this condition. Yet, no candidate genes have been suggested on the X chromosome. We report a patient with a full-brown Hartsfield syndrome phenotype who had a microduplication at Xq24 that involved at least 5 genes: A Japanese boy was born by a vaginal birth at 42 weeks gestation .He had bilateral ectrodactyly of the hands. He had midline cleft lip with aplasia of premaxillary segment, cleft palate, a low nasal bridge. Hypotelorism was minimal. He had micropenis with undescended testes. A CT scan of the brain revealed lobar-type holoproencephary. Cleft lip and palate was repaired a age 2 years and ectrodactyly was repaired at age 3. He had multiple episodes of hyper natremia with serum sodium level as high as 194 mEq/l. His development was mildly delayed: He could stand without support at age 1 year and walked alone at age 18 months. His G-banded karyotype was normal. Array comparative genomic hybridization using the Whole Human Genome CGH array (Agilent)revealed a de novo deletion of 0.3 Mb, on Xq24. The fact that the duplication has never been recognzied as benign copy number variation in various CNV databases does not prove but does suggest that the duplication could be pathogenic. From a clinical standpoint, it is important to note that the proband, who performed relatively well for a patient with holoprosencephaly, went through multiple episodes of potentially life-threatening hypernatremia.

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A Newly Described Mutation of the CLCN7 Gene Causes Neuropathic Autosomal Recessive Osteopetrosis in an Arab family. *A.Y. Edrees[2], A.A Dabbagh[3], J.Y Alaama[1,2].* 1) Department of Genetic Medicine,Faculty of Medicine, jeddah, Saudi Arabia; 2) Princess Al Jawhara Center of Excellence in Resear, jeddah 21533, Saudi Arabia; 3) Department of Pediatrics,Faculty of Medicine,King Abdulaziz University,jeddah,Saudi Arabia.

Autosomal recessive osteopetrosis may be due to mutations in TCIRG1 (50%), CLCN7 (15%) or OSTM1 (2%) CLCN7 mutations can also lead to autosomal dominant or an intermediate form. Neurologic manifestations in osteopetrosis are usually secondary to sclerosis of the skull bones. A rare neuropathic subtype exists that resembles neuro-degenerative storage disorders. This form does not respond to hematopoietic stem cell transplantation. Preliminary reports suggest that this form is more likely caused by mutations in the CLCN7 gene in an autosomal recessive manner. This case report provides further evidence for this phenotype-genotype correlation in that we present a previously unreported mutation in the CLCN7 gene in a Yemeni patient with the neuropathic form. This is also the first report of any mutation in osteopetrosis patients of Arabic ethnicity. A literature review suggests that this type may be more common in Arabs. Thus cascade genetic screening of autosomal recessive-osteopetrosis in patients of early onset. Arabic ancestry may preferably start with CLCN7 rather than TCIRG gene as is routine identifying a mutation in the CLCN7 gene in a patient with early onset autosomal recessive-osteopetrosis may also guide therapeutic decisions, regarding further genetic studies on Arab individuals with osteopetrosis may provide evidence for an alternative pattern of mutations in this sub-group founder effects, and important phenotype-genotype correlation data. Molecular testing of further Arab patients may prove that this type of mutation is recurrent or that the range of mutations causing autosomal recessive-osteopetrosis is different from that reported from the Western and has unique pattern. To our knowledge this is the first report of osteopetrosis in a Yemeni family and the first report of a genetic mutation in an Arabic patient. It is also the first report of this specific mutation in the literature. Keywords: CLCN7; osteopetrosis; autosomal recessive; new mutation; DNA analysis; homozygous; Arab; neuropathic form.

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Ophthalmic findings in the Greek isolate of Cohen syndrome. *S. Douzgou[1], J.R. Samples[2, 3], N. Georgoudi[4], M.B. Petersen[1].* 1) Department of Genetics, Institute of Child Health, "Aghia Sofia" Children's Hospital, Athens, Greece; 2) Rocky Vista University, Parker CO, USA; 3) Oregon Health & Sciences University, Department of Opthalmology, Portland, USA; 4) Department of Mental Health and Social Welfare, Institute of Child Health, Athens, Greece.

Cohen syndrome is a rare condition of mild to moderate developmental delay, characteristic craniofacial features, childhood hypotonia, joint hyperextensibility, neutropenia, and a variety of ophthalmic abnormalities. A high frequency of the syndrome has been observed in a Greek island with 2,000 inhabitants and a high degree of inbreeding. All patients were homozygous for a COH1, exon 6 to 16 deletion, suggesting a founder effect. We present the results of their first systematic ophthalmologic assessment. Myopia and chorioretinal atrophy were present in all patients of this cohort. Yet, in contrast to all groups previously reported, the majority presented corneal changes, independently from age, gender and family history. A pair of sisters, aged 11 and 15 years old, presented bilateral keratoconus. More frequently (86%) than in any other ethnic group, Greek patients presented cataracts that were bilateral and often graded as high as 3, even at a young age. As a whole, the ophthalmic phenotype of the Greek isolate of Cohen syndrome is characterized by the involvement of both the posterior and the anterior eye segment, bilaterally, in the majority of cases (93%). Greek Cohen subjects that share a founder mutation are at a higher risk of developing blindness in respect to those of other ethnicities and genotypes. This study added to the range of visual problems seen in children and adults with Cohen syndrome the finding of thin corneas and highlighted the need for pachymetry measurement as a means of surveillance and prediction of the visual impairment frequently observed.

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Involvement of LTBP4 and FBLN5 mutations in patients with autosomal recessive cutis laxa : clinical and molecular considerations. *F. Malfait, P. Vlummens, T. Van Damme, L. Van Laer, A. De Paepe.* Ctr Med Gen, Ghent Univ Hosp, Gent, Belgium.

Hereditary cutis laxa delineates a heterogeneous group of conditions characterized by abnormalities of the elastic fibers and presenting with loose, sagging and inelastic skin and variable systemic manifestations. Mutations in the fibulin-5 gene (FBLN5) cause an autosomal recessive form of cutis laxa (ARCL) characterized by severe skin laxity, pulmonary emphysema and peripheral pulmonary artery stenosis. Very few FBLN5 mutations however, have been identified so far and the genetic defect remains unknown in a significant proportion of patients. Recently the gene encoding the latent transforming growth factor-beta binding protein 4 (LTBP4) was shown to be implicated in families with an ARCL phenotype. In the current study, we examined a cohort of 16 patients with ARCL. Direct sequencing of FBLN5 in all patients identified 1 known and 1 novel mutation (p.C217R and p.E391X) in 2 probands, whereas molecular analysis of LTBP4 in the 14 remaining probands identified 9 novel loss-of-function mutations (p.R448X, p.C617X, p.S803X, p.Q1221X, p.Q1296X, p.R1377X, c.1263delC, c.4114dupC and c.780+2T>G) and 1 known mutation (c.4127insC) in a total of 8 patients. These results show that LTBP4 mutations are more prevalent than FBLN5 mutations in ARCL. Phenotypic comparison between LTBP4 and FBLN5 mutation positive patients shows overlapping but also distinguishing clinical features, i.e. LTBP4 mutation positive patients have more severe gastro-intestinal and urinary tract involvement. Our results also suggest LTBP4 as the first gene to test in the molecular work up of patients with ARCL.

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***Frem1* as a model for congenital diaphragmatic hernia (CDH) and bifid nose, renal agenesis, and anorectal malformations syndrome.** *T. Beck, M. Wat, B.J. Kim, H. Zaveri, M. Justice, B. Lee, D.A. Scott.* Molec & Human Gen, Baylor College Med, Houston, TX.

Congenital diaphragmatic hernia (CDH) is a sporadic birth defect that affects approximately 1:4000 newborns. In a recessive ENU mutagenesis screen we identified a mouse strain, *eye2*, with several congenital anomalies including retrosternal CDH, lung segmentation defects, kidney agenesis and a propensity to develop rectal prolapse. In addition, test crosses showed that approximately 50% of homozygotes die in utero. Traditional linkage mapping, followed by sequencing of positional candidate genes, revealed the *eye2* mutation to be a homozygous L826X change in the extracellular matrix protein Frem1. Recently, mutations in FREM1 were found in three consanguineous families with bifid nose, renal agenesis, and anorectal malformations (BNAR) syndrome. The clinical features associated with this syndrome have obvious overlaps with the defects seen in our *Frem1* mouse models. Mice deficient in *Slit3*—which encodes a large secreted molecule—also have retrosternal CDH and kidney agenesis. We have shown that an in vivo genetic interaction exists between *Frem1* and *Slit3* with regards to renal agenesis. We hypothesize that FREM1 and SLIT3 work together to promote invasion of the metanephric mesenchyme by the ureteric bud in the developing embryo and that failure of this process leads to the kidney agenesis seen in children with BNAR syndrome.

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Hearing Loss, Inner Ear and Sinuses in Cornelia de Lange Syndrome. *A.D. Kline[1], A. Munitz[2], A. Kimball[1], J. Nogee[4], S. Ishman[3,4].* 1) Harvey Inst Human Gen, Greater Baltimore Med Ctr, Baltimore, MD; 2) Dept of Radiology, Greater Baltimore Medical Center, Baltimore, MD; 3) Division of Pediatric Otolaryngology, Department of Head and Neck Surgery; 4) Johns Hopkins University School of Medicine, Baltimore, MD.

In children with an underlying syndrome and sensorineural hearing loss, there is known to be a higher incidence of a cochlear or vestibular abnormality. Otolaryngologic involvement is common in Cornelia de Lange syndrome (CdLS), particularly hearing loss, both conductive and sensorineural. A prior limited study of temporal bone CT scans in CdLS reported abnormalities including ossicular dysmorphism, hypoplastic cochlea, and dysplastic vestibule, with correlation found between the number of structural findings, hearing loss severity and overall severity of CdLS. In addition, sinusitis and nasal polyposis is more common in CdLS than in the general population, with estimates of 39% and 12% in adults with CdLS. Identification of chronic versus acute sinus disease and the causes and implications of this association in CdLS have not been investigated. Gastroesophageal reflux disease (GERD) has been thought to contribute to chronic sinusitis in the general population, and correlations have been noted, including detection of H. pylori in nasal polyps, although overall the link has been controversial. This is significant, since reflux occurs in over 90% of individuals with CdLS, with complications at a young age. While a direct link has not been shown, patients often manifest a broad spectrum of behavioral difficulties ranging from self-injury, aggression, and anxiety to sleep disturbance, which may indicate a hidden source of pain, such as sinusitis. We have obtained temporal bone CT scans on 8 individuals with CdLS and a history of hearing loss, non-sedated in most. Two (25%) demonstrated ossicular abnormalities and 2 had a hypoplastic cochlea; 1 has had significant benefits from a cochlear implant. Three (38%) were found to have mucous retention cysts versus nasal polyposis; 2 of these had associated sinusitis. Five (63%) had sinus abnormalities despite having no acute symptoms at the time of the CT scan, and 1 of these had mastoid opacification as well as sinusitis. Of these patients, 4 (80%) have GERD and significant behavioral issues. These findings have significance in terms of management and treatment. Cohesin, the protein complex affected in CdLS, is known to bind preferentially to many actively transcribing genes and control gene regulatory sequences; it is likely that there is abnormal regulation of the otic placode, when mutations in genes for cohesin are present.

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Mutations in *ZIC3*, *LEFTYA* and *ACVR2B* are a common cause of heterotaxy and associated cardiovascular anomalies. *L. Ma[1], S. Tierney[2], P. Lanzano[1], W.K. Chung[1].* 1) Division of Molecular Genetics, Department of Pediatrics, Columbia University, New York, NY 10032; 2) Department of Cardiology, Children's Hospital Boston, 300 Longwood Avenue, Boston, MA 02115.

Background- A spectrum of congenital heart disease is caused by left-right asymmetry disturbances and may present as an isolated cardiac anomaly or in association with abdominal situs anomalies referred to as situs ambiguous or heterotaxy. Methods and Results- To study the frequency of mutations in the Zinc Finger Protein of the Cerebellum 3 (ZIC3), Left Right Determine Factor 2 (LEFTYA), Activin A Receptor Type IIB (ACVR2B), and Cryptic Protein (CFC1) in heterotaxy, we screened 50 unrelated patients with heterotaxy associated congenital heart disease by dideoxy sequencing of all the coding exons and splice sites. The study cohort included 48% of subjects with atrial ventricular cushion defects and 70% with mal-position, interruption or absence of the superior or inferior vena cava. 17% had isolated congenital cardiovascular diseases without extra cardiac manifestations. Two novel genetic changes in *ZIC3* and one novel variant in *LEFTYA* were identified. None of these variants was observed in 100 ethnically matched control samples. One previously reported missense mutation in *ACVR2B* was identified in 2 unrelated subjects. The genetic changes identified in ZIC3, LEFTYA and ACVR2B are all located in highly conserved regions and probably affect protein function in left right axis formation and cardiovascular development. Conclusions: Mutations in ZIC3, LEFTYA and ACVR2B were identified in 5/50 subjects with heterotaxy for a yield of approximately 10%. Our results expand the mutation spectrum of monogenic heterotaxy and associated cardiovascular diseases and suggest that there are other causes of heterotaxy yet to be identified. Key words: heterotaxy, left-right asymmetry, congenital cardiac diseases, ZIC3, LEFTYA, ACVR2B, CFC1.

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Dyschromatosis symmetrica hereditaria: consideration of the pathogenesis with dermoscopic features. *N. Oiso[1], I. Murata[2], M. Hayashi[2], T. Suzuki[2], A. Kawada[1].* 1) Dept Dermatology, Kinki Univ Fac Med, Osaka-Sayama, Japan; 2) Dept Dermatology, Yamagata Univ Sch Med, Yamagata, Japan.

Dyschromatosis symmetrica hereditaria (DSH) is characterized by hyperpigmented and hypopigmented macules on the dorsal hands and feet. It is a genodermatosis caused by a mutation in ADAR1 as an autosomal dominant inheritance. We show a 24-year-old Japanese man having a mixture of oval or round, hyperpigmented and pigmented spots 1-7 mm in diameter and irregularly shaped hypopigmented macules on the dorsal hands and feet. The consanguinities had no such pigmentation. To verify the diagnosis precisely, a genetic study was performed. A novel two-nucleotide deletion mutation (c.1096-1097delAA,p.K366fs) was identified. The precise pathogenesis is uncertain. We found extraordinary dermoscopic features, which had not been described previously. In the hyperpigmented macules, round and variously pigmented spots 0.5 to 1.5 mm in diameter were connected to each other, producing oval hyperpigmented macules. Within each hypopigmented lesion, independent spots were sparsely distributed. Dermoscopy showed the different characteristic of each pigmented spot, such as the degree of pigmentation and the epidermal-dermal structure, and suggested that the spots have varied melanocyte dysfunction, aberrant melanocyte and keratinocyte interaction, and impaired construction of rete ridges. The ADAR1 gene encodes adenosine deaminases acting on RNA 1 (ADAR1) which catalyze the conversion of adenosine into inosine in RNA molecules. It is an important post-transcriptional mechanism for generating transcript diversity. We suppose that dysfunction of ADAR1 induces such various pigment appearances due to the dysregulated post-transcriptional system.

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Expression of Mutant RAB23 Proteins of Carpenter Syndrome Ablate Membrane Trafficking with Consequent Cytosolic Accumulation. *W.A. Paznekas[1], J. Dutta[1], V.R. Thrane[1], A. Iacovelli[1], H. Vega[1], N. Elcioglu[2], Y. Kasai[1], E.W. Jabs[1].* 1) Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, NY; 2) Department of Pediatric Genetics, Marmara University, Istanbul, Turkey.

Carpenter syndrome (Acrocephalopolysyndactyly Type II [MIM #201000]) presents with acrocephaly, diamond-shaped facies, brachysyndactyly of the fingers, preaxial polydactyly and syndactyly of the toes, hypogenitalism, obesity, and mental retardation. Jenkins et al. (2007) and Alessandri et al. (2010) have reported recessive RAB23 mutations in this condition. Mutations identified in 16 families were 5 different premature stop codons and the missense C85R. We have identified RAB23 mutations in 6 additional Carpenter patients, one homozygous for the novel Y79del mutation and 5 homozygous for L145X previously seen in 13 families. Of note, we found L145X alleles for the case presented in Temtamy's (1966) publication defining Carpenter syndrome. RAB23 is a member of the Rab GTPase protein family that cycles between active GTP-bound and inactive GDP-bound states to facilitate membrane trafficking and intracellular signaling of specific effectors. Essentially soluble proteins, they are modified at the C-terminus by prenylation a mechanism for membrane association of cytoplasmic proteins through covalent attachment of hydrophobic molecules to terminal cysteine(s). These cysteine(s) are modified by Rab geranylgeranyl transferase when the Rab protein is in complex with Rab escort protein (REP). We hypothesize that RAB23 mutations lead to proteins with loss of prenylation resulting in intracellular mislocalization. The L145X mutation removes the prenylation domain; Y79del may ablate REP association and prenylation; and C85R is predicted to disrupt protein structure. Each mutation is presumed to affect prenylation and thereby protein localization. To determine the possible effect of mutations identified in Carpenter syndrome we cloned wild-type and mutant (Y79del, C85R, and L145X) RAB23 cDNA-GFP constructs and performed transient transfections in HeLa cells. Results show that while cell membrane distribution is observed for over-expression of wild-type RAB23, distribution of each of the three mutant proteins is confined to the cytosol. Our results suggest that loss of prenylation is the underlying mechanism for the pathogenesis of Carpenter syndrome.

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Craniosynostosis Syndromes in Thailand -Twenty years Experience. *P. Wasant.* Dept Ped/Div Med Gen, Siriraj Hosp/Mahidol Univ, Bangkok, Thailand.

The craniosynostoses are etiologically and pathogenetically heterogeneous. Premature closure of cranial sutures may occur as isolated condition or associated with other anomalies. Almost 100 syndromes are known (Gorlin et al, 1990 ; Cohen and McLean, 2000). The most exhaustive review is that of Cohen and McLean (2000). Sagittal synostosis is the most common type. Coronal synostosis occurs less frequently. Most cases of isolated craniosynostosis are sporadic. Metopic and lambdoid synostoses are uncommon. Associated anomalies are more frequent in coronal than sagittal synostosis. Most common anomalies are limb defects, ear anomalies and cardiovascular malformations. Paternal origin of sporadic FGFR mutations is suggested. (Jones et al, 1975 ; Moloney et al, 1996 ; Glaser et al, 2000). Twenty years clinical experience of craniosynostosis at Genetics Clinic, Siriraj Hospital Faculty of Medicine, Mahidol University, Bangkok, Thailand, the largest teaching hospital in Thailand; will be presented. Accurate diagnosis , genetic counseling and early intervention are extremely important in prognosis and subsequent outcome. Clinical/genetic evaluation and a gallery of craniosynostosis syndromes: Crouzon, Apert, Pfeiffer, Saethre-Chotzen, Cole-Carpenter, Antley-Bixler syndrome and nonsyndromic craniosynostoses: Kleeblattschadel, oxycephaly, scaphocephaly, metopic synostosis, will be presented. There is limitation of molecular analysis of craniosynostosis in Thailand.

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Familial congenital diaphragmatic hernia, cystic kidneys and cardiac anomalies: A new X-linked syndrome? *A. Yeung[1], S. Keating[2], R. Silver[3], D. Chitayat[1,3].* 1) Division of Metabolic and Clinical Genetics, Hospital for Sick Children, Toronto, Ontario, Canada; 2) Department of Pathology and Laboratory Medicine, Mount Sinai Hospital , Toronto, Ontario, Canada; 3) The Prenatal Diagnosis and Medical Genetics Program, Department of Obstetrics and Gynaecology, Mount Sinai Hospital, Toronto, Ontario, Canada.

Congenital diaphragmatic hernia (CDH) occurs in approximately 1 in 3000 live births and is associated with significant morbidity and mortality. Etiologically, CDH is heterogeneous with most isolated cases being multifactorial. About 50% of cases are 'non-isolated', with associated extra-pulmonary anomalies. Some of these cases are single gene disorders and 5-10% have a chromosomal etiology.

We describe two male siblings of non-consanguineous Caucasian parents with CDH, distinctive facial dysmorphism, dilatation of the ascending aorta, valvular dysplasia and enlarged cystic kidneys - a constellation of features not consistent with other previously described CDH syndromes. The cardiac findings point to an associated connective tissue disorder.

Karyotype in both siblings was 46, XY and microarray analysis was normal suggesting that a single gene defect is the underlying cause of this syndrome. A maternal uncle had died in infancy as the result of CDH. Thus, the pedigree is strongly suggestive of X-linked inheritance.

We review the features of other known X-linked CDH syndromes which include Simpson-Golabi-Behmel syndrome, Craniofrontonasal syndrome, Thoracoabdominal syndrome and X-linked Cornelia De Lange syndrome. None of these are reconciled by the phenotype in our two cases and indeed, genetic testing for *GPC3* to rule out Simpson-Golabi-Behmel syndrome was negative. We conclude that this family exhibits a new X-linked genetic syndrome of which congenital diaphragmatic hernia is a principal feature.

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Prevalence and clinical features of Costello syndrome and cardio-facio-cutaneous syndrome in Japan. *Y. Abe[1], Y. Aoki[1], T. Niihori[1], H. Ohashi[2], K. Kurosawa[3], N. Okamoto[4], H. Kawame[5], S. Mizuno[6], T. Ogata[7], S. Kuriyama[8], S. Kure[1,9], Y. Matsubara[1].* 1) Dept Med Genet, Tohoku Univ Sch Med, Sendai, Japan; 2) Saitama Children's Med Ctr, Saitama, Japan; 3) Kanagawa Children's Med Ctr, Yokohama, Japan; 4) Osaka Med Ctr & Res Inst for Maternal & Child Health, Osaka, Japan; 5) Ochanomizu Univ, Tokyo, Japan; 6) Aichi Prefectural Colony Central Hosp, Nagoya, Japan; 7) National Res Inst for Child Health & Development, Tokyo, Japan; 8) Dept Epidemiology, Tohoku Univ Sch Med, Sendai, Japan; 9) Dept Pediatr, Tohoku Univ Sch Med, Sendai, Japan.

The RAS/MAPK signaling pathway is critical for human development and plays an important role in the formation and progression of cancers. Recent studies have shown that a group of genetic disorders results from dysregulation of the RAS/MAPK cascade. Costello syndrome and cardio-facio-cutaneous (CFC) syndrome are autosomal dominant congenital anomaly syndromes characterized by a distinctive facial appearance, heart defects, mental retardation and tumor predisposition. Germline mutations in HRAS have been identified in patients with Costello syndrome and those in KRAS, BRAF and MAP2K1/2 (MEK1/2) have been identified in patients with CFC syndrome. In order to assess the prevalence, natural history, prognosis and the rate of tumor development among Japanese patients, we designed a prevalence study of patients with Costello and CFC syndromes. The study consisted of two-tier questionnaire surveys, which were distributed to a total of 1244 hospitals, including randomly selected pediatric clinics at hospitals and institutions for severely-retarded children throughout Japan. The response rate of the first survey was 69% (856/1244). Fifty-six HRAS mutation-positive patients with Costello patients and 61 patients with CFC syndrome, who had mutations with KRAS, BRAF or MAP2K1/2, were reported in the first survey. The comprehensive molecular analysis of additional 35 suspected patients are currently under way. The second survey has been sent out, requesting additional detailed clinical information on each patient identified in the first survey. Of these, four of 27 HRAS-positive patients developed malignant tumors, including rhabdomyosarcoma, ganglioneuroblastoma and bladder carcinoma. Four of 44 patients with CFC syndrome developed hematologic abnormalities, including acute lymphoblastic leukemia, non-Hodgkin lymphoma and juvenile myelomonocytic leukemia. These results suggest the importance of molecular diagnosis and careful observation in patients with Costello syndrome and CFC syndrome. In our study, patients older than 30 years of age have not been identified. A long-term follow-up protocol of patients with Costello syndrome and CFC syndrome would be useful to understand the precise natural history, prognosis and the rate of tumor development.

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Townes-Brocks Syndrome with Congenital Adrenal Hyperplasia. *B. Bozorgmehr, F. Afroozan, v. Hadavi, A. Kariminejad.* Dept Clinical Genetics, K-N Pathology & Genetics Ctr, Tehran, Iran.

Abstract: Townes-Brocks syndrome (TBS) characterized by imperforated anus , dysplastic ears , (over folded superior helices and preauricular tags) frequently associated with sensorineural and/or conductive hearing impairment , and thumb malformations triphalangeal thumbs, duplication of the thumb, preaxial polydactyly and rarely hypoplasia of the thumb. Renal impairment, including end-stage renal disease (ESRD), may occur with or without structural abnormalities (mild malrotation, ectopia, horseshoe kidney, renal hypoplasia, polycystic kidneys, vesicoutereral reflux). Congenital heart disease , genitourinary malformations and Mental retardation occurs in these patients. It is autosomal dominant disease with variability in the severity of expression. We are reporting a 8 year girl with dysplastic ears, deafness, dysplastic thumbs, small kidneys, history of repaired imperforated anus, and rectovaginal fistula. She is also diagnosed with congenital adrenal hyperplasia with congenital hyperplasia. We believe our patient is the first case of Townes-Brocks syndrome with congenital adrenal hyperplasia.

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WIDE CLINICAL SPECTRUM IN ZIMMERMANN-LABAND SYNDROME. *M.G. González-Mercado[1,2], D. García-Cruz[1], A.J.L. Brambila-Tapia[1,2], J. Durán-González[1,2], N.O. Dávalos[1], J.A. Cruz-Ramos[1,2], M.O. García-Cruz[3], R. E'Vega[1], B.E. Ríos-González[1,2], M.G. Zavala-Cerna[4], I.P. Dávalos[1,2].* 1) Genética, CUCS, Universidad de Guadalajara, Guadalajara, Mexico; 2) División de Genética, CIBO, Instituto Mexicano del Seguro Social (IMSS). Guadalajara, Jalisco, México; 3) Clínica de Labio y Paladar Hendido, HGR 46, IMSS, Guadalajara, Jalisco, México; 4) División de Investigación, UMAE, Hospital de Especialidades, CMNO, IMSS, Guadalajara, Jalisco, México.

INTRODUCTION Gingival fibromatosis can be present as an isolated form or be part of a genetic disease. The Zimmermann-Laband syndrome (ZLS) is a rare disorder inherited as an autosomal dominant fashion; clinically characterized by gingival fibromatosis, bulbous soft nose, thick floppy ears, nail dysplasia, joint hyperextensibility, hepatosplenomegaly, skeletal anomalies and occasional mental retardation. CASE REPORT The proposita aged 9 years old, was the product of the 2nd pregnancy from non-consanguineous parents. At 7th months of pregnancy the mother presented polyhydramnios; and birth weight of 4,100 g. Generalized hypertrichosis mainly on limbs was detected at birth, at 5 months hepatomegaly, and at 11 months gingival fibromatosis. When she was 2 years 7 months joint hyperlaxity was evident and also nail hypoplasia particularly on 5th toes. The proposita presented delayed psychomotor development:, mainly in speech. At 3 yr. 6 mo. hypoacusia was diagnosed, the auditive evoked potentials revealed severe left hypoacusia and bilateral cortipathy. Clinically she presented wide forehead, telecanthus, high nasal bridge, bulbous nose, thick anthehelices and auricles, large mouth, thick lower lip, hypertrichosis on arms and legs, nail hypoplasia on 5th toes and hepatomegaly (2cm). Oral examination revealed generalized gingival hyperplasia covering the decidual teeth and leading to delay of dental eruption. The karyotype was normal. Family Data. The parents were clinically examined, and the father presented bulbous nose and thick auricles and lips. CONCLUSIONS We studied a 9 year-old girl with the ZLS and hypoacusia. The father presented minimal facial characteristics of ZLS, supporting a wide clinical spectrum in ZLS. Therefore we are considering ZLS as a contiguous gene syndrome or a consequence of a gene mutation with clinical variable expressivity.

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Genital Abnormalities in Pallister-Hall syndrome: Two Case Report. *Y. Narumi[1], T. Kosho[1], G. Tsuruta[2], M. Shiohara[2], E. Shimazaki[3], T. Mori[3], A. Shimizu[2], Y. Igawa[4], S. Nishizawa[5], K. Takagi[6], R. Kawamura[1], K. Wakui[1], Y. Fukushima[1].* 1) Department of Medical Genetics, Shinshu University School of Medicine, Matsumoto, Japan; 2) Department of Pediatrics, Shinshu University School of Medicine, Matsumoto, Japan; 3) Department of Pediatrics, Nagano National Hospital, Ueda, Japan; 4) Department of Urology, Shinshu University School of Medicine, Matsumoto, Japan; 5) Department of Urology, Nagano Children's Hospital, Azumino, Japan; 6) Department of Obstetrics, Nagano Children's Hospital, Azumino, Japan.

Pallister-Hall syndrome (PHS) is a rare autosomal dominant disorder, characterized by hypothalamic hamartoma, central or postaxial polydactyly, bifid epiglottis, and various visceral anomalies. The disorder is caused by mutations in GLI3, a gene encoding a zinc finger transcription factor that regulates downstream target genes in the sonic hedgehog (SHH) signaling pathway. We describe two PHS patients with genital abnormalities: a female with hydrometrocolpos secondary to vaginal atresia and a male with micropenis, hypoplastic scrotum, and bilateral cryptorchidism. Nonsense mutations in GLI3 were identified in both patients. Clinical and molecular findings of 12 previously-reported patients who had GLI3 mutations and genital abnormalities were reviewed. Genital features in the male patients included hypospadias, micropenis, and bifid or hypoplastic scrotum, whereas all the females had hydrometrocolpos and/or vaginal atresia. No hotspot for GLI3 mutations has been found and genital features in PHS might present with a wide range of severities among patients with the same nucleotide change. Micropenis and cryptorchism in male PHS patients were speculated to be caused by absent or diminished gonadotrophins during fetal development. In our review of the literature, only 2 of 5 male patients had panhypopituitarism. No female patients in our review of the literature had panhypopituitarism. Urogenital and anorectal abnormalities in PHS might be related to dysregulation of SHH signaling caused by GLI3 mutations rather than hormonal aberrations.

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Hematologic abnormalities associated with patients with cardio-facio-cutaneous syndrome. *Y. Saito[1], Y. Aoki[1], T. Niihori[1], A. Ohtake[2], A. Shibuya[2], K. Sekiguchi[3], S. Suenobu[3], T. Izumi[3], H. Muramatsu[4], S. Kojima[4], S. Kure[1,5], S. Tsuchiya[5], Y. Matsubara[1].* 1) Dept Med Genet, Tohoku Univ Sch Med, Sendai, Japan; 2) Dept Pediatr, Saitama Med Univ, Moroyama, Saitama, Japan; 3) Dept Pediatr and Child Neurol, Oita Univ Facul Med, Oita, Japan; 4) Dept. Pediatr, Nagoya Univ Graduate Sch Med, Nagoya, Japan; 5) Dept. Pediatr. Tohoku Univ Sch Med, Sendai, Japan.

Cardio-facio-cutaneous (CFC) syndrome is a multiple congenital anomaly/mental retardation syndrome characterized by a distinctive facial appearance, ectodermal abnormalities and heart defects. Clinically, it overlaps with both Noonan syndrome and Costello syndrome. Mutations in KRAS, BRAF and MAP2K1/2 (MEK1/2) have been identified in patients with CFC syndrome. We have previously reported that two CFC patients with BRAF mutations developed acute lymphoblastic leukemia. A patient with a MAP2K1 mutation, who developed hepatoblastoma, has also been reported. Here we report additional two patients with CFC syndrome who developed non-Hodgkin lymphoma and juvenile myelomonocytic leukemia (JMML) -like myeloproliferative disorder. The first patient developed pneumonia with pleurisy at two months of age and was diagnosed as having non-Hodgkin lymphoma (precursor T-cell lymphoblastic lymphoma) by cytopathologic examination of pleural fluid. He was suspected of having Noonan syndrome because of his facial appearance, webbed neck and cubitus valgus. Precursor T-cell lymphoblastic lymphoma was treated by TCCSG NHL 94-04 protocol. At 9 years of age, he was clinically re-evaluated and diagnosed as having CFC syndrome because of his distinctive facial appearance, multiple nevi and moderate mental retardation. Sequencing analysis revealed a germline p.A246P (c.736G>C) mutation in BRAF previously reported in CFC syndrome. The second patient was delivered by caesarian section at 32 weeks' gestation due to non-immune hydrops fetalis. He had curly hair, low-set ears, atrial septal defect, hepatosplenomegaly, pulmonary arteriovenous fistula and portosystemic shunt. At one month age, the patient showed marked monocytosis and decrease in platelet count. The diagnosis of JMML was made on bone marrow cell culture studies. Hematological abnormalities resolved spontaneously at three month of age. Sequencing of genomic DNA from the patient showed a heterozygous p. T241P (c.721A>C) mutation. This is the first report that a patient with CFC syndrome developed JMML-like myeloproliferative disorder. These results suggest the importance of molecular diagnosis and careful observation in children with CFC syndrome.

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Renpenning Syndrome in Infancy. *S.A. Schrier, H. Feret, E.H. Zackai.* Division of Human Genetics, Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, PA.

Renpenning syndrome is a rare X-linked disorder characterized by the presence of mental retardation, short stature, small testicular volumes, as well as microcephaly, long, narrow facies, and prognathism. Mutations in the Polyglutamine binding protein 1 gene (PQBP1) have been found to be causative. Here, we report the first case in the literature of the youngest individual to be diagnosed with Renpenning. The proband, a 4 week-old male, was brought for evaluation due to concerns that he looked strikingly similar to an older brother with mental retardation, short stature, a "small head," small testes, and a maternal uncle with the same features. Physical examination of the infant at four weeks revealed a height of 50.3 cm (50th %), weight of 3.25 kg (25th %), and head circumference of 34.5 (10th %). The infant showed slight midfacial flattening, an inverted triangular face, and a small chin. Examination of the older brother was suggestive of Renpenning syndrome and therefore, the PQBP1 gene was sequenced in both individuals and the mother. They were found to have a hemizygous four pair deletion in the PQBP1 gene beginning at nucleotide 459 (c459_462del4). Review of the literature of Renpenning syndrome shows the youngest reported patient with the disorder to be 18 months of age. This patient had a rounded face at the time of diagnosis similar to childhood pictures of affected adults. However, in our case, the mother of the patient was able to recognize the facies as comparable to her affected brother and son early on. There is a paucity of data on birth parameters. Of 9 patients' data reported in the literature, 7 had normal birth weight, 2 were <2 %. Our patient's birth weight was normal. Birth length was available on only one and was normal as was our patient's. Birth head circumference on three patients were between 10-25%. Our patient's head circumference at 4 weeks was at the 10th %. Our patient's length at 4 weeks was at the 50th%. Thus, although his head circumference is disproportionate to length, microcephaly seems to be a developing problem with 86% of adults having head size < 3rd percentile. Short stature is not evident early on either and seems to be a progressive problem with 60% of adults being less than <3rd percentile in height. The patient will be followed prospectively to gain further insight into the natural history of this condition.

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Heterogeneity of Mabry syndrome: hyperphosphatasia with seizures, neurologic deficit and characteristic facial features. *M.D. Thompson[1], T. Roscioli[2], M.N. Nezarati[3], E. Sweeney[4], P. Meinecke[5], P.M. Krawitz[6,7], C.C. Mabry[8], D. Horn[7], R. Mendoza[10], H. van Bokhoven[2], F. Stephani[2], C. Marcelis[2], A. Munnich[9], H.B. Brunner[2], D.E. Cole[1].* 1) Lab Medicine, Banting Inst, Univ Toronto, Toronto, ON, Canada; 2) Department of Human Genetics, University Medical Centre St. Radboud, 6525 GA Nijmegen, The Netherlands; 3) Department of Genetics, North York General Hospital, Toronto ON, Canada; 4) Royal Liverpool Children's Hospital, Liverpool, United Kindom; 5) Medizinische Genetik, Altonaer Kinderkrankenhaus, 22763 Hamburg, Germany; 6) Institut für Medizinische Genetik, Charité Universitätsmedizin Berlin, 13353 Berlin, German; 7) Berlin-Brandenburg Center for Regenerative Therapies (BCRT), 13353 Berlin, Germany; 8) University of Kentucky College of Medicine, Lexington, Kentucky, USA; 9) INSERM U781-Université Paris Descartes-Hôpital Necker-Enfants Malades; 10) Division of Clinical and Metabolic Genetics, Hospital for Sick Children, Toronto ON, Canada.

Persistent hyperphosphatasia associated with developmental delay and seizures was originally described in a single family (OMIM#239300) in 1970. However, the condition is rare and the nosology has remained uncertain. Patient recruitment has been conducted globally in order to delineate this distinctive disorder and provides evidence in favor of autosomal recessive inheritance. Common to all patients is facial dysmorphism, particularly hypertelorism, a broad nasal bridge and a tented mouth. All patients have some degree of middle and distal phalangeal shortening that varies in position and degree. The persistent elevation of alkaline phosphatase (ALP) activity without any evidence for active bone or liver disease also varies considerably among patients (from ~1.3 to ~20 times the upper age-adjusted reference limit), but remains constant in any one individual. Identification of mutations in the type V phosphatidylinositol glycan (PIG) anchor biosynthesis (PIGV) gene in some but not all probands, also points toward genetic heterogeneity. The possibility that other genes in the PIG anchor biosynthesis pathway might be involved is an attractive one, since the PIG anchor is a major modulator of ALP expression on the cell surface. The substantial phenotypic variation, including variable degrees and extent of lysosomal storage in different probands, also argues for genetic heterogeneity in Mabry syndrome.

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Hypermobility Syndrome in Japan. *A. WATANABE[1, 2], M. HATAKEYAMA[2, 3], R. TSUNODA[4], K. MATSUMOTO[5], H. KAWAME[3], T. SHIMADA[1, 2].* 1) Deptartment of Medical Genetics, Nippon Medical School, Tokyo, Japan; 2) Division of Clinical Genetics, Nippon Medical School Hospital, Japan; 3) Genetic Counseling Program, Graduate School of Humanities and Scieces, Ochanomizu University, Japan; 4) Department of Orthopedics, Nippon Medical School, Japan; 5) Department of Biosignaling and Radioisotope Experiment, Center for Integrated Research in Science, Shimane University, Japan.

Background: Hypermobility Syndrome (ICD-10 M357) is a connective tissue disorder characterized by generalized joint hypermobility and arthralgia (joint pain) in multiple joints (major criteria in Brighton criteria (2000)), while Ehlers-Danlos syndrome hypermobility type (EDS-HM) is also a connective tissue disorder characterized by generalized joint hypermobility, subluxations, dislocations and chronic pain. As both the conditions are rarely known in Japanese society, patients with the either condition are having much difficulty in getting enough support from those who are around and even from those who are healthcare professionals. The two conditions are treated as the same one here. Our research purpose is to focus on symptomatic status of patients with generalized hypermobility in Japan. Methods: 1. A retrospective chart review of patients seen and diagnosed from 2007 to present. 2. A short form of questionnaire was handed out and following 10 minutes interview per person during the 2010 EDS patients annual meeting. Data sets of general, mucocutaneous, musculoskeletal, cardiovascular, gastrointestinal, urogynecological, and ear-nose-throat symptoms were collected. Results: We have collected data sets from 20 patients. The most dislocated joints were; shoulders and knees. Wheel chairs were used by a number of patients because of the instable knees. Chronic pain even in joints without subluxations or dislocations was significant in most patients. Orthostatic disturbance was reported by more than half of the patients. More than one-third of patients had either cardiovascular or abdominal symptoms. Management of chronic pain was the most urgent issue while the number and the variation of approved indication drug is overly limited compared to the U.S.. Cutaneous findings were not at all common in this population. Conclusions: Here the broad phenotype of Hypermobility syndrome in Japan is described based on data from 20 patients. As many are suffering from symptoms not limited within that of joints, our goal will be to fully cooperate with department involved to maximize the QOL of patients with the chronic, disabling condition, let alone raising awareness in medical healthcare professionals.

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Clinical and Molecular characterization of Children with Rubinstein -Taybi Syndrome from Western Canada. *O. Caluseriu[1], C. Beaulieu[1], C.R. Greenberg[2], F. Bernier[1], D.R. McLeod[1], M.A. Innes[1].* 1) Dept Med Gen, Alberta Children's Hosp, University of Calgary, AB Canada; 2) Departments of Biochemistry & Medical Genetics and Pediatrics & Child Health, University of Manitoba, Winnipeg MB Canada.

Rubinstein-Taybi Syndrome (RTS) is a rare multiple congenital anomaly syndrome, characterized by a distinctive facial gestalt with broad thumbs and great toes. Other features include growth and mental retardation and increased risk of malignancy. RTS is genetically heterogeneous, with approximately 50% of patients having mutations in the CREB binding protein (CREBBP) gene and 3% in its homolog EP300. Both code for transcriptional coactivators that interact with a large number of proteins and act as a potent histone acetyltransferase (HAT). The etiology of RTS in over 40% of patients is currently unexplained. While there are a few cases of children with consanguineous parents or affected siblings born to unaffected parents, RTS is typically autosomal dominant; most patients have confirmed or presumed de novo mutations. We present a group of Canadian children from the Prairie Provinces clinically diagnosed with RTS. Our cohort includes several typically affected patients including a concordant monozygotic twin pair and atypical cases including one with severe short stature and a single central incisor. Investigations planned on this cohort include full CREBBP sequencing and array CGH to exclude microdeletions. To date, one new missense substitution was found in CREBBP c.1982T>A, in a highly conserved region with CBP coactivator properties in one atypical RTS individual with normal stature. Of particular interest to our group is the identification of a number of Hutterite patients with possible RTS. All current Hutterites, an endogamous population, descend from fewer than 100 common founders and we have described over 30 autosomal recessive conditions (many novel) in this population. The first two related RTS Hutterite patients were born to unaffected consanguineous parents. Two further Hutterite patients with some features of RTS not directly related to the previous have also been identified. No mutation in CREBBP gene was found in one of the Hutterite patients and we suspect a possible autosomal recessive 'variant' with significant clinical overlap with RTS. Homozygosity mapping applied to this first patient identified several homozygous regions of interest. Further studies on other family members are needed to refine the candidate region that could reveal a recessive gene responsible for RTS in the Hutterite population. If a shared region of interest is identified, genes with histone acetyltransferase activity would be compelling candidates.

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Negative cystic fibrosis meconium ileus in two Asian newborns: implications for diagnosis and counseling of a pathognomonic condition. *A. Iglesias.* Department of Pediatrics, Divison of Medical Genetics, Beth Israel Medical Center, New York, NY 10003.

Traditionally the presence of meconium ileus (MI) in a newborn is considered pathognomonic of cystic fibrosis (CF). Since screening immunoreactive trypsinogen activity is usually negative, molecular testing is currently use until a sweat test can be performed. Since therapeutic decisions, especially enzyme replacement, are usually done in the first weeks of life, having an early diagnosis is important. The advent of CF sequencing and deletion/duplication studies brings possibility of confirming the diagnosis early on a reality. We are presenting two newborn cases of MI. Both patients were from Asian descent. Patient one was born via c-section at 29 =BD weeks after a pregnancy complicated for maternal hypertension and echogenic bowel. Amniocentesis showed normal chromosomes, 46, XY. Surgical repair of MI was done and the diagnosis was confirmed upon pathology examination. Patient two was born full term after an uncomplicated pregnancy. Prenatal CF maternal screening was negative. MI and perforation were diagnosed at birth and repaired. Molecular testing for CF including sequencing and deletion/duplication studies were negative in both cases. Therefore the chances of these patients to be affected with CF were considered extremely reduced. However, no other etiologies for MI were found in either patient. Interestingly, further sweat testing results were either inconclusive or negative in both cases reinforcing the diagnostic dilemma. Both patients are currently being followed at a pulmonology clinic without special interventions and both are doing fairly well. Two main lessons can be drawn from these cases. First, MI although considered pathognomonic for CF, might be present as a non-CF etiology. Second, negative CF prenatal screening in Asian couples is a practice with very low yield. Accordingly, its use as a decision making tool should be questioned in this context, especially in the first case were echogenic bowel was present. Finally, the search for other etiologies for MI, although unsuccessful in these cases, seems to be prudent while waiting for the results of the molecular testing. Nevertheless, further follow-up with repeat sweat tests and clinical assessments seems to be a prudent practice until the diagnosis of CF can be completely excluded.

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THE THREE FACES OF KABUKI SYNDROME. *A. Tsai.* Sect Clinical Gen & Metabolism, Childrens Hosp, Denver, B300, Denver, CO.

Introdcution: Kabuki syndrome (KS) is first described simultaneously by two groups in Japan. Because the facial features resembled the make-up of actors in traditional Japanese play, Kabuki, the syndrome was thus named. The inheritance was thought to be X-linked or Autosomal Dominant. Milunsky et al., 2003 reported a 3.5Mb duplication of 8p22-8p23 in six unrelated patients by microarray but this finding was not supported by later study. Various chromosomal anomalies were reported to have KS or KS-like phenotype. No single unifying etiology has been found. Material and Method: From 1999-2010, 50 patients age from 1 day to 15 years were ascertained in a tertiary care pediatric hospital/genetic clinic with a "suggestive diagnosis" of Kabuki syndrome. Patient with MCA who later on determined to be Kabuki syndrome was not included in this study. All patients received complete dysmorphology examination and laboratory work up whenever indicated by a single geneticist. 4 categorical diagnoses were delineated. Result: 4 patients with X chromosome anomalies: 45,XO, one large ring mosaicism with XIST present, one small ring chromosome without XIST and one with incontinentia pigmenti (IP) due to exons 4-10 deletion. 2. 4 patient with del/dup involving 8p22-8p23: 3. 8 patients on whom the diagnosis was concurred of unknown etiology. 4. 34 patients on whom the diagnosis were disagreed. Discussion: X chromosome anomalies represent one face of Kabuki syndrome. The long palpebral fissure with the lower lid shadow (eversion of eye lashes) w/ s heart defects tend to prompt this diagnosis. Matsumoto et al. pointed out that those patients with KS-like features and ring X tend to have more severe manifestations which are seen in this study in the patient with a smaller ring. The patient with IP and kabuki features may further narrow down the critical region. While 8p22-8p23 is a highly polymorphic region with CNVs, Shieh et al., 2006 also reported triplication of 8p22-8p23 in a patient with features similar to Kabuki Syndrome; so were the 4 patients ascertained here with duplication; mostly from the heart defects, arched eyebrow and ear anomalies. This represents the second face. Lastly, there is a clear (third) face with clinical constellations consistent with Kabuki syndrome yet etiology to be determined. While exome/large-scale sequencing is a powerful tool in identifying the cause of a mendelian disorder, careful classification of phenotype remain cruicial.

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Quantitative measurement of FMRP as a screening and prognosis approach for the Fragile X Syndrome. *F. Corbin[1], M. Lessard[1], A. Chouiali[1], R. Drouin[2].* 1) Dept Biochemistry; 2) Dept Pediatrics, Facult Med & Hlth Sci, Universite de Sherbooke, Sherbrooke, PQ, Canada.

The fragile X syndrome is the leading cause of inherited mental retardation. In most cases, the syndrome results from a large expansion of CGG repeats in the 5' UTR of the FMR1 (Fragile X Mental Retardation 1) gene along with the methylation of its CpG Island. Classically, Southern blot and PCR analyses are the screening/diagnostic tools used to confirm this sole mutation (expansion and methylation) among affected boys and girls. Methylation of the FMR1 gene leads to transcriptional silencing of the FMR1 gene and the absence of its encoded protein FMRP (Fragile X Mental Retardation Protein) in affected cells. Cognitive functions among fragile X individuals are variable and tends to be higher in female and mosaic patients where FMRP is produced in a certain proportion of their neurons. A screening test based on quantitative FMRP measurement would therefore not only detect the fragile X syndrome but might also predict the cognitive functions of affected individuals. Therefore, a new quantitative approach based on the measurement of FMRP within blood sample components was developed to screen for fragile X syndrome. Levels of FMRP in a control population were first determined following the recruitment of participants of different ages and gender without any intellectual disabilities. The measured levels of FMRP in this population were then compared with those of fragile X syndrome subjects, recruited in our clinic. Cognitive functions were also assessed in these individuals using age-specific Wechsler intelligence scale and the Vineland adaptive behavior scale. Our results show that levels of FMRP in our control population follow a normal distribution and don't seem to be affected by age or gender. In fragile X individuals, levels of FMRP were significantly and sufficiently lower than controls to determine a cut-off value. Therefore a FMRP dosage below this value suggests the possibility of a fragile X syndrome diagnosis. Moreover, a direct proportional relationship was established between global IQ and FMRP level. In fact, as expected, levels of FMRP were higher in females as well as their global IQ scale. Finally, the proposed screening approach would not only discriminate fragile X from normal individuals but also predicts cognitive functions in confirmed fragile X cases. These clinical data would help to provide appropriate support for these patients.

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EXPANDING THE PHENOTYPE OF AAA SYNDROME. *D. Kostiner, P. Himes.* Department of Medical Genetics, Kaiser Permanente, Portland, OR.

Achalasia-Addisonianism-Alacrima syndrome, also known as Triple-A syndrome or Allgrove syndrome, is a rare autosomal recessive condition with a prevalence of less than 1/1,000,000. It causes a triad of achalasia (hypertrophy of the gastroesophageal cardia, preventing food from entering the stomach); ACTH-resistant adrenal insufficiency; and alacrima (lack of tears). Other common features include autonomic dysfunction, anisocoria, decreased saliva, muscle weakness/wasting, and hyperkeratosis of the palms and soles. It often presents in childhood with adrenal crises, and can be lethal if not treated promptly. It is caused by mutations in the AAAS gene, which encodes a WD-repeat protein termed ALADIN. This protein is thought to be involved in cytoplasmic trafficking and/or normal peroxisomal function. The locus is on band 12q13 near the type II keratin gene cluster, which likely explains the hyperkeratosis seen in some patients. Our patient is a 38-year-old woman who presented for preconception counseling regarding her ACTH-resistant adrenal insufficiency. She was healthy at birth, but was diagnosed with non-CAH adrenal insufficiency at 2 years of age. Complications included febrile seizures (likely adrenal crisis), hypoglycemia, and orthostatic hypotension. She developed osteoporosis and poor dentition due to long-term steroid use. At 18, she was diagnosed with achalasia, for which she is treated annually with esophageal dilations and Botox via EGD. Exam findings include petite size (Ht 5'0", Wt 83 lbs, BMI 16), metopic prominence, deep-set eyes, alacrima, anisocoria, thin nose, poor dentition, cleft soft palate (s/p repair), geographic tongue, Buffalo hump (due to overtreatment with steroids), thin limbs, tiny hands, and mild hyperkeratosis of the palms. Intelligence is normal. Molecular testing of the AAAS gene revealed two mutations: Q145X, C->T in exon 5 and R230X, C->T in exon 7. Both are previously-reported nonsense mutations that cause premature truncation of the protein and/or nonsense-mediated mRNA decay. Identifying the correct diagnosis in this patient allowed for accurate preconception counseling, improved treatment regiment by her new endocrinologist, and information about what other complications might arise. This is also the first reported case of cleft soft palate in a patient with AAA syndrome, so oral clefting might be a new feature of the AAA phenotype.

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Compound Heterozygosity of β°/β+-thalassemia (CD41/42-PolyA) with Non-deletional HbH Disease (--^{SEA}/α^{CS}α) - A rare combination in Malaysian Malays. *J.A.M.A. Tan[1], J.L. Kok[2], K.L. Tan[1], Y.C. Wee[1], E. George[3].* 1) Department of Molecular Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia; 2) Pediatrics Department, Hospital Kuching, Sarawak, Malaysia; 3) Hematology Unit, Department of Pathology, Faculty of Medicine and Health Sciences, University Putra Malaysia, Serdang, Selangor, Malaysia.

Preliminary diagnosis of hemoglobin disorders are carried out using hemoglobin analysis and electrophoresis. The co-inheritance of heterozygous β-thalassemia with homozygous α+-thalassemia (-α/-α) or heterozygous α°-thalassemia (--/αα) can present a near-normal hematological picture. Heterozygous β°-thalassemia with co-inheritance of heterozygous α°-thalassemia (--/αα) show normal MCV and MCH values. In contrast, patients with co-inheritance of deletional HbH disease (--/-α) and heterozygous β-thalassemia show anemia with marked hypochromic and microcytic red cells and very low MCV and MCH indices. Co-inheritance of β-thalassemia with non-deletional HbH disease is rare compared with the more common co-inheritance of β-thalassemia with deletional HbH disease. This study presents a unique Malay patient with HbH-CS disorder and β°/β+-thalassemia (CD41/42/PolyA). Molecular analysis was performed using Duplex-PCR and the Amplification Refractory Mutation System. The patient presented with hemolytic anemia and hepatosplenomegaly. Her hemoglobin at 2 months was 7g/dl and she received her first transfusion at sixteen months. She had increased Hb A_2 of 9.7% and absence of HbH inclusion bodies. A preliminary diagnosis of thalassemia intermedia was made in view of the Hb anlaysis and normal ferritin. Molecular studies were recommended to check for a more severe hemoglobinopathy, and compound heterozygous β-thalassemia/HbH-CS disorder was confirmed. The SEA α-gene deletion in *cis* (--^{SEA}/αα) is generally not present in the Malays, who more commonly posses the two α-gene deletion in *trans* (-α/-α). In addition, the β- gene mutation at CD41/42 is common in Malaysian Chinese and not in the Malays. The presence of both the SEA deletion and CD41/42 in the patient suggests an introduction of these two defects into this Malay family by a previous marriage with a Chinese. In conclusion, reliance on Hb analysis alone is not accurate especially in countries with a multi-racial population. The high frequency of different globin gene mutations together with interactions between α- and β-thalassemias can produce asymptomatic to moderate and severe phenotypes depending on the molecular defects involved. Although co-inheritance of β-thalassemia with HbH disease may result in at most, an intermedia thalassemia phenotype, confirmation of the defects by molecular analysis will provide valuable information that will greatly assist in genetic counseling and prenatal diagnosis.

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Studying the Molecular Mechanism of Cornelia de Lange Syndrome (CdLS) by Using Patient-Derived iPS Cells. *D. Xu[1], J. Mills[2], M. Kaur[1], P. Gadue[2,3], D. French[2], I. Krantz[1].* 1) Division of Human Genetics, The Children's Hospital of Philadelphia, Philadelphia, PA; 2) hESC core facility, The Children's Hospital of Philadelphia, Philadelphia, PA; 3) Department of Pathology Center for Cellular and Molecular Therapeutics, The Children's Hospital of Philadelphia, Philadelphia, PA.

Patients with Cornelia de Lange Syndrome (CdLS) display various congenital defects such as cognitive and behavioral impairment, growth delays and multiple structural defects. Approximately 65% of CdLS probands have an identifiable mutation in cohesin or cohesin-related genes such as *SMC1A*, *SMC3*, and *NIPBL*. The mutations in these genes seen in CdLS, results in disruption of the cohesin complexes' ability to regulate dynamic gene expression rather than its canonical role in regulating chromosome segregation. The disruption of cohesin's gene regulatory role is likely the underlying cause of the specific clinical features seen in CdLS. Our group and others have demonstrated a highly-regulated developmental and tissue specific pattern of gene dysregulation in CdLS individuals. The limitations in studying gene regulation during development in human samples in a temporal and tissue-specific manner have prohibited exploring the mechanism of disease during critical stages when these genes are most relevantly active. To overcome this problem, we have generated a series of induced pluripotent stem cell (iPSC) lines from a fibroblast cell line derived from a severely affected CdLS proband with a frame-shift mutation in NIPBL. A set of control iPSC lines were generated in parallel from a fibroblast cell line derived from a healthy person with matched age, race, and gender. In addition to morphology and growth features characteristic of stem-cells, these iPS cells highly express pluripotent genes such as *REX1*, *ABCG2*, and *DNMT3B*, indicating the fully reprogrammed state of these iPSC lines. These reprogrammed iPS cells have been induced into neuronal and cardiac lineages, further suggesting their de-differentiated state. The ongoing differentiation assays for inducing the iPS cells into different types of cells will provide invaluable specimens for studying the basic molecular mechanism of CdLS in distinct organs and systems. These studies are anticipated to lead to the identification of downstream "effector" target genes of cohesin action that may be critical to the development of specific tissues and will themselves be candidates for isolated structural and cognitive differences seen in constellation in CdLS.

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Colon Cancer In RAPADILINO Syndrome. *K. Anyane-Yeboa, A. Burke, E. Guzman.* Dept Pediatrics, Columbia University Medical Center, New York, NY.

RecQ family of genes is named after RecQ protein of E. coli. Where studied, RecQ family members have been shown to be helicases involved in DNA repair after cell damage, except recQL4. Mutations in helicases have been shown to cause genomic instability disorders in man. Germline mutations of three of them five known human recQ helicases are cause autosomal recessive genomic instability disorders with cancer predisposition and/or aging. RecQ DNA has a helicase domain that is conserved across species. Bloom syndrome and Werner syndrome are autosomal disorders with mutations in RecQ helicases. Individuals with Bloom syndrome are growth delayed microcephalic and have increased susceptibility to various cancers including lymphomas and osteogenic sarcoma. Werner syndrome is associated with premature aging and susceptibility to sarcoma. RecQL4 mutations have been documented in three autosomal recessive disorders: Rothmund-Thomson, Baller-Gerold, and RAPADILINO syndromes. All three disorders are associated with radial and thumb hypoplasia, as well as susceptibility to Cancer. RAPADILINO syndrome, the rarest of these syndromes has been reported in 15 Finnish patients and a few other non-Finnish patients. Among the Finnish patients,there have been 2 osteosarcomas and 4 lymphomas, an overall cancer incidence of 40%. We report the first case of colon cancer in RAPADILINO syndrome in a 42-year-old man with history of low birth weight, cleft palate, and neonatal diarrhea, absent patellae, radial hypoplasia, absent thumbs, short stature, club feet, café-au-lait spots, and borderline intelligence, all features of RAPADILINO syndrome. Chromosome breakage studies revealed levels that are higher than for controls but less than for Fanconi Anemia.His cell are not sensitive to mytomycin C or diepoxybutane. He developed adenocarcinoma of the colon at 29 years of age and had right hemicolectomy but subsequently required total colectomy because of recurrence. Parents are Ecuadorian and there is no consanguinity. Sequencing of RecQL4 reveled 2 novel mutations: c.198delC and c.1465G>T (p.V489F) variant. To our knowledge this is the first reported case of colon cancer in this syndrome. We recommend careful follow-up patients with this syndrome, not only for lymphoma and osteosarcoma but also for colon cancer.

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Genetic background of cochlear implant patients, GJB2 (Connexin 26) and GJB6 (Connexin 30) mutation spectrum. *R. Birkenhager, A. Aschendorff, S. Arndt, W. Maier, E. Lohle, R. Laszig.* Otorhinolaryngology H&N Surg, Univ Freiburg, Freiburg, GermanyDepartment of Otorhinolaryngology and Head and Neck Surgery, University Medical Center Freiburg, Killianstrasse 5, D-79106 Freiburg, Germany.

Congenital sensorineural hearing impairment affects approximately 1-3/1000 newborn. Especially mutations in the GJB2 gene connexin-26 are the reason for non-syndromic hearing impairment. In the present study 451 patients, from the cochlear program of the Department of Otorhinolaryngology, University Medical Center Freiburg (06/2003 - 12/2009), with severe to profound hearing impairment and no evidence of any additional syndrome and malformation of the temporal bones and inner ear in high resolution CT, were analyzed for genetic alterations in the GJB2 and GJB6 genes. Individual exon and intron transitions of the coding exons of the GJB2 gene were PCR amplified using standard conditions. Direct automatic sequencing of fragments was performed bidirectional. Additionally analyse of the GJB6 gene deletions was performed. Mutations in the GJB2 gene were found in 194/451 (43,0%) patients. 42/451 patients were heterozygous for GJB2 alterations, no other mutations were detectable (9,3 %). In five cases the mutation delGJB6-D13S1830 was found in combination with the c.35delG mutation in the GJB2 gene. The frequently-causing mutation c.35delG was detected homozygous in 104 patients (23,1 %). Forty different mutations were identified; fifteen of these gene alterations are novel (Met1Ile, Trp24Leu, Asp46Asn, c.146delC, del307-323 17nt, Trp134Arg, Trp134ter, Glu147Arg, Cys169Tyr, Ala171Gly, Ser183Phe, Gly200ter, Tyr220His, Lys221Asn, ter227Tyr). These mutations were detected in most cases in combination with the mutation c.35delG. These results demonstrate that individuals with severe to profound hearing impairment or deafness should be investigated for GJB2 (connexin-26) mutations. In the case of identification of GJB2 mutations genetic consulting can be offered. Additional screening of newborns with suspected hearing impairment can help to find out early patients who require intensive speech therapy, needs hearing aid and might be candidates for cochlear implantation.

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Schnyder's Corneal Dystrophy: A genetic analysis in humans and canines. *B.N. Kostiha[1,2], M.L. Nickerson[1,3,4], M. Dean[1], J.S. Weiss[5].* 1) Cancer and Inflammation Dept., National Cancer Institute, NIH, Frederick, MD; 2) Biomedical Science Graduate Program, Hood College, Frederick, MD; 3) Graduate Partnership Program, NIH, Bethesda MD; 4) Molecular Medicine Program, Institute for Biomedical Science, George Washington University, Washington, DC; 5) Kresge Eye Institute, Departments of Ophthalmology and Pathology, Wayne State University School of Medicine, Detroit, MI.

Schnyder corneal dystrophy (SCD, MIM: 121800) is a rare autosomal dominant disease with abnormal deposits of cholesterol and phospholipids in the cornea. The diagnostic phenotype is a progressive bilateral corneal opacification resulting in a gradual decrease in visual clarity. A novel gene mutated in SCD, *UBIAD1*, was previously identified independently by two groups. *UBIAD1* is predicted to contain a prenyl-transferase domain and eight transmembrane spanning regions. Treatment is limited for the disease with only two types of treatment available, a phototherapeutic keractectomy and penetrating keratoplasty surgery. As a result, an animal model is of interest. Within this study both the analysis of humans and canines is presented to further understand genotypic to phenotypic correlation of the disease. Furthermore, the study is an important step toward determining if a canine would be a suitable animal model to investigate other treatment options. Patient recruitment occurred through referrals from physicians, family members, self, and publication searches. Genomic DNA isolation occurred from blood samples and the DNA was amplified using a touchdown PCR method. When needed TOPO® PCR cloning and colony PCR occurred to isolate region of interest. DNA sequencing was done using the 3730 DNA Analyzer. Analysis for alterations was completed using a sequence viewer. Sequence alignment, homology, and phylogeny were also done using ClustalX and MEGA. This study presents analysis of ten new SCD families. Of these ten, five have novel genetic alterations A97T, D112N, V122E, V122G, and L188H. One family is of Native American ethnicity, this is the first report of SCD in this ethnic group. The other five families all have the putative hotspot mutation of N102S. The mutated amino acids are observed (15/17) to be highly conserved across species, including canines. Four canines underwent analysis which resulted in finding a single nucleotide polymorphism and an intronic variant, neither thought to cause SCD in the canine. Previous SCD analysis demonstrates families who are known to have the disease but have no alterations within the protein coding regions of the *UBIAD1* gene; it is thought that the canine may follow the same suit as these families.

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A SATB2 nonsense mutation causes Cleft Palate and Cognitive Defects through a dominant negative effect. *P. Leoyklang[1], K. Suphapeetiporn[2], M. Huizing[3], WA. Gahl[3], V. Shotelersuk[2].* 1) Biomedical Science Program, Faculty of Graduate School, Chulalongkorn University, Thailand; 2) Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand; 3) Medical Genetics Branch, NHGRI (NIH), Bethesda, MD.

SATB2 (special AT-rich sequence-binding protein2) (*608148) encodes a DNA binding protein, forming a homodimer that specifically binds nuclear matrix attachment regions and is involveds in transcription regulation and chromatin remodeling. In 2007, we found a 36-year-old man with cleft palate, generalized osteoporosis, and profound mental retardation. He had a de novo germ-line nonsense mutation (c.7154C>T; p.R239X) of SATB2 (Human Mutation. 2007; 28: 732-738). Whether the mutation causes disease through haploinsufficiency, a dominant-negative or a gain-of-function effect was not determined. Here, we first show that the mutant protein is stable. Then, using immunofluorescence and subcellular fractionation, we demonstrate that both the wild-type and mutant SATB2 localize to the nucleus. In addition, protein-protein interaction studies show that the mutant truncated SATB2, which retains the dimerization domain, can form a dimer with the wild type SATB2. These findings strongly suggest that the severe clinical features of our patient are caused by a dominant negative effect of the SATB2 nonsense mutation, rather than to a haploinsufficiency, which associated with a milder phenotype.

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A Japanese family of nemaline myopathy which is caused by *TPM2* mutation. *J. Goto, H. Ishiura, Y. Fukuda, Y. Nagashima, J. Shimizu, Y. Takahashi, Y. Ichikawa, S. Tsuji.* Dept Neurology, Grad Sch Med, Univ Tokyo, Tokyo, Japan.

Background: Nemaline myopathy is a heterogeneous group of congenital myopathy which is characterized by presence of nemaline rods in skeletal muscle fibers. To date, six causative genes have been identified; *ACTA1* (NEM3), *TPM2* (NEM4), and *TPM3* (NEM1) for autosomal dominant types, and *TNNT1* (NEM5), *NEB* (NEM2), and *CFL2* (NEM7) for autosomal recessive types. We experienced an autosomal dominant nemaline myopathy family, of which the proband was a male at the age of 64 years old and showed slowly progressive proximal muscle atrophy and weakness, dysphagia, rigid spine, dilated cardiomyopathy and type 2 repiratory failure. **Object:** To identify the genetic cause, the linkage analysis of the family and sequencing of causative genes were performed. **Subjects and Methods:** This study was approved by the University of Tokyo Institutional Review Board. Informed, written consent was obtained from all participants. The family consisted of three generations and 12 individuals. Seven members were affected. The affected father of the proband was deceased. Genomic DNA was extracted from four affected members and two married-in individuals. Genotyping was carried out using Affymetrix SNP Array 6.0 and linkage analysis was performed using Allegro 2.0 through the processing of the SNP data by SNP HiTLink (BMC Bioinformatics 2009,10:121; URL: www.dynacom.co.jp/u-tokyo/snphitlink). Candidate genes were sequenced by Sanger method. **Result:** Estimated maximum LOD score of the family was approximately 0.9. The linkage analysis revealed that zero to two regions every chromosome remained to be candidate regions. *TPM3* was excluded among the three causative genes for autosomal dominant nemaline myopathies. All exons and their flanking regions of *ACTA1* and *TPM2* were sequenced. A single nucleotide substitution, *TPM2* c.349G>A(p.Glu117Lys), was identified as the causative mutation. **Discussion:** The linkage analysis system using a SNP array and SNP HiTLink is a useful and high-throughput system in both research and clinical practice. Myopathies which are caused by mutations of *TPM2* have been reported as different names; nemaline myopathy-4 (NEM4), congenital cap myopathy and distal arthrogryposis (DA2B). They show different clinical features and also some features are overlapping. It is important to elucidate clinical/phenotypical spectrum or genotype-phenotype co-relation of myopathy caused by *TPM2* mutation.

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Germline *DICER1* Mutations and Familial Cystic Nephroma. *A. Bahubeshi[1,2], N. Bal[3], T. Rio Frio[1,4], N. Hamel[1,4], C. Pouchet[1,2], A. Yilmaz[1,2,4], d. Bouron-Dal Soglio[5], M. Tischkowitz[1,2], J.R. Priest[6], W.D. Foulkes[1,2,4].* 1) Program in Cancer Genetics, Departments of Oncology and Human Genetics, McGill University, Montreal, Quebec, Canada; 2) Lady Davis Institute, Segal Cancer Centre, Jewish General Hospital, Montreal, Quebec, Canada; 3) Department of Pathology, Baskent University Faculty of Medicine, Ankara, Turkey; 4) The Research Institute, McGill University Health Centre, Montreal, Quebec, Canada; 5) Department of Pathology, CHU Sainte Justine, 3175 Chemin de la Côte-Ste-Catherine, Montréal, Québec, Canada; 6) The International Pleuropulmonary Blastoma Registry, St. Paul, Minnesota, USA.

BACKGROUND-Multilocular cystic nephroma (CN) is a benign kidney tumor and is part of a family of kidney neoplasms including cystic partially differentiated nephroblastoma and Wilms Tumor (WT). CN is rarely familial or bilateral, but it occurs in approximately 10% of families where pleuropulmonary blastoma (PPB) is present. Recently, germline mutations in *DICER1* were found in familial PPB. We searched for *DICER1* mutations in two families with familial CN; PPB was present in one family. Additionally, we tested germline DNA from 50 children with sporadic WT for *DICER1* mutations.

METHODS-We screened two families for mutations in the DICER1 gene. Also, we investigated cystic nephromas for loss of heterozygosity and DICER1 expression.

RESULTS-Both families with multiple CN were found to have mutations in *DICER1* leading to premature stop codons, predicted to result in loss of the ribonuclease and dsRNA binding domains. These domains are essential to the function of DICER1. No germline mutations were found in any of the 50 children who had developed WT.

CONCLUSION-We have established that *DICER1* mutations cause familial CN and may be implicated in bilateral CN. No germline mutations were found in the WT cases, suggesting that *DICER1* mutations are unlikely to play a major role in the etiology of sporadic WT. Our results provide further evidence implicating miRNA dysregulation in tumorigenesis.

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A case of likely digenic inheritance of oculocutaneous albinism. *C. Dinsmore[1], A. Hamosh[1], P.W. Chiang[2].* 1) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) UCHSC DNA Diagnostic Laboratory, University of Colorado Denver School of Medicine, Aurora, CO.

Oculocutaneous albinism (OCA) is a well-known autosomal recessive disease caused by mutations in one of four genes in the melanin synthesis pathway. Digenic inheritance has been recognized in a few other recessive diseases, such as retinitis pigmentosa and non-syndromic hearing loss. Here we report a case of likely digenic inheritance of oculocutaneous albinism. The proband, a 27 year-old female, presented to our clinic for genetic counseling regarding ocular albinism. Her ophthalmologic history was significant for nystagmus, decreased visual acuity, retinal hypopigmentation, foveal depressions, and iris transillumination. Although fair, her skin and hair color were consistent with the complexion of her first-degree relatives and her Irish-English-Scottish ancestry. Bidirectional sequence analysis identified one mutation in the *TYR* (OCA1) gene, p.T373K:c.1118C>A, and one mutation in the *OCA2* (P) gene, p.N489D:c.1465A>G. Sequence analysis of *TYRP1* (OCA3), *SCL45A2* (OCA4), and *GPR143* (OA1) was negative. Parental testing revealed her father carries the *TYR* gene mutation, and her mother carries the *OCA2* gene mutation. Both of these mutations have been reported as disease-causing mutations in the literature. There is debate in the literature regarding whether the types of OCA represent a spectrum or distinct genotypic, phenotypic, and biochemical entities. This observation of likely digenic inheritance supports the view of OCA as a spectrum. We cannot rule out the possibility that a second unidentified mutation is present in either the *TYR* or *OCA2* gene. However, this result is consistent with several other published cases of autosomal recessive ocular albinism (AROA) in which only one mutation is identified. Recently, we proposed a new disease mechanism for OCA and suggested that multiple genetic factors, including modifiers from other genes, are necessary to cause the condition, especially in cases of AROA. We have also shown a haploinsufficiency effect of OCA2 in a Hispanic family we studied. Therefore, the presence of one mutation in *TYR* and one mutation in *OCA2*, together with the lighter pigmentation profile of the patient, crossed the threshold and caused AROA in this patient. The knowledge of likely digenic inheritance in OCA is clinically important since the recurrence risk in this case is 25% rather than the <1% risk to individuals with autosomal recessive conditions.

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Association of Rubinstein-Taybi Syndrome with a novel point mutation in the EP300 acetyltransferase domain. *D. EL IMAM[1], M.E. Ahearn[2], R. Faugue[3], N. Bishopric[1,3].* 1) Department of Molecular and Cellular Pharmacology, University of Miami, Miami, FL; 2) Department of Genetics, University of Miami, Miami, FL; 3) Department of Medicine, University of Miami, Miami, FL.

Introduction: Rubinstein Taybi Syndrome (RTS) is a rare syndrome characterized by mental retardation, dysmorphic features and skeletal abnormalities. Classical and non-classical forms of RTS have been described. 52 mutations within the CREBBP gene have been linked to RTS, as well as 6 mutations in EP300. CREBBP and EP300 encode paralogous nuclear histone/factor acetyltransferases (HATs) that activate signal-dependent transcription through acetylation of histones and DNA-binding transcription factors. The majority of CREBBP mutations span or lie in the HAT domain and result in CREBBP haploinsufficiency. EP300 has only recently been linked to RTS and studied in a smaller subset of RTS patients; all mutations to date involve deletions or nonsense mutations. It remains unknown whether EP300 mutations are responsible for milder or non- classical forms of RTS, or whether EP300 haploinsufficiency results in unique clinical manifestations. Aims: (1) to develop an RNA-based method for genetic screening in RTS; (2) to study the functional significance of EP300 mutations. Methods: A mother and daughter with clinically diagnosed classical RTS were studied. Immortalized lymphocytes were cultured and used to isolate RNA and DNA. New PCR primers sets were designed to amplify overlapping fragments of the CREBBP and EP300 coding sequence. Transcripts were amplified and sequenced from lymphocyte RNA. Genomic DNA of the probands was amplified using published primers for CREBBP and EP300. Exomic DNA sequencing was used to confirm RNA-derived cDNA sequence analysis. Results: A novel point mutation was found in mother and daughter at position 3386 A>G in exon 15, changing isoleucine to valine. The amino acid substitution lies within the HAT domain, potentially affecting p300 acetyltransferase activity. Genotypes determined from RNA and DNA sequencing were concordant. The mutation was not found in 96 individuals with no evidence of genetic disorders. Conclusions: (1) We have identified a novel point mutation in two patients with RTS in the HAT domain of EP300, the first reported missense mutation in this gene. Our finding confirms the genetic heterogeneity of RTS and the contributing role of EP300 in the phenotypic heterogeneity of this syndrome. The mutation is expected to affect HAT activity and/or p300 protein stability. (2) RNA-based sequencing can be an efficient alternative to exomic DNA sequencing for mutation screening and genotype-phenotype discovery in RTS.

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Comprehensive search for exonic deletions within the CREBBP gene in Rubinstein-Taybi syndrome patients using MLPA. *M.J. van Belzen[1], C.A.C. van der Lans[1], J.G. Dauwerse[1,2], D.J.M. Peters[2].* 1) Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands; 2) Human Genetics, Leiden University Medical Center, Leiden, Netherlands.

Rubinstein-Taybi syndrome (RSTS) is a rare disorder affecting approximately 1/100,000 newborns. The syndrome is characterized by mental and growth retardation, and a particular dysmorphology mainly concerning the face, hands and feet. The most frequent cause of RSTS are mutations in the CREBBP gene, which are found in 30-50% of patients. Mutations in the EP300 gene are less common, with a frequency of <10%. Mutations in CREBBP are primarily nonsense, frameshift and splice site mutations that occur over the entire coding region, although also several pathogenic missense mutations have been identified. In addition, large deletions, mostly of the entire gene, have been detected by FISH. Recently, smaller deletions were detected by quantitative multiplex fluorescent PCR and multiplex PCR/liquid chromatography. We used the multiplex ligation-dependent probe amplification (MLPA) technique, with probes for every exon of CREBBP, to test for the presence of deletions and duplications in a group of 121 patients suspected of having RSTS. For all patients the coding region of CREBBP, including intron/exon boundaries, was completely sequenced and in 39 patients a pathogenic mutation was detected. In 10 of the remaining 82 mutation-negative patients, eight different deletions were detected. Only one deletion comprised the entire gene. The other deletions were much smaller, between 1 and 8 exons, and two deletions had occurred twice. One deletion occurred in mosaic form in both blood and buccal cells of the patient. No exonic duplications were found. In total, deletions were found in 8% of this group of RSTS patients, and they represented 20% of all detected pathogenic CREBBP mutations. Therefore, screening for exonic CREBBP deletions is an important part of mutation scanning in RSTS patients.

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Molecular and Clinical Findings in Type VI Ehlers Danlos with Lysyl Hydroxylase Deficiency. *Z. Xu[1], D. Vats[2], E.M. Blevins[3], J. Yang[1], L.J. Sloper[1,4], M.J. Willis[3], A.M. Slavotinek[2], J.H. Black[5], N.B. McDonnell[1].* 1) National Institute on Aging, Baltimore, MD; 2) Department of Pediatrics, University of California, San Francisco, CA; 3) Navy Medical Center San Diego, San Diego, CA; 4) MedStar Research Institute, Baltimore, MD; 5) Department of Surgery, Johns Hopkins University School of Medicine, Baltimore, MD.

The type VI variant of the Ehlers-Danlos Syndrome (EDS) is a rare, autosomal recessive connective tissue disorder characterized by scoliosis, hypotonia, easy bruising, scleral fragility, and friable, hyperextensible skin. The implicated gene is mutations in *PLOD1*, resulting in deficient activity of lysyl hydroxylase, an intracellular enzyme involved in the formation of mature collagen. Here, we present clinical and molecular findings in four probands with EDS VI who were diagnosed by reduced pyridinum cross-links in the urine. Patient 1, a girl, was born with hypotonia and intracranial bleed. Bruising and scoliosis at 8 months of age led to diagnosis of EDS VI. Patient 2, a boy, was diagnosed at age 5 due to hypotonia, scoliosis and skin fragility. At age 6, he developed two aneurysms in his legs, one of which progressed to rupture. The aneurysms were successfully treated surgically. Patient 3 is a child of consanguineous parents (uncle-niece), who presented with hypotonia, skin fragility and scoliosis in early childhood. Progressive scoliosis was surgically corrected at age 15, followed by development of aortic and subclavian dissections and aneurysms within two weeks of the procedure. Patient 4 is a 6-year-old child of consanguineous parents (half-first cousins), with neonatal hypotonia, multiple intracranial hemorrhages, hyperextensible joints, lax skin, kyphosis and gross motor delay. By bidirectional sequencing of 19 exons of *PLOD1*, we identified six mutations in the forms of compound heterozygotes or homozygotes in these four unrelated patients with EDS VI. Out of the six mutations, four were novel and five were premature termination mutations. This is the first report of an initiation codon mutation of human *PLOD1*. Using quantitative real-time PCR, we analyzed the relative amount of *PLOD1* mRNA transcripts in the fibroblast cells from two patients, providing evidence for the reduced *PLOD1* expression in these patients. The findings suggest that in children with hypotonia, type VI Ehlers Danlos should be suspected especially in the setting of consanguinity. All our patients had undergone evaluations for suspected neuromuscular disorders prior to the correct diagnosis. Also notable are clinical features of intracranial bleed and relatively good outcomes with vascular surgeries. Scleral rupture was not found in any of the probands to date. Molecular testing has a high yield and allows for prenatal diagnosis.

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Atypical Autism Profile in Children with Cornelia de Lange Syndrome. *S. Srivastava[1], A.D. Kline[2], M. Grados[3].* 1) Johns Hopkins University, Baltimore, MD; 2) Harvey Institute for Human Genetics, Greater Baltimore Medical Center, Baltimore, MD; 3) Johns Hopkins University, Baltimore, MD.

Purpose: Cornelia de Lange Syndrome (CdLS) is a monogenic disorder characterized by growth retardation, craniofacial abnormalities, deficits in limb development, and intellectual disability. Also commonly noted are autistic features, including avoidance of social interaction and physical contact, self-stimulatory movements, and deficits in expressive language. However, the specific delineation of autistic features in CdLS are not well understood. Given the description of autism traits in other monogenic disorders, we seek to define the autism profile for children with CdLS and examine its association with a range of maladaptive behaviors and adaptive functioning measures. Methods: Children with CdLS 5-17 years of age were recruited from specialized clinics and family support group (CdLS Foundation). The clinical diagnosis of CdLS was ascertained using the CdLS Diagnostic Checklist (Kline). A cross-sectional sample of 41 ascertained children with CdLS ages 5-17 years were then evaluated using the interview-based Childhood Autism Rating Scale (CARS; Schopler), interview-based Vineland Adaptive Behaviors Scales (VABS; Sparrow), and the parent-report Aberrant Behavior Checklist (ABC; Aman) in order to assess autistic features, adaptive functioning, and maladaptive behaviors, respectively. Results: For the 41 children with CdLS, 3 autism severity groups were formed based on CARS scores: no significant autism features (n=7; 18%); moderate-mild autism features (n=17; 41%); severe autism features (n=17; 41%). Frequently endorsed CARS items included abnormal emotional response, abnormal body use (self-injury, stereotypies), poor object use, lack of adaptation to change (rigidity), and lack of verbal communication. Relatively spared domains included forming relationships and nonverbal communication skills. Autism severity was associated with temper outbursts and mood swings (Irritablity scale ABC), repetitive behaviors (Stereotypy subscale ABC) and low mood/social avoidance (Apathy subscale ABC). Adaptive skills were also impacted negatively by autism severity scores. Conclusions: Autism features in CdLS children are characterized by deficits in verbal skills and repetitive behaviors with relatively spared social cognition. Further, autism traits are associated with maladaptive behaviors and lower adaptive skills. As in other autism-related monogenic disorders, an atypical autism profile is present in children with CdLS.

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VMD2-related autosomal dominant vitreoretinochoroidopathy: Range of developmental ocular anomalies. *A. Vincent, C. Mcalister, E. Heon.* Department of Ophthalmology, Hospital for Sick Children, Toronto, Ontario, Canada.

Purpose: To explore the spectrum of phenotypic characteristics of autosomal dominant vitreoretinochoroidopathy (ADVIRC) in a family with p.V86M mutation in *VMD2*. Methods: Six affected subjects across three generations underwent ophthalmological evaluation and electrophysiological testing. Goldmann visual field analysis was performed on four. Five family members including three affected were directly sequenced for mutations in the *VMD2* gene. Results: A heterozygous change, p.V86M (c.256G>A), was identified in *VMD2* gene in three affected subjects and was shown to co-segregate with the disease. The best corrected visual acuity ranged from ≥ 20/25 (4 cases; age range 27 - 61 years) to absent perception of light (case 3; 63 years). Color vision was normal in all four tested. Angle closure glaucoma was observed in two subjects whereas shallow anterior chambers and microcornea was noted in one. Iris dysgenesis with abnormal sphincter pupillae was observed in two subjects. Cataracts were observed in three (cases 3, 5 & 6; ages 61, 32 and 28 years respectively) and a history of prior cataract surgery was noted in one (case 1). The classical peripheral concentric band of retinal hyperpigmentation was noted in five. One subject (Case 3 - mother of proband) was blind with dense cataracts at diagnosis precluding retinal evaluation but had anterior segment anomalies, angle closure glaucoma, abnormal electroretinogram (ERG) and the V86M mutation. Bilateral optic nerve hypoplasia was noted in at least one (case 2). Goldmann fields were normal in three (cases 2, 4 & 5; age range 27 -61 years) but showed only central 10^0 preservation in one (case 1; 42 years). Full-field ERG responses were near normal in two subjects (cases 2 & 5; ages 27 & 32 years respectively), moderately reduced in two (cases 4 & 6; ages 61 & 28 years respectively) and non-detectable in two others (cases 1 &3; ages 42 & 63 years respectively). Electro-oculography showed borderline Arden's ratio in case 2 but was not performed in others. Optical coherence tomography showed normal macular thickness in one (case 2; 27 years) and severe thinning in one (case 1; 42 years). Conclusion: Marked intra-familial phenotypic variability is demonstrated in ADVIRC. Developmental ocular anomalies being potential risk factors for development of glaucoma, should be recognized in ADVIRC. Optic nerve hypoplasia and iris dysgenesis are novel observations that extend the ocular phenotype in ADVIRC.

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Chronic lung disease in patients with cutis laxa. *R. Westman[1], S. Henger[1], A. Shifren[2], R.D. Yusen[2], R.P. Mecham[2,3], F. Sciurba[4], Z. Urban[1].* 1) Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA; 2) Department of Medicine, Washington University of School of Medicine, St. Louis, MO; 3) Department of Cell Biology and Physiology, Washington University of School of Medicine, St. Louis, Mo; 4) Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA.

Cutis laxa (CL) is a genetically and phenotypically heterogeneous group of disorders characterized by loose, inelastic, and redundant skin. The different types of CL are classified by inheritance, clinical, and molecular findings. Genes found to cause CL include ELN, FBLN4, FBLN5, ATP6V0A2, LTBP4, PYCR1, RIN2, and ATP7A. Patients with CL often develop pulmonary involvement that may consist of emphysema or respiratory distress. This study aimed to characterize the pulmonary phenotype of the different types of CL based on mutational status. METHODS: The study collected clinical and genetic data of patients with CL. RESULTS: Of the 26 participants, 10 were heterozygous for an ELN mutation, 4 were heterozygous for a LTBP4, 6 were homozygous or compound heterozygous for LTBP4 mutations, 4 were homozygous or compound heterozygous for ATP6V0A2 mutations, and 2 were homozygous for FBLN4 mutations. Of the ELN patients, 5/10 had no significant pulmonary involvement and 3/10 had obstructive lung disease. For LTBP4, 2/4 heterozygotes had no pulmonary involvement, 1/4 had a history of asthma and wheezing, and 1/4 had COPD with bronchiectasis and tracheal diverticulum, and 6/6 LTBP4 homozygotes and compound heterozygotes had significant pulmonary involvement including neontatal respiratory distress, emphysema, tachypnea, pneumonia, herniated lungs, and/or diaphragmatic hernia. In ATP6V0A2 patients, 4/4 had mild to moderate pulmonary involvement with bronchiectasis, diaphragmatic hernia, history of pneumonia, or moderate obstructive disease. In FBLN4 patients, 1/2 had emphysema and diaphragmatic hernia and 1/2 died from respiratory failure. CONCLUSION: Patients with CL have a heterogeneous type and severity of pulmonary complications that do not clearly correlate with genotype. Future studies of patients with CL will require a larger sample size and additional data collection to establish genotype-phenotype correlations.

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Ulnar-Mammary syndrome: Expending the phenotype to include ankyloglossia and cleft lip and palate. *J. Jessen[1], B.H.Y. Chung[1,2], B. Fernandez[3], C. Li[4], D. Chitayat[1,2].* 1) Dept Prenatal Diagnosis, Mount Sinai Hosp, Toronto, ON, Canada; 2) Clinical and Metabolic Genetics, Department of Paediatrics, Hospital for Sick Children, Toronto ON, Canada; 3) Disciplines of Genetics and Medicine, Memorial University of Newfoundland, St. John's, Newfoundland, Canada; 4) Clinical Genetics Program, McMaster Children's Hospital, McMaster University, Ontario, Canada.

The ulnar-Mammary syndrome is an autosomal dominant condition with intrafamilial variability extending from non-penetrant to the known clinical manifestaions, seen within the same family. The main features are ulnar ray defects with missing digits or post-axial polydactyly, ano-genito-urinary abnormalities and hypoplasia of the apocrine glands and breasts. New clinical manifestations are being added in almost every publication making the diagnosis very difficult. We noted two additional findings: ankyloglossia and cleft lip and palate. CASE REPORT: The proband parents were first seen for abnormal fetal ultrasound at 15 weeks gestation, showing bowed and shortened humeri, single forearm bones, bilateral elbow and wrist contractures and bilateral oligodactyly. He also had fenestrated ASD, ankyloglossia, cleft lip and palate, short clavicles with slopped shoulders and unpalpable pectoralis major, laterally displaced inverted nipples and undescended left testis with hypoplastic left hemiscrotum. Karyotype and BAC microarray analysis were normal and DNA analysis for TBX5, EDA1, and TP63 genes showed no detectable mutation. .Detailed family history suggested that his paternal aunt had right upper limb findings similar to the proband and was previously diagnosed as having Holt-Oram syndrome (HOS). Further investigation showed ankyloglossia, and in her recent experience as a mother she had difficulty in lactation from her right breast. TBX3 testing was first done in her and subsequently in our proband and his asymptomatic father detected a familial heterozygous p.R131X mutation in the TBX3 gene. CONCLUSION: UMS is a rare autosomal dominant disorder caused by TBX3 mutations. The diagnostic challenge is related to the poor genotype-phenotype correlation and intrafamilial and interfamilial variability in clinical manifestations. We report two additional clinical observations: ankyloglossia and cleft lip and palate not previously reported in UMS. Different members of the family are dispersed geographically and assessed by different geneticists, making it difficult for any individual geneticist to make the diagnosis. In fact this family was reported as a new syndrome by Li et al., 2009. This illustrates the importance of detailed history and examination, inter-professional communication and sharing of investigation findings in clinical genetics.

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Arylsulfatase E deficiency: functional analysis of novel mutations in patients with CDPX1 and examination of phenocopies. *C. Matos-Miranda[1], G. Nimmo[1], B. Williams[2], S. Bale[2], N. Braverman[1].* 1) Montreal Children's Hospital Research Institute, Department of Human Genetics, McGill University, Montreal, Quebec, Canada; 2) GeneDx, Gaithersburg, Maryland, USA.

X-linked recessive brachytelephalangic chondrodysplasia punctata (CDPX1) is a disorder of bone development characterized clinically by chondrodysplasia punctata (CDP), nasomaxillary hypoplasia and brachytelephalangy. There is a wide range of clinical severity in affected males. CDPX1 is caused by inherited deficiency of Arylsulfatase E (ARSE), a Golgi enzyme whose natural substrate is unknown and, is inhibited by warfarin, a drug that reduces vitamin K levels. Nevertheless, almost half of the patients with CDPX1 phenotypes do not have identifiable ARSE mutations. Although this could be due to undetected ARSE mutations or genetic heterogeneity, some could be phenocopies due to fetal deficiency of vitamin K in the first trimester of pregnancy. This has been observed with maternal warfarin use and maternal small bowel disease, pancreatitis and severe hyperemesis gravidarum. We propose that fetal vitamin K deficiency could inhibit a normal ARSE enzyme, thus generating CDPX1 phenocopies. Through the NIH-CETT program, we identified 20 novel missense variations in ARSE and 17 potential phenocopies. In order to determine the effect of the variations on protein function, we engineered all ARSE mutant constructs and transiently expressed them in COS1 cells, which do not have high interfering sulfatase activities. ARSE activity was measured using the fluorogenic artificial substrate, 4-MU. Our results showed that most mutant alleles had negligible ARSE activity, suggesting that they were pathological. However, alleles located in the C-terminal region showed potential residual activity, indicating an association between mutation position in the protein domains and loss of activity. As part of the CETT program, we also collected clinical data on all patients and we are currently evaluating it to determine genotype-phenotype correlations amongst patients with ARSE mutations, and to identify maternal effects in mutation negative patients.

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P2RX7 rs1718119 and External Apical Root Resorption during Orthodontia. *J.K. Hartsfield[1,2,3,4], T. Moremi[1,2], G. Falcão-Alencar[1,2,5], L.A. Morford[1,2], D.W. Fardo[2,6], J.V. Macri[3], G.T. Kluemper[1].* 1) College of Dentistry, Univ Kentucky, Lexington, KY; 2) Hereditary Genomics lab, Univ Kentucky, Lexington, KY; 3) School of Dentistry, Indiana Univ, Indianapolis, IN; 4) School of Medicine, Indiana Univ, Indianapolis, IN; 5) Universidade De Brasilia, Laboratorio ge Genetica, Departamento de Genetica e Morfoligia, Brasilia, Brazil; 6) College of Public Health, Univ Kentucky, Lexington, KY.

Objective: External Apical Root Resorption (EARR) concurrent with orthodontic treatment is a complex trait. Previous studies have demonstrated that variation in the human *IL-1*β gene is associated with increased EARR in orthodontic patients. The purinergic receptor P2X, ligand-gated ion-channel-7 (*P2RX7*), is an upstream mediator of IL-1β production in both monocytic and osteoclastic cells. This study investigates the association of a non-synonymous (Ala348Thr) gain-of-function SNP in the human *P2RX7* gene, rs1718119, with moderate to severe EARR of the maxillary incisors in orthodontic patients. The null-hypothesis states that variation in *P2RX7* is not associated with EARR. The alternative-hypothesis states that variation in *P2RX7* is associated with EARR. Methods: DNA was isolated from the buccal swabs of 279 Caucasian orthodontic patients (55 EARR cases, 224 controls). Taqman®-based genotyping was utilized for the allelic discrimination of rs1718119 on the Roche LightCycler480. Pre/post-treatment radiographs were analyzed qualitatively by three investigators for EARR of the maxillary-incisors. Subjects were considered to be "affected" when at least two-of-three investigators agreed that EARR was moderate to severe based on graphic standards on at least one of the maxillary incisors. The Cochrane-Armitage trend test assuming an additive mode of inheritance was used to evaluate the association of the SNP and susceptibility to EARR. Results: All rs1718119 genotypes were in Hardy-Weinberg equilibrium. The Cochrane-Armitage Trend Test revealed a p-value of 0.076. Conclusion: While the null-hypothesis could not be rejected for rs1718119 (*P2RX7*) and EARR during orthodontia, the data are suggestive that analysis of additional subjects could reveal a small effect of *P2RX7* on EARR. If demonstrated, this would indicate that *P2RX7* (or a locus in linkage disequilibrium with it) plays a role in EARR of maxillary-incisors during orthodontic treatment. Currently, additional subjects are being analyzed along with analysis of rs208294 *P2RX7* genotypes. This research was supported in part by the Indiana U. Bixler Fund for Genetics Research and Education, the U. of Kentucky E. Preston Hicks Endowed Chair, and the Southern Association of Orthodontics.

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Bardet-Biedl syndrome: A milder phenotype in a female with a proven BBS1 mutation. *K. Bermudez Wagner[1], E. Heon[2], J. Duncan[3], A. Slavotinek[1].* 1) Department of Pediatrics, Division of Genetics, University of California, San Francisco , CA; 2) Department of Ophthalmology and Vision Science. The Hospital for Sick Children Research Institute, Ontario, Canada; 3) Retinal Degenerations Clinic. Department of Opthalmology. University of California, San Francisco, CA.

Bardet-Biedl syndrome (BBS) is usually diagnosed clinically; however, less severe phenotypes may be under-diagnosed and mutation analysis is useful in such situations. We describe a mildly affected 14 year old female who was homozygous for the p.Met390Arg mutation in the BBS1 gene. At birth, she had postaxial polydactyly on both feet and postaxial skin tags on both hands which were removed at 3 months. At age 7, she presented with a refractive eye disorder and was diagnosed with retinitis pigmentosa at 12 years and Bardet-Biedl syndrome at 14 years of age when she had marked visual deterioration (corrected visual acuity 20/200 bilaterally) and night blindness. On examination at 14 years, height was 156.2 cm (10-25%), weight was 64.1 kg (75-90%) and BMI was 26 mg/m2 (overweight defined as > 25 mg/m2; her BMI is much reduced from the mean BMI in BBS females of 31.5 mg/m2). She had slight truncal adiposity, bitemporal narrowing, short and upslanting palpebral fissures, mild retrognathia with a high and narrow palate, brachydactyly with single palmar creases, a sandal gap between the 1st and 2nd toe and pes planus. A renal ultrasound showed mild pelviectasis of the left kidney with persistent microhematuria but normal renal function tests, and she had had normal menarche at 12 years. She had met all of her developmental milestones appropriately and was in regular school, although she had recently been noted to have mild learning disabilities. Genetic testing showed homozygosity for p.Met390Arg in BBS1; both parents were heterozygous for the same mutation. Ethnicity was Nicaraguan and there was no known consanguinity or other relatives who were similarly affected. Although mutations in BBS1 are commonly associated with a 'classical' BBS phenotype, this child was diagnosed at a relatively late age compared to the average age at diagnosis of 9 years (Beales et al., 1999) and she had relatively minor manifestations, having achieved good grades in school until the time of visual deterioration. A milder BBS phenotype in association with p.Met390Arg was also observed in two males with rod-cone dystrophy and polydactyly but without significant obesity or learning difficulties, similar to this child (Cannon et al., 2008). The mild presentation in this girl is consistent with the wide range of phenotypic variability in BBS, and emphasizes the importance of molecular testing.

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Treacher Collins-like mandibulofacial dysostosis with X-linked recessive inheritance. *A. Bytyci Telegrafi, N.L. Sobreira, A. Hamosh, M. Gunay-Aygun.* Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD.

Treacher Collins syndrome (TCS) (MIM #154500) is a form of mandibulofacial dysostosis characterized by symmetrical hypoplasia of the zygomatic bones, downslanting palpebral fissures, lower eyelid colobomas, absent eye lashes, micrognathia, external ear abnormalities and conductive hearing loss. Inheritance pattern is typically autosomal dominant; although autosomal recessive inheritance was also suggested. TCOF1, is the only gene currently known to cause TCS; presence of several TCS patients without TCOF1 mutations suggests locus heterogeneity. TCOF1 encodes Treacle, a nucleolar protein probably involved in nucleolar-cytoplasmic transport. We present a family with a TCS -like phenotype with apparent X-linked recessive inheritance. The proband's prenatal ultrasound at 36 weeks showed micrognathia and polyhydramnios. Amniocentesis showed a normal 46 XY fetal karyotype. Postnatal examination revealed a full term male infant with normal growth parameters. He had symmetrical facial dysmorphism characterized by downslanting palpabral fissures, bilateral zygomatic hypoplasia, midline U-shaped cleft palate and severe micrognathia. There was no coloboma of the eyelids; lower eye lashes were partially absent. External ears were normally formed and placed; ear canals were patent. There was one small ear pit on the right. He had bilateral preauricular hair displacement. There were no limb abnormalities; digits were well formed. Remainder of the exam was unremarkable. Hearing was normal on the right and indeterminate on left ear. Echocardiogram revealed a structurally normal heart. Family history revealed 4 other males with micrognathia, zygomatic hypoplasia, and cleft palate. These included a maternal uncle, and 3 male cousins on the maternal side. All but one of these other affected males died within the first months of life secondary to respiratory distress; a 2-month-old cousin, who is alive, had coloboma of the lower eyelids and bilateral atresia of the external ear canals in addition to the above phenotype. Mother and maternal grandmother had normal facial features. The X-linked recessive TCS-like phenotype in this family may be caused by mutations in a gene on the X chromosome, probably encoding a protein involved in the same functional pathway as Treacle. Alternatively, this might be a sex-limited expression of a gene located elsewhere in the genome. Molecular and clinical studies of the affected and unaffected members of this family are underway.

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A paradoxical genotype-phenotype correlation for *EFNB1* mutations: worse outcome in mosaic than constitutionally-deficient males. *S.R.F. Twigg[1], C. Babbs[1], A. Goriely[1], H.G. Brunner[2], H.V. Toriello[3], I.M.J. Mathijssen[4], A.J.M. Hoogeboom[5], B.R. Pober[6], M.R. Passos-Bueno[7], S.A. Wall[8], A.O.M. Wilkie[1,8,9].* 1) Clinical Genetics, Weatherall Institute of Molecular Medicine, University of Oxford, UK; 2) Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 3) Michigan State University's College of Human Medicine, MI, USA; 4) Department of Plastic and Reconstructive Surgery, Erasmus Medical Center, Rotterdam, The Netherlands; 5) Department of Clinical Genetics, Erasmus Medical Center, Rotterdam, The Netherlands; 6) Center for Human Genetics, Massachusetts General Hospital, Simches Research Building, Boston, MA, USA; 7) Centro de Estudos do Genoma Humano, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil; 8) Department of Plastic and Reconstructive Surgery, Oxford Radcliffe Hospitals NHS Trust, Oxford, UK; 9) Department of Clinical Genetics, Oxford Radcliffe Hospitals NHS Trust, Oxford, UK.

Craniofrontonasal syndrome (CFNS) is an X-linked disorder caused by loss-of-function mutations of *EFNB1*, which exhibits an unusual sex reversal in phenotypic severity: females characteristically have frontonasal dysplasia, craniosynostosis and additional minor malformations, but males are usually mildly affected with hypertelorism only. The proposed mechanism to explain the paradoxically worse CFNS female outcome is termed cellular interference. *EFNB1* is randomly X-inactivated in females, generating two cell populations that express on their cell surfaces either the mutant or wild type protein, ephrin-B1; as ephrin-B1 is normally involved in cell-cell attraction/repulsion, the interaction of these two cell types is thought to lead to the formation of abnormal tissue boundaries, a process that does not occur in hemizygous males. A subset of males, however, has more severe features overlapping those of females. To investigate these unexplained observations, we studied five sporadically affected males diagnosed with the full-blown CFNS phenotype.

Hypothesizing that the more severe male phenotype might also be due to cellular interference, we examined different tissue samples from each male through a combination of Wave DHPLC, sequencing (both dideoxy and massively parallel) and multiplex ligation-dependent probe amplification (MLPA) analysis. We identified mosaic mutations of *EFNB1* in each male, comprising 3 missense changes, 1 gene deletion, and a novel point mutation within the 5' untranslated region. The latter variant (-95T>G), mutates the STOP codon of a small 5' upstream open reading frame (uORF). A nucleotide change at the same position was also found in a classically affected CFNS female (-95T>C). We demonstrate, by luciferase assay, that mutation of -95T is likely to interfere with translation of the wild type protein, probably because the mutant uORF now overlaps the downstream *EFNB1* ORF.

This study, in demonstrating that mosaic *EFNB1* mutations in males are paradoxically associated with a more severe phenotype than fully hemizygous mutations, has provided support for the proposed pathogenic mechanism of CFNS. Furthermore, this work has led to the discovery of a mutation hotspot within the 5' untranslated region of *EFNB1*, likely to interfere with translation of the wild type protein.

920/F

Novel compound mutations in the CLNC7 gene causing Osteopetrosis. *E. GUZMAN, H.H. SHUHAIBER, A. BURKE, K. ANYANE-YEBOA.* COLUMBIA UNIVERSITY MEDICAL CENTRE, NEW YORK, N.Y.

Osteopetrosis ("marble bone disease") is a descriptive term that refers to a group of rare, heritable disorders of the skeleton characterized by increased bone density on radiographs. The overall incidence of these conditions is difficult to estimate but autosomal recessive osteopetrosis (ARO) has an incidence of 1 in 250,000 births, and autosomal dominant osteopetrosis (ADO) has an incidence of 1 in 20,000 births. Osteopetrotic conditions vary greatly in their presentation and severity, ranging from neonatal onset with life-threatening complications such as bone marrow failure (e.g. classic or "malignant" ARO), to the incidental finding of osteopetrosis on radiographs (e.g. osteopoikilosis). We describe one neonate with DNA molecular testing showed two compound mutations in the CLNC7 gene(c.1504T>C; Cys502Arg) and (c.2011C>T; Gln617Term). Testing reveals two dIscrete changes in the CLCN7 gene. Neonate suffered from mutiple bone fractures, pale fundus eye exam. Further testing of parents showed both parents are carriers of the mutation. The child eventually expired. The severe infantile forms of osteopetrosis are associated with diminished life expectancy, with most untreated children dying in the first decade as a complication of bone marrow suppression. Life expectancy in the adult onset forms is normal. It is anticipated that further understanding of the molecular pathogenesis of these conditions will reveal new targets for pharmacotherapy.

921/F

NIIKAWA-KUROKI SYNDROME, A NEW CASE MEXICAN. *L. Hernandez-Gomez[1], S. Juárez-García[2], E. Hernández Gómez[3], D. Gómez Torres[4].* 1) Dept Audiologia, Instituto Nacional de Rehabilitacion, México; 2) Dept Neuropsicología, Instituto Nacional de Rehabilitacion, México; 3) Biología, Facultad de Estudios Superiores Iztacala; 4) Instituto Nacional de Rehabilitacion, México.

Kabuki syndrome is a congenital mental retardation syndrome with additional features, including postnatal dwarfism, a peculiar facies, skeletal anomalies in 92%, dermatoglyphic abnormalities in 93%, mild to moderate mental retardation in 92%; postnatal growth deficiency in 83% and congenital heart defects. Ophthalmologic and otologic problems were common, particularly recurrent otitis media. The incidence of the disorder in Japanese newborns was estimated at 1 in 32,000. All cases were sporadic. The sex ratio was even, and there was no correlation with birth order. Consanguinity was not increased among the parents, and no exogenous agent could be incriminated. Three of the 62 patients had a Y chromosome abnormality involving Yp11.2. In general, the findings of Niikawa et al. (1988) were considered compatible with an autosomal dominant disorder in which each patient represents a fresh mutation We present a new case: female mexican child 10 years old of non consanguineous parents, pregnancy without adversities prenatal and postnatal ,with recurrent otitis media Physical exploration: female with auditory conduct normal, voice and language without alterations. facies characterized by long palpebral fissures with eversion of the lateral third of the lower eyelids arched eyebrows with sparse or dispersed lateral one-third, prominent ears, depressed nasal tip, severe maxillary recession, malocclusion, microdontia, high-arched palate, small dental arch, midfacial hypoplasia. Spine with scoliosis. Audiometric test showed normal audition right, left without answer. Speech audiometric test showed normal right, left without answer. Timpanogram curves As of Jerger bilateral. Stapedial reflex present in right, absent left. Psychology test mild mental retardation Computed Tomografhy of the petrous bones. No middle ear abnormalities were identified, left dysplasia of the inner ear (hypodysplasia of the cochlea, vestibule, and semicircular canals).

922/F

Genetic variants associated with acute toxic hepatitis. *A. Krumina[1], L. Piekuse[1], M. Kreile[1], J. Keiss[1, 2], V. Sondore[2], A. Chernushenko[2].* 1) Dept Biol & Microbiol, Riga Stradins Univ, Riga, Latvia; 2) Infectology Center of Latvia, Riga, Latvia.

Acute toxic hepatitis (ATH) is an inflammation of the liver caused by various toxic chemicals. The role of genetic factors in different outcome of the disease has not yet been clearly defined. The aim of the research was to investigate whether particular mutations in the genes *GSTT1, GSTM1, GSTP1, GSTA1, MTHFR, and UGT1A1* coding for six liver detoxification enzymes are associated with clinical outcome in patients with ATH. Objects of the study were 58 patients with ATH (18 lethal cases, 40 - non-lethal cases) caused by drinking of illegally produced alcohol, and 224 healthy individuals. Genomic DNA was isolated from peripheral blood samples. Mutations were analysed using multiplex polymerase chain reaction (PCR), PCR - RFLP, and direct DNA sequencing. Differences between patients (lethal and non-lethal cases) and control subjects were analysed with SPSS statistical package. Frequencies of mutation C677T in the gene *MTHFR, GSTT1* null genotype, and *GSTT1/GSTM1* double null genotype were significantly higher in ATH patient group compared to control subjects. These frequencies were also higher in ATH lethal cases compared to non-lethal cases. Conclusion: there are genetic variants of liver detoxification enzymes predisposing to more severe clinical manifestation of acute toxic hepatitis.

923/F

Amyoplasia revisited. *J.G. Hall.* Dept Pediatrics and Medical Genetics, University of British Columbia, Vancouver, BC, Canada.

In the early 1980s, we identified Amyoplasia as a specific entity from within the heterogeneous group of disorders described at that time as Arthrogryposis Multiplex Congenita (multiple congenital contractures). It represented one third of cases within a large study (e.g., 135 of 350 patients) of individuals with multiple congenital contractures who survived the newborn period. In the subsequent 30 years, we have accumulated almost 600 cases of confirmed Amyoplasia which greatly expands the variability of phenotype and natural history. About 60% of cases have “classical” four limb involvement with symmetric characteristic positioning of limbs at birth. The affected individuals are sporadic and of normal to high normal intelligence. Prognosis is dependent on the amount of functional muscle and early physical therapy to loosen contractures. Additional subtypes of Amyoplasia which have been identified include: 1) only upper limb involvement, 2) only lower limb involvement, 3) three limb involvement in a predictable pattern, 4) severe involvement with flexed elbows and markedly decreased muscle mass, 5) severe hyperextension of the spine such that the feet touch the head at birth, 6) trismus of the jaw with variable types of contractures at birth. 15% of affected individuals have additional major anomalies related to vascular compromise. 6% of cases of Amyoplasia represent only one of a MZ twin pair (20 times increased above expected). Affected limbs lack normal growth ending up 10%-20% shorter than normal. We continue to postulate vascular compromise between 6 and 11 weeks of embryonic development plays a role in producing the Amyoplasia phenotype. It is important to distinguish Amyoplasia from genetic forms of arthrogryposis.

924/F

The middle interhemispheric variant of holoprosencephaly in a series of 23 patients. *K.E. D'Aco[1], B.D. Solomon[2], N.J. Clegg[3], M.R. Delgado[3], J.S. Hahn[4], L.K. Conlin[5], E.H. Zackai[1], M. Muenke[2].* 1) Division of Human Genetics, Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, PA; 2) Medical Genetics Branch, National Human Genome Research Institute, National Insitututes of Health, Bethesda, MD; 3) Department of Neurology, Texas Scottish Rite Hospital for Children, Dallas, TX; 4) Departments of Neurology and Diagnostic Radiology, Stanford University School of Medicine, Palo Alto, CA; 5) Depatment of Pathology and Laboratory Medicine, Children's Hospital of Philadelphia, Philadelphia, PA.

Syntelencephaly, or middle interhemispheric variant (MIHV), is a rare subtype of holoprosencephaly (HPE) manifesting with incomplete formation of the interhemispheric cerebral fissure, affecting separation of the posterior frontal and/or parietal lobes. MIHV clinically overlaps classic HPE subtypes (alobar, semilobar, lobar) with comparable incidence of seizures and dystonia, but has milder neurocognitive deficits and lower prevalence of endocrinopathies. The etiology of classic HPE is extremely heterogeneous, including multiple genetic and teratogenic causes. Here we report 23 new cases of MIHV with their associated genetic workup, focusing on one patient whose HPE etiology supports the involvement of a key HPE candidate gene. From a separate, unselected cohort of approximately 1,000 probands with HPE we identified the MIHV subtype in 22 probands (0.2%). All 22 probands were tested for mutations in SHH, ZIC2, TGIF and SIX3. One patient had a maternally inherited mutation in SIX3, one had a de novo whole-gene deletion of ZIC2, and three had intragenic mutations in ZIC2 (one de novo, one paternally inherited, and one unknown). There is strong functional evidence for the pathogenicity of all implicated variants. No other patients had identified causes for their phenotypes. Compared to the larger classic HPE cohort diagnosis was slightly later; only one patient was a deceased infant. All patients had developmental delay, though with less cognitive impairment than in classic HPE, and facial malformations were typically less severe in the MIHV group. There was a non-statistically significant over-representation of females, making up 64% of the MIHV cohort (p=0.201). Other findings traditionally associated with HPE, such as cleft lip/palate and hydrocephalus, were common manifestations. Case 23 was diagnosed with MIHV as a neonate secondary to IUGR, microcephaly and hypertonia. He did not have hypotelorism or midline facial defects. No mutations were found in SHH, ZIC2, TGIF or SIX3. His karyotype revealed ring chromosome 21 in 85% of cells with a 10.21Mb deletion within 21q22.13q22.3 that includes the well-established critical region 21q22.3 associated with HPE1. Case 23 demonstrates a third identified genetic etiology for MIHV-type HPE and highlights the importance of continued work to identify the gene corresponding to HPE1. Our cohort as a whole demonstrates that the MIHV subtype represents a small but important fraction of HPE patients.

925/F

Follow up: hydranencephaly with renal agenesis in a girl with normal intelligence. *T. Wegman-Ostrosky, D. Garcia-Cruz, J. Sanchez-Corona, JE. Garcia-Ortiz.* CIBO, IMSS, Guadalajara, Jalisco, México.

Hydranencephaly is a rare CNS abnormality occurring in less than 1 per 10,000 births worldwide. It is defined as a complete or incomplete brain destruction with a bilateral internal carotid artery distribution, resulting in a fluid replacement of the cerebral hemispheres covered with leptomeninges and dura The brain damage generally occurs in the second trimester of gestation, after formation of brain and ventricles. The pathogenesis of hydranencephaly is vascular, but also has been associated to intrauterine infections, particularly toxoplasmosis and viral infections, or in utero drugs exposure. Hydranencephaly has a poor prognosis; most affected patients die after birth and rarely, may survive the teen age. Renal agenesis has been frequently associated to hydranencephaly even the etiology is unknown. Here, we present a 11 years old female patient with hydranencephaly, renal agenesis and normal neurological development. She was the first product of healthy non consanguineous parents. Her family background was unremarkable. She was delivered via Caesarean section, her birth weight was 3550 grams, her Apgar score was 9/9 .No MCA were observed at neonatal period. Her development milestones were normal. At age of 4 years she was hospitalized for urinary tract infection, incidentally left renal agenesis was observed with USG. Due to observed macrocephaly a cranial CT scan was ordered and hydranencephaly was diagnosed. She did or did not presented seizures, frequent migraine episodes have been presented since this diagnosis. Currently she is years-old and her school achievement is completely normal. To our knowledge, there are 5 published cases in the literature with the association of hydranencephaly with renal agenesis, all of them showing from severe to moderate developmental delay. Our patient would be the only one showing a normal intelligence to date.

926/F

Advanced maternal age increases the proportion of UPD in Prader-Willi syndrome. *T. Nagai[1], K. Matsubara[1,2], T. Ogata[2], K. Obata[1], S. Sakazume[1], Y. Kido[1], N. Murakami[1].* 1) Dept Ped, Koshigaya Hosp, Dokkyo Univ Sch Medicine, Koshigaya Saitama, Japan; 2) National Research Institute for Child Health and Development, Department of Molecular Endocrinology, Tokyo, Japan.

INTRODUCTION: Generally genetic causes of Prader-Willi syndrome (PWS) are known that microdeletion of the paternally inherited 15q11-13 and upd(15)mat occur around 70% and 25% of patients, respectively. However, Whittington et al. (2007) have reported an increase in the frequency of upd(15)mat among the children aged under 5 years in the UK. This phenomenon may be due to advanced maternal age that is widely observed in developed countries, because upd(15)mat usually result from trisomy rescue, and advanced maternal age is a risk factor for the development of trisomies such as Down syndrome. To examine this possibility, we studied Japanese patients with PWS. METHODS: We performed methylation test, FISH, MLPA, and microsatellite analysis for 153 patients with PWS with normal karyotype (F:M 96:57, ages; 0-53 years), and classified them into four types: (1) deletion type, (2) trisomy rescue type disomy, (3) monosomy rescue type disomy, and (4) other imprinting defects. Then, we compared the data from 103 patients born at or before 2002- (GrI) and 50 patients born in the period 2003-2009 (GrII). RESULTS: In GrI, 85 (83%) had microdeletions, 14 (13%) had trisomy rescue type disomy, and 3 (3%) had monosomy rescue type disomy. In GrII, 30 (60%) had microdeletion, 14 (28%) had trisomy rescue type disomy, and 3 (6%) had monosomy rescue type disomy. The frequencies of microdeletion and trisomy rescue type disomy were significantly different between the two groups (P=0.001). There was significant difference in the maternal age at birth between the two groups (median 30, range 19-48 vs. median 35, range 23-45, P=0.0004). Between microdeletions and trisomy rescue type disomy, maternal age at birth was similar (P= 0.063) in GrI but was significantly different in GrII (P=2.5E-06). DISCUSSION: These results suggest that advanced maternal age is a risk factor for the development of trisomy rescue type upd(15)mat in GrII. Although the difference in the maternal age at birth was significantly different between the two different time periods, further investigations will be required to assess the maternal age effect on the development of trisomy rescue type upd(15)mat.

927/F

Recurrent erythema nodosum in a patient carrying a mutation in the Mediterranean fever gene (MEFV). *M. Michelson-Kerman[1,2], C. Vinkler[1,2], M. Yanoov-Sharav[1,2], I. Nezer[1], D. Lev[1,2].* 1) Inst Med Genetics, Wolfson Medical Ctr, Holon, Israel; 2) Maccabi Health Sevice, Wolfson Medical Center, Holon. Israel.

Erythema nodosum (EN)is a painful disorder of the subcutaneous fat, the common type of panniculitis. The disorder is characterized by tender, nodular lesions on the anterior aspects of the lower extremities. The process is considered to be an immunologic response and may serve as a marker for systemic diseases such as inflammatory bowel disease, tuberculosis, sarcoidosis, bacterial or deep fungal infection and cancer. Certain antibiotics, oral contraceptives and pregnancy may also be associated. The condition is usually self-limiting and specific treatment is seldom needed in uncomplicated cases. We present an unusual recurrent course of erythema nodosum in a 35 year old woman who is a carrierof a mutation in the MEFV gene. A 35 year old, generally healthy, non-pregnant, woman from Sephardic Jewish ancestry, had suddenly developed painful palpable nodules on the anterior aspects of the lower extremities. Diagnostic evaluation included normal blood count, erythrocyte sedimentation rate and normal liver functions. C-reactive protein, antistreptolysin O-titer, blood cultures and tuberculosis test were negative. Chest radiography was normal. The symptoms exacerbated despite treatment with nonsteroidal anti-inflammatory drugs. Clinical improvement appeared after two courses of corticosteroid therapy, which is unusual for common erythema nodosum course. Mutational analysis of the MEFV gene revealed a heterozygous E148Q mutation. Familial Mediterranean fever (FMF) is an autosomal recessive disease characterized by recurrent attacks of fever with serosal inflammation. The FMF gene (MEFV) encodes the protein pyrin that plays an important role in modulating the innate immune response. MEFV mutations have been identified primarily in patients from Mediterranean populations and in Israel the carrier state is as high as 1 in 5. Although the clinical spectrum and genetic alteration in FMF is variable, erythema nodosum has never been described as a presenting sign of FMF especially in patients with a heterozygous mutation. Various heterozygous mutations in the MEFV gene have been described in association with certain diseases such as Henoch-Schonlein purpura, rheumatoid arthritis and other auto inflammatory disorders and are are considered to be modifying factors. We suggest that MEFV, and particularly the E148Q mutation, is an important susceptibility factor and an independent modifier for the clinical manifestations of erythema nodosum in our case.

928/F

CAUDAL DUPLICATION. A CASE REPORT. *I.P. Davalos[1,2], T. Wegman-Ostrosky[1,2], R. Alferez-Morfin[3], M.G. González-Mercado[1,2], M.L. Ramírez-Dueñas[1], J.A. Cruz-Ramos[1,2], N.A. Vázquez-Cárdenas[1,2], A.J.L. Brambila-Tapia[1,2], B.E. González-Ulloa[3].* 1) División de Genética, CIBO, Instituto Mexicano del Seguro Social (IMSS). Guadalajara, México; 2) Doctorado en Genética, IGH, CUCS, Universidad de Guadalajara. Guadalajara, México; 3) Servicio de Radiología e Imagen , UMAE, Hospital de Especialidades, CMNO, IMSS. Guadalajara, México.

INTRODUCTION We present a case of caudal duplication syndrome, this disorder is associated with partial or complete duplication of different organs in the caudal region spe=ACcially gastrointestinal and urogenital tracts. CASE REPORT We describe a 21 year old female patient. She was born at full-term by normal delivery, weight of 2500. There was no history of neonatal distress. Physical examination at birth revealed imperforate anus sigmoid corrected surgically by colostomy at 10 days and was detected the presence of 3 labia mayora. At birth was diagnosed Caudal Duplication (bladder, uterus and vagina). She has normal psychomotor development. She had no family history of other similar case or congenital anomaly. She suffers from chronic constipation and recurrent infection urinary track. Recently, she presented a severe massive fecal impaction (fecaloma). The RNM revealed double bladder cavity, with well defined borders, normal bladder filling, two uterus, each one with an ovary, with cystic imagens and a calcified fecaloma. DISCUSION/CONCLUSIONS .The pathogenesis of the caudal duplication anomaly is still unclear. It has been proposed that this malformation resulted from an insult to the caudal cell mass and hindgut at approximately the 23rd through the 25th day of gestation. Hypermethylation of the AXN1 gene has been implicated in caudal duplication anomalies. We present a mexican female case of caudal duplication and calcified fecaloma.

929/F

Amelogenesis imperfecta, severe mental retardation, epilepsy (Kohlschutter-Tonz syndrome): questioning the autosomal recessive inheritance, about a new family. *S. Mercier[1], M. Blayau[2], L. Pasquier[1], C. Dubourg[2], C. Henry[3], C. Allaire[4], D. Bonneau[5], M. Tardieu[6], J.L. Sixou[7], S. Odent[1].* 1) Genetique clinique, CHU Rennes, RENNES, France; 2) Laboratoire de Genetique Moleculaire, CHU Hopital Pontchaillou, Rennes, France; 3) laboratoire de Cytogenetique, CHU Hopital Pontchaillou, Rennes, France; 4) Service de Pediatrie, CHU Hopital Sud, Rennes, France; 5) Service de Genetique, CHU Angers, Angers, France; 6) Service de neurologie pediatrique, CHU Bicetre, Le Kremlin-Bicetre, France; 7) Faculte Odontologie, Rennes 1, CHU Rennes, Rennes, France.

Amelogenesis imperfecta ("yellow teeth") is a rare condition. Its association with severe and progressive developmental delay and epilepsy was described for the first time in 1974 as Kohlschutter-Tonz syndrome (OMIM 226750). The 26 published cases from nine different families strongly suggested an autosomal recessive mode of transmission because of the context of consanguinity and / or several cases of recurrence in siblings with normal parents. We report the case of a family with two sons and a daughter born to two different unions of the mother. Both fathers had no known relationship and a panel of nine microsatellites confirmed that the first affected boy and the girl had different fathers. The brothers, from the first marriage, died respectively at 15 years and 14 years. They both presented a severe progressive encephalopathy, seizures, dental anomalies with oligodontia, enamel dysplasia, dental malpositions, and progressive microcephaly. A nonspecific cerebral atrophy was described on MRI. The karyotype, the FISH analysis of telomeres, and metabolic evaluation were normal. During the second marriage, genetic counseling was first reassuring, with a strong probability of autosomal recessive or X-linked disease, especially as the fetus was a female during a new pregnancy. Unfortunately, this patient also had a developmental delay in the first months, seizures at six months, and dental anomalies similar to those of her half-brothers. The first eruption of teeth was delayed, occurring at the age of 5 years. The head circumference curve bent at 3 months. At 8 years, she did not sit, had no language, and was dysmorphic (round face, large mouth, short philtrum). The mitochondrial respiratory chain activity and mitochondrial DNA were normal in lymphocytes and fibroblasts. CGH array study (Agilent chip 4x44K) was normal. There was no skewed X-inactivation in the mother. In conclusion, the etiology of Kohlschutter-Tonz syndrome remains unknown, and this pedigree is consistent with an autosomal dominant pattern of inheritance with a recurrence due to germline mosaicism in the mother. This report emphasizes the importance of detailed dental examination in the assessment of children with severe epilepsy and developmental delay.

930/F

Overgrowth with increased proliferation of fibroblast and matrix metalloproteinase activity related to reduced TIMP1: A new syndrome? *V. Shah[1], B.H.Y. Chung[2,3], A. Hinek[4], D. Chitayat[2,3].* 1) Pediatrics, Mount Sinai Hospital, Toronto, ontario, Canada; 2) The Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital, Toronto, Ontario, Canada; 3) Division of Clinical and Metabolic Genetics, Hospital for Sick Children, Toronto, Ontario, Canada; 4) The Heart Centre - Cardiovascular Research, Hospital for Sick Children, Toronto, Ontario, Canada.

We report on a child with prenatal onset overgrowth associated with facial dysmorphism, thick, excessive wrinkled skin, cleft palate, Chiari malformation and polymicrogyria. His clinical features do not resemble any reported overgrowth syndromes. Genetic investigations tests including testing the possibility of epigenetic alterations of 11p15 region and mutations of the CDKN1C gene (Beckwith-Wiedemann syndrome), mutations and dosage changes of GPC3 gene (Simpson-Golabi-Behmel syndrome), mutations of HRAS gene (Costello syndrome) and chromosomal rearrangement by karyotype and oligo-array. Using immunohistochemistry, we found that cultured skin fibroblasts obtained from this patient demonstrated normally assembled elastic fibers and normal pattern of chondroitin sulfate-deposition with defective deposition of Collagen I fibers. In addition, the fibroblasts revealed high levels of immuno-detectable metalloproteinase (MMP) and undetectable tissue-inhibitors of metalloproteinase (TIMPs). The defective collagen deposition in the fibroblast culture can be reversed by broad spectrum MMP inhibitor - doxycycline. The fibroblasts of this patient also had an increased rate of cellular proliferation. We propose that this is a previously unreported syndrome with overgrowth associated with increased cellular proliferation and defective collagen I deposition due to an imbalance between MMP and TIMP in fibroblasts.

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A Genome-Wide TDT analysis in children with idiopathic intellectual disability. *M. Yazdanpanah[1,2], S. Adam[1,2], F.R. Zahir[2,3], T. Tucker[2,3], M.A. Marra[3,4], J.M. Friedman[1,2,3].* 1) Department of Medical Genetics, University of British Columbia, British Columbia Clinical Genomics Network, Vancouver, Canada; 2) Child & Family Research Institute, Vancouver, Canada; 3) Department of Medical Genetics, University of British Columbia, Vancouver, Canada; 4) Genome Sciences Centre, British Columbia Cancer Agency, Vancouver, Canada.

BACKGROUND Intellectual disability (ID) is etiologically heterogeneous. Genomic imbalance, mendelian conditions and teratogenic exposures are responsible in some cases, but the cause remains unknown in most instances. Multifactorial inheritance has been suggested as an important cause of ID, but the genetic factors involved are unknown. OBJECTIVE: To identify susceptibility genes for idiopathic ID in affected trios in a genome-wide association study. METHODS We used a multistage design to perform a genome-wide transmission disequilibrium test (TDT) analysis in children with idiopathic ID and both of their unaffected parents. The children had moderate-to-severe mental retardation or developmental delay of unknown cause after complete clinical evaluation. In our first stage (discovery phase), we performed genome-wide SNP genotyping with 500 K Affymetrix Gene Chip® arrays on 154 ID trios. We included the SNPs that passed the quality control filter including 90% genotype call rate, MAF ≥1%, and HWE p<10-4 for both the affected children and their parents. Association analysis was performed using PLINK software. RESULTS In our preliminary analysis, the overall genotyping rate was 0.95%. We identified a set of top-ranking SNPs based on a significance threshold criteria of p<10-4. We observed an excess of independent regions associated at p<10-4, suggesting that common susceptibility variants for idiopathic ID exist,but our initial study did not have sufficient statistical power to identify them definitively. Next we will replicate the top-ranking SNPs found in the discovery phase in an independent cohort of 55 ID trios.

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LEUKOCYTE HYPERADHESIVENESS: A NOVEL SYNDROME CHARACTERIZED BY DELAYED SEPARATION OF THE CORD AND RECURRENT SKIN INFECTIONS. *O. Abdul-Rahman[1], L. Svensson[2], A. McDowell[2], K. Yarbrough[1], N. Hogg[2].* 1) Division of Medical Genetics Department of Pediatrics, Univ Mississippi, Jackson, MS; 2) Leukocyte Adhesion Laboratory, Cancer Research UK London Research Institute, London, UK.

Leukocyte adhesion deficiency-1 (LAD-1) is an autosomal recessive disorder characterized by recurrent bacterial infections, defective neutrophil mobility, and delayed separation of the cord caused by mutations in the beta-2 (CD18) subunit common to three integrins. We report a patient with features of LAD-1 in whom a newly identified defect in leukocyte adhesion was detected representing a potentially novel condition. The patient's umbilical cord did not separate until 6 weeks of age, followed by omphalitis. Over the first two years of life, the patient developed multiple episodes of skin infections. Leukocyte adhesion deficiency was initially suspected. However, sequencing of the β2 (CD18) subunit was normal. Adhesion assays on ICAM-1 and fibronectin substrates revealed that the patient's T cells were highly activated with a background adhesion level that was as high as stimulated control cells. Migration assays evaluated the patient's T cells migrating on ICAM-1 using time-lapse microscopy. The cells were extremely adherent and polarized when compared with control T cells, migrating at a rate of 4.05 μm/min compared to 16.07 μm/min for the controls. Our patient demonstrated normal integrin expression, but significantly high adhesion to integrin substrates resulting in abnormally slow migration rates. Since sequencing of the beta-2 subunit was normal (LAD-1), it suggests there may be a problem in the cytoskeletal machinery that regulates integrin turnover. However, this pathway has not been well-characterized. Further studies of other cell lines such as platelets and neutrophils are currently underway to establish the extent of abnormal cellular adhesion.

933/F

Molecular analysis suggests loss of IgA-plasma barrier in intestinal transplant rejection. *C. Ashokkumar[1], M. Ningappa[1], S. Ranganathan[1], L. Schmitt[1], B.W. Higgs[1], Q. Sun[1], C. Kim[2], H. Hakonarson[2], R. Sindhi[1]*. 1) Surgery, Children's Hospital of Pittsburgh , Pittsburgh, PA; 2) Center for Applied Genomics, Children's Hospital of Philadelphia, PA.

Background: Acute cellular rejection (ACR) limits graft survival after intestinal transplantation (ITx). Purpose/Methods: To identify potential mechanisms, differentially expressed genes in allograft tissue with ACR (n=9, rejectors) were characterized with Affymetrix 1.0 ST gene arrays using case-controlled comparison with rejection-free biopsies (n=8 non-rejectors). Replication testing included qRT-PCR and immunohistochemistry (IHC) of biopsy tissue from 8 rejectors and 4 non-rejectors for protein products of candidate genes. Results: Among differentially expressed genes, down regulated CCR10 (negative fold-change 1.275, p=4.95E-04) confirmed by qRT-PCR replication (p=0.012) in rejecting intestine allografts was of interest because in the intestine CCR10 is only co-expressed with IgA, which is present on >70% of normal intestinal plasma cells. Serial biopsy tissue further showed downregulation of the CCR10 gene in rejectors within 4 hours of graft reperfusion (p=NS). CCR10 expression normalized to levels seen in non-rejectors by months 4-12. These changes mirrored serial changes in counts/hpf for mature CD138+plasma cells in IHC studies. Average CD138+plasma cell counts/high power field were lower in biopsies showing ACR compared with rejection free biopsies early after ITx (2.8 vs 27 median cells/hpf, p=0.042). By months 4-12, CD138+plasma cell counts were similar in both outcome groups. Donor-specific alloreactivity measured by allo-antigen-specific CD154+T-cytotoxic memory cells is enhanced during ACR, and was negatively correlated with CD138+plasma cells (Spearman's r= -0.689, p=0.040) corroborating downregulation of the CCR10 gene during ACR. Colocalization studies with confocal microscopy of sequential biopsies from 2 rejectors and 2 non-rejectors showed fewer IgA+cells and more numerous IgG+cells within 4 hours of allograft reperfusion and during ACR among rejectors, compared with non-rejectors. Conclusions: During intestine allograft rejection, downregulation of the CCR10 gene indicates a replacement of the mature CD138+plasma cell population of intestinal allografts with inflammatory IgG+plasma cells. These changes may contribute to antibody-mediated intestine allograft injury.

934/W

Investigating the Role of Fragile X Related Proteins in Mammalian Circadian Behaviors. *J. Lumaban, D. Nelson.* Molec & Human Gen, Baylor College Med, Houston, TX.

Fragile X syndrome, the most common form of inherited mental retardation, results from the absence of the *fragile X mental retardation 1 (FMR1)* gene product FMRP. *FMR1* has two paralogs in vertebrates: *fragile X related gene 1* and *2* (*FXR1* and *FXR2*). One of the behavioral symptoms observed in fragile X patients is the increased occurrence of sleep disorders. Mice that are lacking in both *Fmr1* and *Fxr2* (double knockout) exhibit complete loss of rhythmic activity in both the light:dark cycle and total darkness, and display significant alterations in the cyclical patterns of abundance of core clock component messenger RNAs in the liver, but not the suprachiasmatic nucleus. These findings suggest that Fmrp and Fxr2p are acting downstream of the central clock to control rhythm in mice, but it is unclear how the FXRs affect the peripheral outputs such as in the liver. Locomotor assays with restricted feeding demonstrate that the *Fmr1/Fxr2* double KO mice were able to adjust their rhythm to food availability, and developed food anticipatory activity (FAA), which is an increase in wheel-running activity 3-4 hours prior to a scheduled food presentation. The entrainment response, however, is not as strong as that of the other mice tested: WT, *Fmr1* KO, *Fxr2* KO and even the *Fmr1* KO/*Fxr2* heterozygous (KOH) mice. Current efforts are underway to test whether this entrainment is also observable in the cycling of mRNA expression of clock genes in the liver. Since the *Fmr1/Fxr2* double KO mice are able to entrain to food as a circadian input, and thus have a functional clock, we are also testing whether the arrhythmic behavior previously observed is due to circadian defect or sleep disorders, using both home cage behavior analysis and electroencephalography. The *Fmr1/Fxr2* double KO mice also had a significantly higher mortality rate in the first three days of restricted feeding, and could indicate a difference in their physiological response to food restriction. The liver clock is known to contribute to glucose homeostasis, and efforts are underway to test for the specific involvement of FXRs in this system. The close association of food input to the circadian system and the timing of sleep and wakefulness, together with the typical disturbances of circadian behavior and sleep in Fragile X syndrome, opens up a new perspective for the investigation and treatment of patients suffering from this disorder.

935/W

Association mapping for age related hearing impairment using a mouse model. *J. Ohmen[1], X. Li[1], C. White[1], A. Lusis[2], R. Friedman[1].* 1) Molecular Biology and Genetics, House Ear Institute, Los Angeles, CA; 2) Department of Medicine Dept of Microbiology and Immunology and Molecular Genetics UCLA, Los Angeles, CA 90095.

Age-Related Hearing Loss or AHL (sometimes referred to as presbycusis), is the most prevalent sensory impairment in the elderly. ARHI is a complex disease caused by an interaction between environmental and genetic factors. While the environmental factors conferring altered risk for ARHI have been extensively studied, investigations into the genetic risk factors have only recently been initiated. We have recently initiated a genetic study of AHRI in the mouse. The study design uses a recently developed association mapping technique that has been termed the Hybrid Mouse Diversity Panel, or HMDP. The HMDP consists of over 100 inbred and recombinant inbred strains of mice for which genome-wide SNP data is available. Phenotypic measurements are made in all 100 strains and when combined with the genotype data, uses association mapping to map genes at single gene resolution. The program used to measure the association is called EMMA, and includes features that compensates for population stratification within the different strains of mice used. We have initiated our study of ARL in this collection of mice and present data that confirms it's utility by the confirmation of previously identified AHL loci. Novel loci identified and the positional candidate genes are discussed.

936/W

A Mouse Model for Testing the Influence of Maternal Obesity on the Offspring Health. *H. Miller[1], D. Buchner[2], J. Nadeau[2], D. Serre[1].* 1) Genomic Medicine Institute, Cleveland Clinic, Cleveland, OH; 2) Department of Genetics, Case Western Reserve University, Cleveland, OH.

Several studies have shown the maternal obesity during pregnancy increases the risk for the offspring to develop metabolic diseases in adulthood. However, the molecular basis underlying these life-long consequences remain poorly understood. Here we present a mouse model for testing the influence of maternal obesity during pregnancy on the health of the offspring in adulthood. We fed C57BL/6J female mice on either a low-fat (LF) diet or a high-fat (HF) diet for 5 weeks prior to mating and maintained them on these diets throughout pregnancy and lactation. Offspring from LF and HF mice were separated after weaning and assigned on either the HF or LF diet for 6 weeks. We showed that maternal diet during pregnancy significantly affects birth weight and several metabolic traits in male and female adult offspring. In addition, for some of these traits the influence of maternal diet was exacerbated by feeding the mice a high fat diet postnatal. We are now extending this model to include analysis of older animals (26 weeks of age) as well as embryos and pups in order to better understand the etiology and consequences of maternal obesity during pregnancy.

937/W

Association of peroxisome proliferator-activated receptor (PPAR)-gamma2 gene Pro12Ala missense mutation in Kuwaiti patients with primary knee osteoarthritis. *M. Haider[1], K. Al-Jarallah[2], D. Shehab[2].* 1) Dept Pediatrics, Fac Med, Kuwait Univ, Safat, Kuwait; 2) Dept of Medicine, Fac Med, Kuwait Univ, Safat, Kuwait.

Peroxisome proliferator-activated receptors (PPARs) play an important role in a number of cellular and metabolic functions. This study was undertaken to determine the prevalence of a missense mutation (Pro12Ala) in the PPAR-gamma2 gene in Kuwaiti Arab patients with primary knee osteoarthritis (OA) and healthy controls with the aim of identifying a possible association. The prevalence of PPAR-gamma2 gene Pro12Ala missense mutation was determined in 104 Kuwaiti Arab patients with primary Knee OA and 111 ethnically matched healthy controls by PCR-RFLP of the genomic DNA. The prevalence of this Pro12Ala missense mutation was also determined in clinical subgroups of OA patients divided on the basis of age of onset, function and radiologic grading. Significant difference was detected in the prevalence of PPAR-gamma2 gene Pro12Ala genotypes in the subgroups of patients classified on the basis of age of onset and on functional assessment using Lequesne's functional index and radiological grading using Kellgren-Lawrence (K-L) grading. Our data from Kuwaiti Arabs show that the possession of Pro-Pro genotype of the PPAR-gamma2 gene mutation constitute a risk factor for development of primary knee osteoarthritis.

938/W

Familial Idiopathic Scoliosis in Males: Localization to Chromosome. *C.M. Justice[1], K. Swindle[2], S. Cook[2], J. Dunn[2], N.H. Miller[2].* 1) Genometrics Section, IDRB, NHGRI, NIH, Baltimore, MD; 2) University of Colorado, The Children's Hospital, Denver, Colorado.

Idiopathic scoliosis is present in 2 to 3% of children or adolescents and is defined by a lateral curvature of the spine ≥ 10° for which the cause is unknown. For curvatures of 10 degrees, the proportion of affected females to affected males is approximately 1:1, but the proportion of affected females to affected males increases dramatically as the magnitude of the scoliotic curve increases [Roach 1999]. In our initial population of 202 families, there were approximately 7 affected females for every affected male at curvatures ≥ 30°. Lateral curvatures of 30 degrees are less prevalent in the pediatric population, with a prevalence of approximately 0.2 percent [Miller 1999]. The goal of this study was the identification of genetic determinants in families that include males with severe lateral curvatures. The males with severe curve subset was comprised of 25 families (207 individuals) in which at least one male was diagnosed in adolescence with a ≥30° lateral curvature. There were 123 scoliotic individuals (48 male; 75 female), and 85 unaffected individuals (45 male; 40 female) in this subset. A genomic screen was performed with a modified CHLC v.9 marker set. Fine mapping was done with a custom SNP panel and ABI Taqman methodology on an ABI 377 platform. The initial genome-wide screen and subsequent analyses were analyzed by model-independent linkage analysis using SIBPAL (S.A.G.E. v5.1). The genome-wide linkage analysis for the qualitative and quantitative traits resulted in significant p-values (2 adjacent markers with p-values < 0.01) on chromosomes 2, 16 and 22. The most significant p-value was obtained for the qualitative analysis (threshold set at ≥ 10°) for d22s689 (p-value= 4.2 x10-8), for which an adjacent marker, d22s685 (p-value = 3.8 x x10-4) was also significant. Fine mapping with SNPs confirmed this region as being linked to FIS in our subset of families with adolescent males with curvatures ≥30°. Significant SNPs lie primarily in the introns of the LARGE gene, integral to the development and maintenance of skeletal muscle, and SFI1, responsible for the integrity of the chromosomal centromere complex. Future goals include association and sequencing analyses of this region. FIS is a complex genetic disorder, and utilizing clinical criteria may aid in decreasing the heterogeneity of our large familial idiopathic study population, and enhance the successful identification of specific genes responsible for this disorder.

939/W

Association study on HAPLN1 and MMP genes and osteoarthritis. *A. Nakki[1,2], A. Harilainen[3], K. Tallroth[3], P. Leino-Arjas[4], M. Heliövaara[2], J. Kaprio[1,5], L. Peltonen[1], J. Saarela[1], U. Kujala[6].* 1) Institute for Molecular Medicine Finland FIMM, University of Helsinki, Helsinki, Finland; 2) National Institute for Health and Welfare, Helsinki, Finland; 3) ORTON Orthopedic Hospital, Invalid Foundation, Helsinki, Finland; 4) Finnish Institute of Occupational Health, Helsinki, Finland; 5) Department of Public Health, University of Helsinki, Helsinki, Finland; 6) University of Jyväskylä, Jyväskylä, Finland.

Background. Osteoarthritis (OA) is a degenerative joint disease common in the elderly and the heritability ranges between 40-60 %. The disease progresses by the breakage of the extracellular matrix of the cartilage formed by collagens, aggregans and hyaluronic acid. Matrix metalloproteinases (MMP) are a group of enzymes breaking down the collagen network and aggrecans. Our aim was to study the putative role of four MMP genes and hyaluronan and proteoglycan link protein 1 gene (HAPLN1) in OA, all potentially playing a role in the stability of the cartilage. Methods. We genotyped a total of 37 tagging SNPs covering the MMP3, MMP8, MMP9, MMP13 and HAPLN1 genes in 134 severe radiological familial hand OA cases, 113 unrelated radiological bilateral primary knee OA cases and 2436 population based control samples. The Pseudomarker program was used to monitor for association. For evaluating the strongest association findings, two of the studied SNPs were genotyped in 398 clinician diagnosed knee OA cases and 2708 healthy controls. Results. Eight variants in MMP8, MMP9 and HAPLN1 genes provided nominal evidence for association with OA (p<0.05). The strongest association signals were observed for rs1940475 in MMP8 with combined radiological hand and knee OA (p=0.008) and for rs17577 in MMP9 in radiological hand OA (p=0.004). The SNPs did not associate with the clinician diagnosed knee OA (p=0.10, p=0.72 respectively). Discussion. Initial evidence for association was observed between the MMP8, MMP9 and HAPLN1 genes and severe radiological hand OA and combined hand and knee OA. The SNPs showing the strongest evidence for association with hand OA and combined hand and knee OA did not associate with clinician diagnosed knee OA. The role of the genes might be stronger in hand OA than in knee OA but further conclusions cannot be made without a replication of the results. Analyses between all the studied SNPs in 75 clinician diagnosed knee OA cases are ongoing.

940/W

Association of CYP19A1-CYP17A1 gene polymorphisms with serum steroid hormone levels of Turkish osteoporotic postmenopausal women. *M.B. Yilmaz[1], A. Pazarbasi[1], A.I. Guzel[1,3], S. Kocaturk-Sel[1], I.F. Urunsak[2], S. Basaran[4], M. Kasap[1], H. Kasap[1], O. Demirhan[1], D. Alptekin[1].* 1) Cukurova University, School of Medicine, Dept of Medical Biology and Genetics, Adana, Turkey; 2) Cukurova University, School of Medicine, Dept. of Obstetrics and Gynecology, Adana, Turkey; 3) Rize University, School of Medicine, Dept of Medical Biology, Rize, Turkey; 4) Cukurova University, School of Medicine, Dept. of Physical Therapy and Rehabilitation, Adana, Turkey.

Osteoporosis, a common disease characterized by a generalized reduction in bone mineral density (BMD) and disruption of bone architecture, is a very important public health problem. Sex steroids are the most intensively focused physiologic regulators of the bone mass. Therefore, genes regulating sex steroid production and metabolism are possible candidates for osteoporosis and genetic polymorphisms seen in these genes most likely affect osteoporosis susceptibility in an individual. Of these genes CYP17A1 and CYP19A1 encodes the enzymes catalyzing the rate limiting steps of estrogen biosynthesis. CYP19A1 catalyze the conversion of testosterone to biologically active estradiol. Previously, it has been shown that a silent polymorphism (G®A) in exon 3 of CYP19A1 gene was associated with osteoporosis. The other rate limiting enzyme CYP17A1 catalyzes the conversion of progesterone and pregnenolone into precursors of potent androgens. In the 5 promoter region of the CYP17A1 gene, a T®C substitution has been described to alter CYP17A1 gene expression. In the present study, we tested the association of CYP17A1 and CYP19A1 polymorphisms with BMD in postmenopausal women with osteoporosis in Turkey. We found that GG genotype frequency of CYP19A1 is higher than that of AA and GG and the estradiol concentrations were found to be higher in AA compared to GG and GA genotypes. As for CYP17A1 gene, we did not find any significant association between T®G polymorphism and serum steroid concentrations. In conclusion, our results may suggest that postmenopausal women having CYP19A1 gene GG and GA genotypes comprise a higher risk for osteoporosis.

941/W

Whole genome expression analysis of amniocytes from anencephalic and control fetuses. *C.A. Markunas, K. Soldano, A. Byrd, H. Cope, P. Xu, A.E. Ashley-Koch, S.G. Gregory.* Center for Human Genetics, Duke University Medical Center, Durham, NC, USA.

Anencephaly is a devastating neural tube defect (NTD) affecting roughly 1/4000 babies in the US. Anencephaly occurs when the neural tube fails to close in the cranial region, which results in an absence of the brain and skull. NTDs, such as anencephaly, are thought to have a complex etiology, resulting from both genetic and environmental factors. One approach to identifying novel candidate genes is to examine gene expression during early stages of development in Anencephalic and normal fetuses. Using Illumina HT-12 expression beadchips (48793 probes), we performed whole genome expression analysis using amniocytes from Anencephalic (N=4) and control (N=4) fetuses. Whole genome expression data were pre-processed as follows: 1) quality control assessment and background subtraction using Illumina's GenomeStudio, 2) variance stabilizing transformation and quantile normalization using the R package, Lumi, 3) adjustment for batch effects using ComBat, and 4) non-specific filtering in order to remove the least variable probes. Out of the remaining 4840 probes, we identified 512 probes that were significantly differentially expressed (Welch's t-test p<0.01, FDR q<0.10). Sixty three percent of these were down-regulated in cases compared to controls. Further analyses were performed using DAVID in order to identify biological pathways and gene ontology (GO) terms that were enriched for in our results. We identified two significant KEGG pathways (FDR q<0.05), the ECM-receptor interaction and Pathways in cancer, as well as several significant GO terms, including developmental process, cell differentiation, regulation of cell death, regulation of cell proliferation, protein binding, and others. To further validate our findings and identify novel candidate genes for follow-up, we compared our results to previous findings from an Anencephaly family-based genetic association study (N=45 families), as well as an expression study which identified genes which were differentially expressed in rostral neural tube tissue during Carnegie stages 12 (time of neural tube closure (NTC)) and 13 (NTC complete). One gene, Spectrin, beta, non-erythrocytic 1 (SPTBN1), was nominally significant (p<0.01) in all three studies and an additional forty five genes were significant in at least two of the studies. Interestingly, SPTBN1 was down-regulated in amniocytes of Anencephalic cases and up-regulated during early Carnegie stage 12 compared to 13, suggesting SPTBN1 may play a role in NTC.

942/W

The Apolipoprotein E (APOE) polymorphisms influence on sleep parameters in Obstructive Sleep Apnoea Syndrome. *R. Pellegrino, DR. Manzzotti, CS. Guindalini, R. Santos-Silva, LR. Bittencourt, S. Tufik.* Psicobiologia, Univ Fed Sao Paulo - UNIFESP, Sao Paulo, Brazil.

The majority of sleep disorders are known to be a result of a complex interaction between environmental factors and individual genetic susceptibility. OSAS (Obstructive Sleep Apnoea Syndrome) is characterized by repeated episodes of declines in breathing (hypopneas) or interruption of breathing (apneas).The APOE is a lipoprotein commonly known to directly act in cholesterol transport and plasma lipoprotein metabolism. It has been suggested that APOE isoforms may be involved in OSAS physiopathology through mechanisms related not only to alteration in lipid metabolism, but also to the binding to beta-amyloid proteins, which can eventually lead to neurofibrillary plaques and tangles in the brain respiratory control center. The aim of this study was to evaluate the association between APOE genotypes and sleep parameters in a population based sample from São Paulo, Brazil. APOE genotypes were evaluated in 885 individuals participating in the Epidemiologic Sleep Study of Sao Paulo city, a population-based survey adopting a three-stage randomized cluster sampling. The volunteers were subjected to a full-night polysomnography. Diagnosis of OSAS was performed according to the International Classification of Sleep Disorders (ICSD-2). No significant association was found between APOE genotypes/alleles and OSAS diagnosis. Nevertheless, individuals with OSAS and carrying the APOE E2 allele showed longer sleep latency, lower sleep efficiency, higher number of brief arousals and arousals/hour, when compared to APOE E3 allele homozygous and individuals carrying the APOE 4 allele (p£0.02). APOE E2 allele carriers also spent significantly more time awake (p=0.01), showed higher percent of stage 1 (p= 0.03) and low percent of stage 3 and 4 (p=0.02) compared to APOE E3 homozygous. No significant differences were found between APOE E3 homozygous and APOE E4 allele carriers. In individuals without OSAS, sleep parameters did not differ among the APOE genotypes. Our data showed that the presence of APOE E2 allele is significantly associated with a decrease of sleep quality only in patients with OSAS, suggesting the APOE gene polymorphisms and its regulatory region may contribute in the molecular basis of OSAS physiopathology. Further studies are necessary to fully characterize the role of APOE cluster in sleep architecture and associated phenotypes.

943/W

Association of methylenetetrahydrofolate reductase gene polymorphisms with sporadic amyotrophic lateral sclerosis. *A. Sazci[1], E. Ergul[1], M.D. Sozuguzel Ozel[1], H.A. Idrisoglu[2].* 1) Dept Medical Biology and Genetics,Faculty of Medicine, Kocaeli University, Kocaeli,Umuttepe,41380, Turkey; 2) Department of Neurology, Faculty of Medicine, University of Istanbul, Capa, 34280, Istanbul, Turkey.

Amyotrophic lateral sclerosis (ALS) is a rare progressive and eventually fatal neurodegenerative disorder where motor neurons of the motor cortex, brainstem,and spinal cord degenerate, causing muscle wasting, weakness and profound paralysis. The 5,10-methylenetetrahydrofolate reductase (MTHFR) is the only enzyme in Hcy metabolism, which catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the predominant circulating form of folate in blood. The MTHFR gene has at least two functional polymorphisms, 677C>T and 1298A>C.The MTHFR 677T allele is associated with reduced enzymatic activity, decreased concentrations of folate in serum, plasma, and red blood cells, and mildly increased plasma total Hcy (tHcy) concentrations.We genotyped 398(245 male; 153 female) sporadic amyotrophic lateral sclerosis(SALS)and 439(276 male;163 female) healthy controls to show whether there is an association between MTHFR polymorphisms and SALS. In female SALS, 677T allele is associated with SALS(X2=3.610; P=0.057; OR=1.536; 95%CI=0.986,2.395). TT genotype is also associated with SALS in females (X2=4.649; P=0.031; OR= 2.426; 95%CI=1.062,5.543).CCAA compoud genotype is also protective against SALS in females(X2=4.841;P=0.028; OR=0.426;95%CI= 0.195,0.927). TTAA compound genotype is also associated with SALS in females(X2=5.697; P=0.017; OR=2.747; 95%CI= 1.165, 6.478). In conclusion, MTHFR gene polymorphisms are associated with female SALS in a gender specific manner.

944/W

Association analysis of SMOC2 gene Variant in Jordanian Arab Vitiligo Patients. *A. Alkhateeb[1], F. Qarqaz[2], N. Marzoqa[1].* 1) Dept Biotechnology and Genetics, Jordan Univ Science & Tech, Irbid, Jordan; 2) Dept Dermatology, Jordan Univ Science & Tech, Irbid, Jordan.

Generalized vitiligo is a common autoimmune disorder, characterized by patchy loss of pigmentation due to melanocyte death. It is a multifactorial disorder in which multiple genes and environmental triggers contribute to the expression of the phenotype. Different genetic variants can have varying effects on having vitiligo. Recently, an SMOC2 variant (rs13208776) was reported to be associated with vitiligo in Caucasian patients from an isolated founder population. In this study, we investigate the association of SMOC2 variant with Jordanian Arab vitiligo patients. Forty-four patients with generalized vitiligo and 151 matched normal controls were recruited. DNA samples were obtained from patients and controls and samples were genotyped for SMOC2 variant by restriction fragment length polymorphism. Allelic frequency of the less common allele (A allele) was 29.5% in patients compared to 19.6% in the controls (p=0.27). Genotypic frequency for AA was 4.5% in patients and 7.9% in controls while heterozygous genotypes were 50% for patients and 33.1% in controls. Genotypes did not show statistical difference in patients versus control (p=0.12). Our data shows that the variant rs13208776 in SMOC2 gene does not play a major role in increasing the risk of vitiligo in Jordanian Arab patients. This is in contrast to the previous association reported for Caucasian patients from an isolated patient population in Romania. This signifies genetic differences in the two populations.

945/W

Identification of susceptibility variants for maxillary lateral incisor agenesis: a case-control study. *M. Alves-Ferreira[1,2], T. Pinho[3], A. Sousa[1,4], J. Sequeiros[1,2,4], C. Lemos[1], I. Alonso[1,2].* 1) UnIGENe, IBMC, University of Porto, Portugal; 2) CGPP, IBMC, University of Porto, Portugal; 3) CICS, ISCS-N/CESPU, Portugal; 4) Department of Population Studies, ICBAS, University of Porto, Portugal.

Agenesis of maxillary lateral incisors (MLIA) is the absence of formation of deciduous or permanent lateral incisors. Individuals with MLIA may present phonetic and masticatory dysfunction and also emotional development impairment. MLIA is the most frequent form of hypodontia with prevalence, estimated by us, of 1.3% in the Portuguese population. In a previous study, we found evidence of familial aggregation of MLIA in a sample of Portuguese families suggesting the presence of a strong genetic component for this trait. Odontogenesis is a complex mechanism mediated by genetic factors and is responsible for the determination of the position, number, shape and tooth size. Several genes have been identified as being expressed during odontogenesis and variations in these genes could lead to abnormalities in odontogenesis, resulting in tooth agenesis. Mutations in several genes have been identified in various forms of tooth agenesis or in syndromes in which tooth agenesis is a regular feature. New findings on the function of these genes that are mutated in certain human syndromes further strengthen the important role of these genes in tooth agenesis. Therefore, our aim was to study the association of polymorphisms in seven odontogenesis-related genes with MLIA susceptibility. Association studies are an essential approach in the research of candidate genes involved in MLIA susceptibility in our patients, allowing the identification of common variants associated with this trait. We are currently performing a case-control study, with a case:control ratio of 1:2, to increase the statistical power of our study. We focused on a candidate gene strategy, selecting genes based on their function on odontogenesis and possible involvement in MLIA susceptibility. The selection of the tagging single nucleotide polymorphisms (SNPs) was performed based on the degree of linkage disequilibrium (LD) between the SNPs. This study will comprise a total of 44 SNPs, which will be genotyped by SNaPshot, using a multiplex approach. We are starting the genotyping of 40 SNPs in the 300 individuals ascertained. Next, we will optimize the remaining SNPs and proceed to statistical analysis, comparing SNPs' frequencies in the two groups, in order to identify variants associated with MLIA susceptibility. The data obtained from this study will be useful to better understand the genetic basis of dental agenesis and the pathways underlying MLIA susceptibility.

946/W

Analysis of the variety of sequence variants of SOS1 gene in cleft lip and/or palate patients of Lithuania. *L. Ambrozaityte[1], I. Uktveryte[1], A. Timinskas[2], A. Utkus[1], A. Matuleviciene[1], V. Kucinskas[1].* 1) Human & Med Gen, Vilnius Univ, Vilnius, Lithuania; 2) Institute of Biotechnology, Vilnius, Lithuania.

Orofacial clefting (OFC) is developmental malformation during embryogenesis. It is one of the most common birth defects. Incidence of OFC varies from 1/500 to 1/1000 births and in the population of Lithuanian it is 1/700 - 800 that is akin to Caucasian (European) populations statistics. There are different genes and environmental forces involved in the formation of cleft lip with or without cleft palate (CL/P) and isolated cleft palate (CPO). Up to date more than 20 candidate genes are known which may contribute to non-syndromic CL/P (NS - CL/P). The goal of this study was to identify sequence variants of SOS1 gene (son of sevenless homolog 1 (Drosophila)) that may contribute to NS - CL/P phenotype in Lithuanian population. According to the performed bioinformatic analysis SOS1 gene interacts with 4 CL/P and 50 non CL/P genes. For the detection of risk factors all 23 exons of SOS1 gene were sequenced for 84 patients with NS - CL/P and 84 controls to find variants not only in coding but also in flanking sequences as NS - CL/P risk factors most probably are non patogenic mutations which usually cause complex phenotypes. 19 different variants were identified, 15 of which are SNPs and four in/del polymorphisms. According to the $\chi 2$ test results variant 100711insGTG (c.1075-98_1075-97 insGTG; rs56299761) showed statistical significance ($\chi 2$=4.841; p=0.028), 127470 -/insA (c.2792-51_2792-50 insA) was close to significance ($\chi 2$=3.388; p=0.066). Calculation of partial identity of population using hypergeometrical distribution did not confirm previous results but showed 11 sequence variants or its combinations statistically significant in different NS - CL/P subtypes: 139450G/G (p= 0.041 and p=0.024); 66325C/C (p=0.0179); 66325C/G (p=0.0179); 135314A/G-135350C/T-135389delT-135488T/C (p=0.0179); 135350C/T (p=0.0179); 135488T/C (p=0.0179); 70008C/C-70182C/T-70357G/G (p= 0.0259); 70182C/T (p=0.0259); 112515C/G-112516C/T (p=0.039); 112516C/T (p=0.039). Results of this study demonstrate association of SOS1 gene with NS - CL/P or it's subtypes. SOS1 gene can be considered as candidate gene for NS - CL/P according to this study results and known interaction with other CL/P candidate genes FGFR1 (fibroblast growth factor receptor 1), ERBB2 (v-erb-b2 erythroblastic leukemia viral oncogene homolog 2), EGFR (epidermal growth factor receptor) confirms SOS1 gene's importance to NS - CL/P phenotype.

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Association analysis of Adiponectin and Sulphonylurea Receptor gene polymorphisms with Type 2 Diabetes (T2D) in North Indian Punjabi population. *A.J.S. Bhanwer, K. Matharoo.* Department of Human Genetics, Guru Nanak Dev University,Amritsar, Punjab, India.

Type 2 diabetes (T2D) is emerging as a major health concern in the North Indian Punjabi population which has undergone major change in the life style due to rapid growth in economy after green and white revolution; urbanization and industrialization. There is dearth of data pertaining to molecular genetic studies on predisposition to T2D in this population. To fill the existing lacunae the present case-control study was initiated to analyse single nucleotide polymorphisms (SNPs) in the adiponectin (APM) and sulphonylurea receptor (SUR1) genes with susceptibility to T2D in Punjabi population. 200 T2D patients and 200 non-diabetic age and sex matched healthy controls were recruited for the study with informed consent. Genomic DNA was extracted by phenol-chloroform method and PCR-RFLP procedure was used for genotyping of APM 45 (T®G) and SUR1 Exon16 (-3C®T) & Exon18 (C®T) SNPs. The distribution of APM 45 genotypes (TT, TG and GG) was 45.5%, 51% and 3.5% in T2D and 61.5%, 34% and 4.5%, in controls, respectively. The frequency of T and G allele was 71% and 29% in T2D and 78.5% and 21.5% in controls, respectively. The difference in the genotypes (p=0.003) and alleles was statistically significant [p=0.02; OR=0.67 (0.49-0.93)]. Analysis as per genetic models revealed a protective association of TT genotype against T2D [(p=0.034; OR=0.61 (0.39-0.96)] adjusted for age, sex, BMI. TG-genotype appeared to provide increased risk [p= 0.014; OR=1.78 (1.12-2.82)]. The resulting population attributable risk (PAR) of the G-allele was 9.55% (1.69-18.56). In the analysis of Exon 16 polymorphism, the frequency of C and T-allele in the T2D cases (56.8% and 43.2%) and controls (57.5% and 42.5%) was not significantly different (p=0.83). The frequency of T-allele of Exon-18 was very low in both cases and controls (1.8% and 0.8%, respectively) with insignificant difference (p= 0.20). Adiponectin stimulates glucose uptake and fatty acid oxidation. In the present study, the presence of G allele confers an increased risk to T2D while T-allele provides protection against T2D. Lower adiponectin levels are considered strong predictors for future development of T2D in Asian Indians. Probably, the SNP45 in linkage disequilibrium with other functionally relevant SNPs might affect the levels of protein. Thus, the study suggests that the APM 45 (T®G) polymorphism in the adiponectin gene seems to play a role in susceptibility of T2D in the Punjabi population.

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Analysis of candidate genes for generalized vitiligo in a GWAS dataset supports additional susceptibility loci. *S. Birlea, Y. Jin, P. Fain, R. Spritz.* Human Medical Genetics Program, University of Colorado School of Medicine, Aurora, CO.

Generalized vitiligo (GV) is the most frequent pigmentation disorder, in which white patches of skin, hair, and mucous membranes result from autoimmune loss of melanocytes from involved areas. We previously carried out a genomewide association study (GWAS) of GV in Caucasians, identifying 13 loci involved in disease susceptibility. To identify additional GV susceptibility loci not detected by the GWAS, we re-analyzed the genomewide dataset (1392 cases, 2629 controls) to specifically test association of 20 candidate genes previously suggested to be involved in GV pathogenesis, followed by meta-analysis incorporating the current data and any previous published data. We confirmed association of three of these candidate genes at a Bonferroni-corrected significance threshold P < 2.50E-03. At 5q22.1 we detected association with *TSLP* (rs764916, P = 3.03E-04, OR = 1.60; combined P for published SNP rs3806933 = 3.15E-03, OR= 1.13) encoding thymic stromal lymphoprotein, which induces release of T cell-attracting chemokines from monocytes and enhances the maturation of CD11c(+) dendritic cells. At 22q12.1 we detected association of *XBP1* (rs6005864, P = 3.65E-04, OR = 1.17; combined P for published SNP rs2269577 = 9.50E-09, OR = 1.21), encoding a bZIP transcription factor that regulates immune function and expression of *HLA-DRA*. At Xp11.23 we detected association of *FOXP3* (rs11798415, P = 7.26E-04, OR = 1.19), which encodes a forkhead box transcription factor that is the master regulator of regulatory T cell development and is the gene underlying the IPEX multiple autoimmune disease syndrome. We found no apparent association of GV with the candidate genes *FOXD3, GSTM1, PTGS2, IL10, FBXO11, NFE2L2, ESR1, MBL2, FAS, CAT, VDR, C12orf10, ACE, CLEC11A, AIRE, COMT* and *GSTT1*. Our findings thus extend our previous GWAS results, supporting involvement of *TSLP*, *XBP1*, and *FOXP3* in the pathogenesis of GV and implicating new immunologic pathways in disease pathogenesis.

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Study of association of K121Q and A-G1044TGA polymorphisms of ENPP1 gene with clinical markers of insulin resistance and obesity-related phenotypes in Mexican-mestizo children and adolescents. *A. Del Toro-Valero[1], MG. López-Cardona[6,3], E. Romero-Velarde[2], Y. Saldaña-Alvarez[4], M. Escalante Pulido[5], L. Aguirre-Salas[2,3], L. Orozco-Orozco[4], G. Salas[4], D. Carmona-Navarro[6], N. Davalos-Rodriguez[6,3], J. Armendariz-Borunda[6].* 1) Doctorado en Genetica Humana, CUCS. Universidad de Guadalajara, Guadalajara, Jalisco, México; 2) O.P.D. Hospital Civil de Guadalajara, Dr. Juan I. Menchaca. Guadalajara, Jalisco, México; 3) HR "Dr. Valentín Gómez Farías", ISSSTE. Zapopan, Jalisco, México; 4) Instituto Nacional de Medicina Genómica. DF, México; 5) Unidad Metabólica, CMNO, IMSS, Guadalajara, Jalisco, México; 6) CUCS, Universidad de Guadalajara, Guadalajara, Jalisco, México.

Background: Glucose metabolic disturbances and insulin resistance (IR) are closely related to obesity, diabetes mellitus and cardiovascular disease. The etiology involves cultural and environmental factors, as well as strong genetic predisposition, presenting high global prevalence. Recent reports state that ENPP1 gene encodes a protein that inhibits the alfa subunit of the insulin receptor, suggesting that the ENPP1 gene could be a candidate for IR. Objective: To analyze the relationship of K121Q and A-G1044TGA polymorphisms of ENPP1 gene with clinical markers of IR and obesity-related phenotypes in Mexican children and adolescents. Material and methods: We selected 123 children and adolescents with obesity (BMI $\geq$ 97th percentile) from 2-19 years old and 154 healthy adults. We analyzed the presence of acanthosis nigricans, glucose and insulin serum levels, HOMA index, only in the obese. The SNPs K121Q and A-G1044TGA were genotyped with allelic discrimination by TaqMan. Applying x2, OR and ANOVA for statistical analysis. Results: Of the 123 obese individuals, 48% were female and 52% male. 64% were between 11 and 19 years, 33% between 6 and 10 years and 3% between 2 and 5 years. 76% of patients had acanthosis nigricans. Serum glucose levels equal or greater than 100 mg / dl in 4% of the individuals. Over 70% had insulin levels >15 μU/ml and HOMA index >3. The genotype frequencies presents in patients for SNP K121Q were AA 0.62, AC 0.36 and CC 0.2; and for A-G1044TGA were AA 0.16, GA 0.57 and GG 0.27. For A-G1044TGA SNP in obese individuals the mean HOMA index for homozygous GG was significantly higher (6.6), unlike the genotypes AA and AG (5.9 and 4.7 respectively), with significant differences of p=0.029. The rest of the variables were not statistically significant. The genotype frecuency between healthy adults and obese individuals was no significant. Conclusions:Only GG genotype from A-G1044TGA polymorphism, present in obese children and adolescents was related to higher HOMA index, no relationship was found with acanthosis nigricans, insulin and glucose elevated serum levels. On the other hand, no association was found between K121Q polymorphism and the four variables. The presence of GG genotype from A-G1044TGA polymorphism could be associated with a higher risk of IR in Mexican obese children and adolescents.

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Genome-wide linkage and association analyses in uterine leiomyomata reveals candidate gene on chromosome 17. *S.L. Eggert[1], R. Kavalla[2], K.L. Huyck[4], P. Somasundaram[2], E.A. Stewart[5], A. Lu[6], J.N. Painter[7], G.W. Montgomery[7], S.E. Medland[7], S.A. Treloar[7], L. Rose[8], P.M. Ridker[8,9], D.I. Chasman[8,9], N.G. Martin[7], R.M. Cantor[6], C.C. Morton[2,3,9].* 1) Department of Genetics, Harvard Medical School, Boston, MA; 2) Department of Obstetrics, Gynecology and Reproductive Biology, Brigham and Women's Hospital, Boston, MA; 3) Department of Pathology, Brigham and Women's Hospital, Boston, MA; 4) Department of Pathology, University of Vermont, Burlington, VT; 5) Department of Obstetrics and Gynecology, Mayo Clinic, Mayo Medical School, Rochester, MN; 6) Department of Human Genetics, David Geffen School of Medicine at UCLA, Los Angeles, CA; 7) Queensland Institute of Medical Research, Brisbane, Australia; 8) Division of Preventive Medicine, Brigham and Women's Hospital, Boston, MA; 9) Harvard Medical School, Boston, MA.

Uterine leiomyomata (UL), commonly known as fibroids, are the most prevalent pelvic tumors in women. UL pose a major public health problem given their prevalence (>70%) and rate of symptoms (~20-25%) in women of reproductive age and their indication for >200,000 hysterectomies in the U.S. annually. A genetic basis for tumor development is supported by the observation of recurrent chromosomal aberrations. Further, analyses of ethnic predisposition, twin studies, and familial aggregation indicate a genetic component to UL predisposition, but no genome-wide study for heritable variants has been reported. A genome-wide SNP linkage panel was applied to 261 white UL sister pair families from the Finding Genes for Fibroids study. Two significant linkage regions were detected; the highest LOD score discovered is in 10p11 (LOD=4.15) while the second significant peak is in 3p21 (LOD=3.73). Five additional linkage regions were identified with a suggestive LOD score over 2.00. These regions are in 2q37 (LOD= 2.41), 5p13 (LOD=2.13), 11p15 (LOD=2.53), 12q14 (LOD=2.62), and 17q25 (LOD=2.15). Fine mapping was performed with an independent sample of white women from the Women's Genome Health Study (WGHS) and 39,115 SNPs positioned under the linkage regions. Cases were defined as women who reported being diagnosed with fibroids under age 40 years, and/or had a hysterectomy, and have a mother or sister also diagnosed with fibroids. A standard case/control analysis was performed with 746 cases and 4487 controls; 44 SNPs were identified with p-values £ 0.001. Replication was performed with the 44 suggestive SNPs in a third independent sample of white women from an Australian twin cohort in which cases are defined as women who have been diagnosed with fibroids. A standard case/control analysis was performed with 484 cases and 610 controls; seven SNPs were identified with p-value £ 0.05. Of the seven candidate SNPs, six are in linkage disequilibrium with each other in 17q25.3 and are located in *CCDC57*. The p-values of the six SNPs in *CCDC57* reach genome-wide significance after meta-analysis of the WGHS and Australian samples. Functional studies will be performed to determine the possible role of these candidate SNPs in UL predisposition. Further understanding of genetic variants associated with UL will provide insight into the biology of tumor development and may lead to improved management or novel therapy.

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Support for involvement of CIITA gene variants in rheumatoid arthritis in a Norwegian material and in an updated meta-analysis of 15 cohorts. *M.C. Eike[1], B. Skinningsrud[1,2], T.K. Kvien[3], A. Stormyr[2], D.E. Undlien[1,2], B.A. Lie[4].* 1) Dept of medical genetics, University of Oslo, Oslo, Norway; 2) Dept of medical genetics, Oslo university hospital, Ullevål, Oslo, Norway; 3) Dept of rheumatology, Diakonhjemmet hospital, Oslo, Norway; 4) Inst of immunology, Oslo university hospital, Rikshospitalet, Oslo, Norway.

The class II major histocompatibility complex (MHC) transactivator (CIITA) is a crucial regulator of MHC class II gene expression. Due to the established role of MHC class II in autoimmunity, CIITA is therefore an excellent candidate for involvement in autoimmune diseases. Accordingly, the rs3087456 A>G promoter SNP in this gene has been reported associated with rheumatoid arthritis (RA) and the intronic rs8048002 T>C SNP with autoimmune Addison's disease (AAD). In this study, we genotyped 819 Norwegian RA patients and 2149 controls for both of these SNPs. The rs3087456 GG genotype was significantly associated with RA in a recessive model, both in our material (OR 1.50 [95% CI 1.10-2.05], P=0.0093) and in an updated meta-analysis of 15 cohorts, totalling 13154 RA patients and 16351 controls (OR 1.15 [95% CI 1.01-1.31], P=0.031). However, no significant association was found at the allele level (P>0.05). Moreover, the recessive association appeared to be restricted to Scandinavian cohorts (P=0.0011 vs. 0.24 in other studies combined). The rs8048002*C allele was suggestively associated with RA both at the allele level (OR 1.25 [95% CI 1.00-1.56], P=0.046) and in a dominant model (OR 1.30 [95% CI 1.03-1.63], P=0.026) in our material. Linkage disequilibrium (LD) between the rs3087456 and rs8048002 SNPs was estimated to D'=0.98 and r^2=0.21. Haplotype analysis revealed that rs8048002*C was virtually always carried together with rs3087456*G on a susceptible haplotype (P=0.050), while the latter allele also occurred on non-risk haplotypes. Together with results of regression analyses, this indicates that the RA association of rs3087456 can be explained by LD with rs8048002. CIITA is the only known gene in the LD block harboring rs8048002 in European HapMap populations. Moreover, both rs3087456 and rs8048002 were in low LD with the rs6489169 SNP in the neighbouring C-type lectin domain family 16 member A (CLEC16A) gene previously associated with RA in our material (D'<0.12, r^2<0.002), indicating that the CIITA associations were independent of this effect. Our results provide support for the involvement of CIITA gene variants in RA, but suggest that variants other than the rs3087456 promoter SNP are involved in disease susceptibility. This provides a possible explanation for the diverging results for the rs3087456 SNP in previous studies of RA patients, and warrants follow-up studies that aim to genotype a spectre of SNPs tagging the CIITA harboring LD block.

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Further evidence for the important role of apoptosis in clubfoot. *A.R. Ester[1], S. Richards[2], D. Barnes[3], C. Alvarez[4], S.H. Blanton[5], J.T. Hecht[1,3].* 1) Univ Texas Medical Sch, Houston, TX, 77030; 2) Texas Scottish Rite Hospital for Children, Dallas, TX 75219; 3) Shriners Hospital for Children, Houston, TX, 77030; 4) University of British Columbia, Vancouver, BC, Canada; 5) Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL 33136.

Isolated clubfoot, a common birth defect occurring in more than 135,000 live births worldwide each year, is associated with significant health care and financial burdens. Clubfoot is a complex defect caused both genetic and environmental factors, most of which are yet to be defined. Brewer et al. (1998) identified six chromosomal deletion regions associated with syndromic clubfoot, and suggested that genes in these regions may contribute to isolated clubfoot. Previously, we found evidence for association with genes in the mitochondrial-mediated apoptotic pathway. The goal of this study was to further explore the association between apoptotic genes and clubfoot. Fifty-eight SNPs in eight mitochondrial-mediated apoptotic genes were genotyped in our expanded nonHispanic white (NHW) and Hispanic simplex and multiplex families, as were one hundred and ninety-two SNPs spanning twenty-nine apoptotic genes from the six clubfoot deletion regions. The mitochondrial-mediated apoptotic gene SNPs showed moderate association similar to that previously reported. For the chromosomal deletion region apoptotic genes in the NHW group, a SNP in TNIP2 showed the most significant association while SNPs in NFAT2 were most significant in Hispanics. Importantly for both ethnic groups, gene interactions involving many of the same genes and pathways were identified. Many of these SNPS were in promoter regions and would affect expression of the gene. Apoptosis is involved in muscle development, and the non-rotation of the clubfoot to plantar grade may be due to the disruption of normal muscle development. These results support a significant biologic mechanism for clubfoot that involves a high-risk haplotype composed of different genetic variants that create a higher genetic liability that may increase susceptibility to environmental influences.

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***LOXL1* promoter region SNPs modify risk of exfoliation syndrome and exfoliation glaucoma.** *B.J. Fan[1], L.R. Pasquale[1], D. Rhee[1], J.L. Haines[2], J.L. Wiggs[1].* 1) Department of Ophthalmology, Harvard Medical School, Massachusetts Eye and Ear Infirmary, Boston, MA; 2) Center for Human Genetic Research, Vanderbilt University School of Medicine, Nashville, TN.

LOXL1 is necessary for elastogenesis and elastin homeostasis and is a major risk factor for exfoliation syndrome (ES), an ocular condition predisposing to glaucoma. Two highly associated *LOXL1* SNPs are missense changes in exon 1 (R141L, G153D). The risk allele frequency for both SNPs varies among different populations; the R141L risk allele flips in Japanese and Chinese and the G153D risk allele flips in the black South Africans, suggesting that these variants are not biologically causative but are in linkage disequilibrium with other variants affecting gene expression or protein function. To identify other ES-predisposing *LOXL1* variants, we evaluated SNPs across the whole gene region for association in a U.S. sample. **16** *LOXL1* tag SNPs, and six 5' SNPs near transcription factor binding sites, were genotyped in 196 Caucasian cases and 201 controls. Single-SNP association was initially analyzed using the chi-square test and further evaluated using logistic regression after adjusting for the effects of age and the R141L, G153D missense variants. **13** *LOXL1* SNPs were initially associated with ES after correction for multiple testing, however after adjusting for the effects of age and the R141L and G153D variants only rs12914489 remained associated (p=0.04). Logistic regression showed an additive effect between rs12914489 and G153D with an increased risk of ES (OR=20.40) in individuals carrying risk alleles in both SNPs compared to individuals carrying only the G153D risk allele (OR=8.73) suggesting that variants in the region 5' to the gene may independently influence disease progression. Of the 6 significant SNPs located in the 5' region rs16958477 demonstrated the best association ($p=6.4\times10^{-7}$). The haplotype consisting of risk alleles for rs16958477 and G153D was significantly associated with ES ($p=1.1\times10^{-7}$). rs12914489 is located approximately 30kb from the transcription start site in a conserved region containing at least one transcription factor binding site. Joint analyses with the G153D variant suggest that rs12914489 may contribute to ES risk as an independent additive factor. rs16958477 is located near the transcription start site, and previously the rs16958477 A allele has been shown to have significantly less transcription activity than the C allele. Our results showing association between the A allele and ES suggests that rs16958477 could contribute to ES by reducing *LOXL1* gene expression. **Grant support:** NEI R01EY015872, P30EY014104; RPB, MLERF.

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ALX4 Variants in Nonsyndromic Sagittal Craniosnosynostosis. *A.S. Ghuman[1], L. Qi[2], J. Liu[1], G. Yagnik[1], E. Cherkez[1], M.L. Cunningham[3], V. Kimonis[4], S.A. Boyadjiev Boyd[1].* 1) Dept Pediatrics, SOM, University of California Davis, Sacramento, CA; 2) Rowe Program in Human Genetics, University of California Davis, Davis, CA; 3) Dept Pediatrics, Division of Craniofacial Medicine, University of Washington, Seattle, WA; 4) Dept Pediatrics, University of California Irvine, Irvine, CA.

Premature fusion of one or more cranial sutures is defined as craniosynostosis (CS) and this defect occurs in one of 2,000 live births. Nonsyndromic cases occur when the suture synostosis is an isolated anomaly (85% of reported cases, vs. 15% syndromic). Sagittal craniosynostosis (SCS) comprises 50% of all nonsyndromic CS patients. ALX4 plays the general role as a transcription factor involved in skull development and specifically, in controlling the proliferation-differentiation balance in the coronal suture. Loss of function (LOF) mutations of ALX4 result in parietal foramina. Similarly, LOF mutations of MSX2 result in parietal foramina, while gain of function (GOF) of MSX2 causes craniosynostosis. Based on that, we hypothesize that ALX4 GOF polymorphisms will result in SCS. Sequencing of ALX4 in 140 probands with nonsyndromic SCS identified three rare missense variants that are not present in the existing genetic databases and involve highly conserved residues. These polymorphisms are then to be tested for expression levels via dual luciferase assay. In addition, thirteen variants were identified in a larger SCS cohort (n=229) of which seven were present in controls and six were novel. Tests for associations documented statistically significant over representation of two non-synonymous variants in probands, suggesting that they confer increased susceptibility to SCS. In summary, our data suggests that unique and common ALX4 variants may contribute to a proportion of SCS cases through a GOF effect.

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Gene-targeted (by MLPA) quantitative analysis in Autism Spectrum Disorders. *F. Gurrieri, N. Cannelli, G. Neri.* Genetica Medica, Univ Cattolica, Rome, Italy.

Autistic disorders (ASD) are a group of childhood neurodevelopmental disorders characterized by difficulties in socialization and communication and stereotypic behaviors. Array-CGH has recently allowed the identification of CNVs in 10% of ASD cases, often non randomly. Nevertheless, this high-throughput approach is limited by the resolution and assembly of the array-chip used in which some genes or genomic regions, still involved in ASD, may not be represented. In addition, alterations can be detected in regions not known to be related to ASD and therefore difficult to interpret as meaningful results. With the aim to develop a targeted quantitative genetic test for ASD we built an "in-house" MLPA assay with probes covering 9 candidate genes for ASD and tested it on 115 ASD patients. METHODS: All patients tested negative for Fragile-X syndrome, Rett syndrome and SHANK3 deletions/mutations. Candidate genes included in the MLPA assay were: FOXP2 (7q31), UBE2H (7q32), CALU (7q32.1), GRM8 (7q31), GRM5 (11q14), DOC2B (17p13), HOMER1 (5q14), NLGN3 (Xq13), FHIT (3p14). Probes were designed for each gene, according to MRC-Holland on-line manual and MLPA reactions were performed using EK1 reagent kit (MRC Holland, Amsterdam). Results were analysed by a developed spreadsheet in Microsoft Excel. All test/reference ratio values below 0.7(0.35 for NLGN3 in males) or exceeding 1.3 (0.65 for NLGN3 in males) were considered as CNA. The assay was first validated on 30 controls. RESULTS:We found a CNV in 4 patients (3.5%). In two of those, brother and sister, we detected an extra copy of NLGN3 probe. In another female patient we found a value of 0.57 in NLGN3 probe and in an additional unrelated female we found a similar value for the CALU gene, both values suggestive of a probable allele loss. These probes are not included in the array-chips currently in use in our laboratory (44K and high-definition 105K from Agilent). CONCLUSIONS:Our results indicate that a targeted MLPA assay could allow detection of intragenic CNVs that might have been missed with array-CGH, even at high resolution. This approach needs to be validated in higher cohorts of patients and other combinations of candidate genes might be assembled for a targeted diagnosis in ASD patients. Whereas NLGN3 alterations confirm already known associations of neuroligins to autism, the CALU alteration is interesting and can open further future investigations about its role in autism pathogenesis.

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Fine Mapping of Regions Linked to Type 2 Diabetes and Admixture Mapping of Type 2 Diabetes in African American Families: The American Diabetes Association GENNID Project. *S. Hasstedt[1], C.L. Hanis[3], S.C. Elbein[2].* 1) Dept Human Genetics, Univ Utah, Salt Lake City, UT; 2) Section on Endocrinology and Metabolism, Dept of Medicine, Wake Forest Univ. School of Medicine, Winston-Salem, NC; 3) Human Genetics Center, School of Public Health, University of Texas Health Science Center at Houston, Houston, TX.

The Genetics of NIDDM (GENNID) study was established by the American Diabetes Association as a resource for the discovery of genes related to diabetes and its complications. Autosomal linkage analysis of type 2 diabetes (T2D) and age of diagnosis (AOD) on a subset of the GENNID sample, which included 1344 (1082 with T2D) members of 524 African-American families, identified 4 candidate regions for T2D susceptibility genes (chromosomes 2 at 68-121 MB, 7 at 50-79 MB, 13 at 19-32 MB, and 18 at 33-67 MB) [Elbein et al Diabetes 58:268, 2009]. To follow up on these findings, we selected 10,650 single nucleotide polymorphisms (SNPs) to tag each gene under the 4 linkage peaks, in addition to 1600 SNPs from a panel of admixture informative markers. Genotyping was performed by CIDR. Admixture linkage analysis using ADMIXMAP identified 4 SNPs with suggestive P-values ($p<0.0005$) on chromosomes 13 (2 SNPs; 10 cM; 23.9 Mb, PARP4 gene), 12 (138 cM; 115 Mb; intergenic region), and 16 (0 cM; 0 Mb; MPG gene), but no SNP achieved genome-wide significance. The two SNPs on chromosome 13 fell within the region of linkage. We tested tag SNPs under each linkage peak for association with T2D, T2D adjusted for BMI, and age of diagnosis using jPAP, and also tested the change in LOD score for each associated SNP. Nominally significant associations were found for 14 SNPs on chromosome 2, 8 on chromosome 7, 13 on chromosome 13, and 7 on chromosome 18. Although no SNP reached experiment-wide significance, we found suggestive associations of rs7349979 (CALN1 gene) and rs2691529 (MAGI2 gene) with T2D and age of diagnosis on chromosome 7, and rs1893430 in transcription factor TCF4 with T2D on chromosome 18. The largest changes in LOD score were for rs7582241 (chromosome 2, CTNNA2; 0.51), rs3789073 (chromosome 2, ACOXL, 0.59), rs9318100 (chromosome 13, MIPEP, 0.66), and rs4770868 (chromosome 13; ATP8A2, 0.57). Given the paucity of T2D loci in African Americans, these studies are an important step in mapping susceptibility genes for African American diabetes. Additional analyses and testing in additional populations is needed to confirm the suggestive findings.

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Investigation of European-derived adiposity loci in African Americans. *J.M. Hester[1,2,3], M.R. Wing[1,2,3], N.D. Palmer[1,2,4], P.J. Hicks[1,2,4], B.H. Roh[1,2], C.D. Langefeld[5,6], B.I. Freedman[7], D.W. Bowden[1,2,4,7], M.C.Y. Ng[1,2].* 1) Center for Diabetes Research, Wake Forest University; 2) Center for Genomics and Personalized Medicine Research, Wake Forest University; 3) Program in Molecular Genetics and Genomics, Wake Forest University; 4) Department of Biochemistry, Wake Forest University; 5) Department of Biostatistical Sciences, Wake Forest University; 6) Division of Public Health Sciences, Wake Forest University; 7) Department of Internal Medicine, Wake Forest University.

Obesity is a major public health problem in the United States with substantial racial differences in prevalence. To date, many GWAS have been performed to identify novel adiposity-associated variants in different European-derived populations, but few have attempted to replicate these associations in African Americans. We have conducted a genome-wide association study (GWAS) on body mass index (BMI) in 1715 unrelated African Americans (816 healthy subjects and 899 subjects with type 2 diabetes and nephropathy) using the Affymetrix 6.0 platform. Single SNP association analyses were conducted on 746,626 good quality autosomal SNPs with adjustment for age, gender and first principal component reflecting admixture proportion. Based on the GWAS results from previous studies in European populations, we have compiled a list of 23 adiposity loci for evaluation in our African American population. Of the 58 known index variants reported in European populations, only rs17782313, rs477181, and rs17700633 in *MC4R* and rs7498665 located between *SH2B1* and *ATP2A1* showed evidence of nominal association in our samples ($0.01<p<0.05$). A locus-wide analysis was performed by examining associations at a ±10kb flanking region around the genes or ±50kb flanking the intergenic index variants. This approach takes advantage of the fact that association signals may vary among different populations, and the smaller LD blocks in our African American population may help to fine map variants that could be causally related to disease. Using this approach, 577 SNPs were nominally associated with BMI ($p<0.05$). The strongest association was observed at rs2887941 ($p=2.5x10^{-4}$) located in the *NRXN3* gene. Nominal associations were also observed at *PARD3B* (rs12622484, $p=2.69x10^{-4}$), *MC4R* (rs639193, p=0.0013), *TFAP2B* (rs9349560, p=0.0018), *NEGR1* (rs7552295, p=0.0019), *MAF* (rs7195737, p=0.0019), *PRL* (rs9366426, p=0.003), and *MTCH2* (rs4752783, p=0.003). These results suggest that common genetic variants at loci that modulate adiposity in European-derived populations have only modest impact in African Americans. The ethnic differences in genetic susceptibility may partly account for the ethnic disparities in obesity-related traits. These putative associated SNPs warrant further investigation in additional African American populations.

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Association of *ADAM33* polymorphisms with asthma in African Americans. *E.Y. Jingwi[1], L. Ricks-Santi[2,3], M. Abbas[2], T. Mason[2], C. Jackson[2], B. Arrey[2], V. Apprey[2,4], G. Bonney[2,4], A.A. Adeyemo[6], C.N. Rotimi[6], C.M. Lee[1], G.M. Dunston[2,5], M.U. Faruque[2,4].* 1) Department of Biology, Howard University, Washington DC; 2) National Human Genome Center, Howard University, Washington DC; 3) Cancer Center, Howard University, Washington DC; 4) Department of Community and Family Medicine, Howard University College of Medicine, Washington DC; 5) Department of Microbiology, Howard University College of Medicine, Washington DC; 6) Center for Research on Genomics and Global Health, National Institutes of Health, Bethesda, MD.

Background and Purpose: Asthma is a chronic inflammatory disease of the airways that has reached epidemic proportions in the US and other parts of the world. It appears to vary phenotypically according to ancestry. African Americans show higher incidences and clinically more severe asthma, with different patterns of allergic sensitization, higher IgE levels and bronchial hyperresponsiveness, when compared with Americans of European ancestry. Asthma is caused by the interaction of multiple genes and environmental factors. Our laboratory uses common variants to interrogate the biology of health disparities like asthma. Single nucleotide polymorphisms (SNPs) in *ADAM33* gene have been consistently associated with asthma in European and Asian populations in independent studies. We studied the association of SNPs in *ADAM33* with asthma in African Americans. Materials and Methods: A total of four single nucleotide polymorphisms (rs2787094, rs612709, rs2280091, rs2280090) were examined in 496 African Americans including 296 asthma cases and 200 geographically matched non-asthmatic controls from the Washington DC metropolitan area. Association was tested by logistic regression analysis under an additive genetic model with adjustment for age and gender. A replication p-value of <0.05 was considered significant. Results: rs2787094 in the 3' untranslated region of the *ADAM33* gene was significantly associated with asthma (OR = 1.51, 95% CI: 1.03 -2.20; p = 0.032). None of the other three SNPs (rs612709, rs2280091, rs2280090) was significantly associated with the disease risk. Conclusions: Out of four SNPs previously reported to be associated with asthma in non-African populations, one was replicated in this study of African Americans. This finding shows the functional significance of common variants in dissecting the biology of health disparities and may be instructive in genotype-based diagnosis and target intervention in the emergence of personalized genomic medicine.

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Identification of a novel systemic lupus erythematosus susceptibility locus at 11p13 near *CD44* in a multi-ethnic study. *C.J. Lessard[1,2], I. Adrianto[1], J.A. Kelly[1], K.M. Kaufman[1,2,3], M.E. Alarcón-Riquelme[1,4], J.M. Anaya[5], S.C. Bae[6], S.A. Boackle[7], E.E. Brown[8], L.A. Criswell[9], J.C. Edberg[10], B.I. Freedman[11], P.K. Gregersen[12], G.S. Gilkeson[13], C.O. Jacob[14], J.A. James[1,15], R.P. Kimberly[10], J. Martin[16], J.T. Merrill[17], T.B. Niewold[18], B.A. Pons-Estel[19], A.M. Stevens[20,21], B.P. Tsao[22], T.J. Vyse[23], C.D. Langefeld[24], C. Gray-McGuire[1], J.B. Harley[2,3], R.H. Scofield[1,2,3], P.M. Gaffney[1,2], K.L Moser[1,2].* 1) Arthritis and Immunology Research Program, Oklahoma Medical Research Foundation; 2) Department of Pathology, University of Oklahoma Health Sciences Center; 3) US Department of Veterans Affairs Medical Center, Oklahoma; 4) Center for Genomics and Oncological Research (GENyO), Granada, Spain; for the BIOLUPUS Network; 5) Center for Autoimmune Diseases Research (CREA), Universidad del Rosario, Bogota, Columbia; 6) Department of Rheumatology, Hanyang University Hospital for Rheumatic Diseases, Seoul, Korea; 7) Division of Rheumatology, University of Colorado Denver; 8) Department of Epidemiology, University of Alabama at Birmingham; for PROFILE; 9) Rosalind Russell Medical Research Center for Arthritis, University of California San Francisco; 10) Department of Medicine, University of Alabama at Birmingham; 11) Department of Internal Medicine, Wake Forest University Health Sciences; 12) The Feinstein Institute for Medical Research, North Shore Long Island Jewish Health System, Laboratory of Genomics and Human Genetics; 13) Division of Rheumatology, Medical University of South Carolina; 14) Department of Medicine, University of Southern California; 15) Department of Medicine, University of Oklahoma Health Sciences; 16) Instituto de Parasitologia y Biomedicina Lopez-Neyra, Consejo Superior de Investigaciones Cientificas; 17) Clinical Pharmacology, Oklahoma Medical Research Foundation; 18) Internal Medicine and Rheumatology, University of Chicago; 19) Department of Genetics and Pathology, Rudbeck Laboratory, Uppsala University; 20) Seattle Children's Hospital, Rheumatology and Pediatric Rheumatology; 21) Department of Pediatrics, University of Washington; 22) Rheumatology and Arthritis, University of California Los Angeles; 23) Division of Medicine, Imperial College London; 24) Department of Biostatistical Sciences,Wake Forest University Health Sciences.

SLE is a chronic, heterogeneous autoimmune disorder characterized by inflammation, loss of tolerance to self-antigens and dysregulated interferon responses. In this study, we sought to replicate a putative association at 11p13 from a genome-wide association (GWA) study not yet exceeding the stringent threshold for genome-wide significance (typically $P<5x10^{-8}$). Genotyping was performed using Illumina iSelect technology. Stringent quality control measures were applied for Hardy-Weinberg proportions, proportion of missing genotypes and missingness between cases and controls. After quality control filtering, 3562 SLE cases and 3491 controls of European ancestry, 1527 cases and 1811 controls of African-American (AA) descent and 1265 cases and 1260 controls of Asian origin were included in the replication analysis. Dominant, recessive and additive genetic models were computed under a logistic regression model, adjusting for gender and three principal components (to adjust for admixture). Stouffer's weighted Z_{trend} scores were calculated for a meta-analysis between the GWA and replication results. Our GWA scan identified two SNPs in strong linkage disequilibrium (LD, $r^2=0.94$) located ~74kb telomeric to *CD44* showing suggestive evidence of association with SLE in cases of European descent (rs2732552, $P=0.004$, OR=0.78, 95%CI=0.69-0.93; rs387619, $P=0.003$, OR=0.78, 95%CI=0.68-0.91). We observed independent replication at both rs2732552 ($P=9.03x10^{-8}$, OR=0.83, 95%CI =0.77-0.88) and rs387619 ($P=7.7x10^{-7}$, OR=0.83, 95%CI=0.77-0.90) in the European samples with a $P_{combined}=1.82x10^{-9}$ for rs2732552. The AA and Asian SLE cases also demonstrated association at rs2732552 ($P=5x10^{-3}$, OR=0.81, 95%CI=0.70-0.94 and $P=4.3x10^{-4}$, OR=0.80, 95%CI=0.70-0.91, respectively). The Asian SLE cases were associated with rs387619 ($P=0.001$, OR=0.8 95%CI=0.70-0.91), but not the AA SLE cases, consistent with differences in the haplotype patterns between racial groups. The meta-analysis at rs2732552 for all 4 ethnic groups produced $P_{combined}=3.00x10^{-13}$. We have established genetic association with SLE to a haplotype near *CD44*. Imputation and trans-ethnic mapping focus the effect on a ~14kb haplotype in a region of strong regulatory potential that may influence expression of the centromeric gene *CD44*. This locus contains multiple regulatory sites that could potentially affect expression and functions of *CD44*, a cell-surface glycoprotein influencing immunologic, inflammatory and oncologic phenotypes.

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CYP4F2 genetic variant and the risk of stroke. *A. Munshi[1], V. Sharma[1], S. Kaul[2], A. Jyothy[1].* 1) Molecular Biology, Institute of Genetics and Hospital for Genetic Dis, Hyderabad, Andhra Pradesh, India; 2) Nizams Institue of Medical Sciences, Panjagutta , Hydearbad, India.

Stroke is a complex disease caused by combination of multiple risk factors. Recent findings have suggested that stroke has a strong genetic component. Evidence suggests that variations in CYP4F2 gene may influence stroke risk. CYP4f2 is an enzyme of cytochrome P450 enzymes which metabolize arachidonic acid to 20-hydroxyeicosatetraenoicacid (20-HETE).On the CYP4f2 gene , a guanine to adenine missense transition at1347 nucleotide results in valine to methionine amino acid substitution at residue 433(rs2108622). The present study was carried out to investigate the role of this gene variant with stroke and stroke subtypes in a South Indian population from Andhra Pradesh. Five hunderd patients with ischemic stroke and four hundred and eighty control subjects were enrolled in this case-control study. Ischemic stroke subtypes were classified according to TOAST (Trial of Org 10172 in Acute Stroke Treatment) classification. The results will be discussed.

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Analysis of common *SCN9A* polymorphisms in clinical pain phenotypes. *S. Paciga[1], R. Lowe[2], J. Wheeler[2], G. Johnston[3], S. John[1], K. Tan[4], D. Williams[5], C. Hyde[6].* 1) Molecular Medicine, Pfizer Global Research & Development, Groton, CT; 2) Quanticate Ltd, Hitchin, UK SG5 1LH; 3) Molecular Medicine, Pfizer Global Research & Development, Sandwich, UK, CT13 9NJ; 4) Translational Medicine, Pfizer Global Research & Development, Sandwich, UK, CT13 9NJ; 5) Pain Research Unit, Pfizer Global Research & Development, Sandwich, UK, CT13 9NJ; 6) Clinical Research Statistics, Pfizer Global Research & Development, Groton, CT.

Previous research has mapped familial cases of congenital inability to experience pain to loss of function mutations in the gene *SCN9A*, which encodes the α-subunit of $Na_V1.7$, a sodium channel expressed in peripheral nerves. Subsequently, common polymorphisms in *SCN9A* have been reported to be associated to pain perception and sensitivity in general populations of patients with osteoarthritis and other pain conditions. We genotyped 32 single nucleotide polymorphisms spanning the *SCN9A* locus, including 5 markers previously reported by Reimann *et al.* (PNAS 107:5148-5153, 2010), in 2461 subjects of European ancestry with a clinical diagnosis of osteoarthritis (OA), Post-Herpetic Neuralgia (PHN), or Diabetic Peripheral Neuropathy (DPN). All patients were recruited into clinical trials where pain was self-reported at baseline or upon entry to the clinical trial. Each quantitative pain phenotype was analyzed independently, and within each phenotype a meta-analysis was conducted across studies. None of the 32 SNPs were significantly associated with pain severity in any of the three phenotypes after adjustment for multiple testing. The missense SNP rs6746030, with a reported functional impact, had the following unadjusted association results: for PHN, p=0.658; for OA, p = 0.54; and for DPN, p=0.038. This study had 94-98% power to nominally replicate the previous findings for the 5 markers in the OA pain phenotype, but all had unadjusted p > 0.1, and the effect estimate in rs6746030 for OA was in a direction opposite to what was previously reported. These data do not provide additional evidence supporting the role for common polymorphisms in *SCN9A* in osteoarthritis-induced pain. Further, we were unable to detect any significant associations in neuropathic pain when accounting for multiple testing. The ability to detect such associations may have been reduced due to the inclusion criteria of the studies eliminating subjects with lower pain scores. Additional comprehensive genotyping in clinically well characterized data sets will be required to determine if common alleles in this gene are associated with pain severity.

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Genetic susceptibility profiles are different between pulmonary and extrapulmonary injury-related acute respiratory distress syndrome. *P. Tejera[1], F. Chen[1], C.C. Sheu[1], R. Zhai[1], Z. Wang[1], L. Su[1], E. Bajwa[2], A. Ahasic[2], P. Clardy[3], D. Gallagher[3], M. Gong[4], A.J. Frank[2], T.B. Thompson[2], D.C. Christiani[1,2].* 1) Environmental, Harvard Sch Pub Hlth, Boston, MA; 2) Massachusetts General Hospital, Boston, MA; 3) Beth Israel-Deaconess Hospital, Boston, MA; 4) Montefiore Hospital, Albert Einstein School of Medicine, New York, NY.

Rationale: Acute respiratory distress syndrome (ARDS) can result from a direct (pulmonary) injury or an indirect (extrapulmonary) injury. Previous studies have suggested that the pathophysiological, biochemical, radiological, and mechanical patterns between ARDS derived from these two types of injury are different. However, genetic backgrounds underlying the differences between ARDS resulting from pulmonary and extrapulmonary injury are unknown. This work is intended to identify genetic variants that contribute to susceptibility to pulmonary and extrapulmonary injury-related ARDS. **Methods**: 1717 Caucasian critically ill patients at risk for ARDS were included in the present study. Patients with pneumonia, aspiration, pulmonary contusion or sepsis and/or bacteremia from pulmonary sources as risk factor for ARDS were categorized as having pulmonary injury. Patients with extrapulmonary injury were those with trauma, multiple transfusion or sepsis and/or bacteremia originated from extrapulmonary sources. Patients with both types of lung injury were excluded from the study. Genotyping was carried out using Illumina HumanCVD BeadChip. SNPs with MAF < 0.05 were removed. Genetic association with ARDS risk was analyzed using multivariate logistic regression (additive model) adjusted for age, gender, and APACHE III score. **Results**: 417 ARDS cases (126 with pulmonary injury and 291 with extrapulmonary injury) and 1300 controls (741 with pulmonary injury and 559 with extrapulmonary injury) were included in the study. After quality control, 31530 SNPs were analyzed. Top SNPs associated with pulmonary injury-related ARDS were found in *PRKAG2* (rs7807769; p = 1.6 x 10-5, rs7801616; p = 4.4 x 10-5) and *BVES* (rs1190286; p = 5.3 x 10-5). Two SNPs in *VWF* and two SNPs on chromosome region 19q13.3-13.42 (*KLK2-ISG15*) were associated with extrapulmonary injury-related ARDS at p < 4 x 10-4 (top SNP rs198977, p = 2.1 x 10-4). Interestingly, none of the 29 SNPs (in 21 genes) associated with pulmonary injury-related ARDS (p < 10-3) was associated with extrapulmonary injury-related ARDS (p > 0.200). On the other hand, none of the 27 SNPs (in 19 genes) associated with extrapulmonary injury-related ARDS (p < 10-3) was associated with pulmonary injury-related ARDS (p > 0.054). **Conclusion**: Our study suggests that injury-related genetic variants may contribute to ARDS risk. Further validation of our findings in independent populations is ongoing. FUNDED BY:NIH (HL60710, ES00002).

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Evaluation of MMP-1 and MMP-3 Gene Promoter polymorphisms in Chronic Pancreatitis. *A. Venkateshwari[1], K. Srimanjari[1], D. Krishnaveni[1], A. Vidyasagar[2], K. Prabhakar[3], P. Nallari[4], A. Jyothy[1].* 1) Cell Biology, Institute of Genetics and Hospital for Genetic Diseases,Osmania University, Hyderabad; 2) Department of Gastroenterology,Gandhi Hospital ,Hyderabad; 3) Department of Gastroenterology,Osmania General Hospital,hyderabad; 4) Department of Genetics ,Osmania University, Hyderabad.

Chronic Pancreatitis is a progressive disease characterized by irreversible destruction of exocrine pancreatic tissue and extensive fibrosis. Matrix metalloproteinases (MMP) belong to a large group of proteases capable of breaking essential components of extracellular matrix. Polymorphism in the promoter region of MMP1 and MMP3 gene leads to a variation in its level of expression and found to be associated with various disorders. The aim of study is to investigate possible genetic associations of matrix metelloproteinase-1(MMP1) and matrix metelloproteinase-3 (MMP3) gene polymorphism with chronic pancreatitis. A total of 96 chronic pancreatitis patients and an equal number of unrelated age and gender matched control subjects were included in the present study. MMP1 (-1607) 1G/2G and MMP3 (-1171) 5A/6A polymorphisms were determined using standard Polymerase chain reaction- Restriction fragment length polymorphism and ARMS -PCR methods respectively. Differences in allele and genotype distributions were analyzed using appropriate statistical methods. A significant increase in the frequency of 2G/2G genotype of MMP1 polymorphism was observed in patients compared to control subjects, whereas there was no significant difference for the MMP3 5A/6A polymorphism. These findings suggest the possible association of MMP 1 gene polymorphism in the development of disease.

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Association between Common Polymorphisms of COCH and Primary Open-angle Glaucoma in a U.S. Caucasian Population. *D.Y. Wang[1], L.R. Pasquale[1], D. Rhee[1], J.L. Haines[2], J.L. Wiggs[1].* 1) Department of Ophthalmology, Harvard Medical School, Massachusetts Eye & Ear Infirmary, Boston, MA; 2) Center for Human Genetic Research, Vanderbilt University School of Medicine, Nashville, Tennesse.

Purpose: Primary open-angle glaucoma (POAG) is the second leading cause of blindness worldwide and is characterized by degeneration of the optic nerve that is usually associated with elevated intraocular pressure. Twenty genetic loci have been linked to POAG, from which three causative genes are identified, however mutations in these genes only account for about 10% of patients with POAG. Genome-wide association studies have identified several single nucleotide polymorphisms (SNPs) associated with POAG but no annotated genes are linked to these SNPs. Our previous genome-wide scan of Caucasian siblings with POAG identified a linkage locus on 14q11, and fine-mapping of this region has identified a 3.5 Mb region likely to harbor a susceptibility gene. Twelve genes are located in this region and of these, the coagulation factor C homolog (COCH) gene has been shown to have increased expression in glaucoma in mouse and human eyes. The purpose of this study is to investigate common COCH polymorphisms for association with POAG. **Methods**: Fifteen tagging SNPs in the vicinity of COCH, capturing 100% of alleles with mean r-square of 0.95 were genotyped in 539 Caucasian patients with POAG and 336 controls, as well as SNPs located in the 5' promoter region (rs3759777 and rs8015095). SNP association analysis was performed for POAG by Fisher's exact test and Wald test. **Results**: All SNPs followed Hardy-Weinberg equilibrium in both cases and controls. No significant association was found between the tagging SNPs or the promoter region SNPs and POAG overall ($p>0.16$). However, subgroup analysis showed that rs8015095 was significantly associated with higher intraocular pressure and increased disease severity ($p\text{-trend}=0.0058$). **Conclusions**: Previous studies have suggested that transcriptional upregulation of COCH may occur in glaucoma. SNP rs8015095 is located 500 bp from the transcription start site and about 1500 bp from conserved binding sites for transcription factors FOXO3, FREAC4 and FREAC2. Our results suggest that rs8015095 may influence COCH gene expression or may be in linkage disequilibrium with other variants that could affect promoter efficiency. Further studies of COCH promoter activity in glaucoma patients could define a role for Cochlin in the disease. **Grant support**: NEI Grants R01EY015872 and P30EY014104, Research to Prevent Blindness and The Massachusetts Lions Eye Research Fund.

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Association of variants in the NF-kB regulatory pathway loci with systemic lupus erythematosus in multiple populations. *F. Wen[1,21], G. Wiley[1,21], I. Adrianto[1,21], K.M. Kaufman[1,2,3], J.M. Anaya[4], M.E. Alarcón-Riquelme[1,5], S.C. Bae[6], S.A. Boackle[7], E.E. Brown[8], L.A. Criswell[9], J.C. Edberg[8], B.I. Freedman[10], P.K. Gregersen[11], G.S. Gilkeson[12], C.O. Jacob[13], J.A. James[1,2], R. Kimberly[8], J. Martin[14], J.T. Merrill[1,2], T.B. Niewold[15], B.A. Pons-Estel[16], R.H. Scofield[1,2], A.M. Stevens[17], B.P. Tsao[18], T.J. Vyse[19], C.D. Langefeld[20], J.B. Harley[2,3], K.L. Moser[1,2], C. Gray-McGuire[1,22], P.M. Gaffney[1,22].* 1) Arthritis and Immunology, Oklahoma Medical Research Foundation, Oklahoma City, OK; 2) The University of Oklahoma Health Sciences Center, Oklahoma City, OK; 3) Oklahoma City VA Medical Center; 4) Universidad del Rosario, Colombia; 5) Center of Genomics and Oncological Research (GENyO), Granada, Spain; on behalf of the BIOLUPUS and GENLES Networks; 6) Hanyang University, Seoul, Republic of Korea; 7) University of Colorado Denver; 8) University of Alabama at Birmingham; 9) Rosalind Russell Medical Research Center for Arthritis, University of California, San Francisco; 10) Wake Forest University Baptist Medical Center; 11) The Feinstein Institute for Medical Research, North Shore Long Island Jewish Health System; 12) Medical University of South Carolina; 13) University of Southern California Keck School of Medicine; 14) Instituto de Parasitologia y Biomedicina López-Neyra, CSIC, Granada, Spain; 15) University of Chicago; 16) Sanatorio Parque, Rosario, Argentina; 17) Seattle Children's Hospital; 18) University of California, Los Angeles; 19) Imperial College London; 20) Wake Forest University Health Sciences; 21) Co-first authors; 22) Co-senior authors.

The transcription factor NF-kB is an important factor in inflammation and the immune response. Unrestrained NF-kB response has previously been associated with autoimmune disease, sepsis, and some cancers. It is therefore not surprising that NF-kB signaling is tightly regulated within the cell through a pathway of protein-protein interaction and post-translational protein modification. One key component of this pathway is the *TNFAIP3* complex consisting of *TNFAIP3*, *TNIP1*, *TNIP2*, *TAX1BP1*, *ITCH*, and *RNF11*. This complex, along with the ubiquitin-conjugating enzyme *UBE2L3*, deactivates NF-kB pathway proteins through deubiquitation of K63 polyubiquitin chains and subsequently targets those proteins for degradation via K48 ubiquitination. Recent GWAS have revealed that genetic variants in the *TNFAIP3*, *TNIP1*, and *UBE2L3* regions are associated with systemic lupus erythematosus (SLE) in subjects of European and Asian ancestry. SLE is an autoimmune disease characterized by loss of tolerance to self-antigens and dysregulated interferon responses. To further characterize and localize the effect of the *TNFAIP3* regulatory complex, we genotyped and imputed single-nucleotide polymorphisms (SNPs) within and flanking *TNIP1* on 5q33, *TNIP2* on 4p16, *UBE2L3* on 22q11, *TAX1BP1* on 7p15 in multiple populations: African-Americans (1,569 cases, 1,893 controls), Asians (1,328 cases, 1,348 controls), Europeans (4,248 cases, 3,818 controls), Gullah (155 cases, 131 controls), and Hispanics enriched for Amerindian-European admixture (1,622 cases, 887 controls). Using the Illumina iSelect system, we genotyped a total of 231 SNPs in and around those loci and 343 ancestry-informative markers (AIMs). We then imputed untyped SNPs at those loci using HapMap Phase III and 1000 Genomes Project data. We assessed single marker association to SLE using logistic regression with sex and global ancestry adjustments. We observed strong associations between SLE and multiple SNPs within *TNIP1* in Europeans, Hispanics, African-Americans, and Asians ($P_{combined}<5 \times 10^{-8}$) with the strongest signal at rs7708392 ($P_{combined}=2.53 \times 10^{-19}$). We also identified strong associations within *UBE2L3* in Europeans, Asians, African-Americans and Hispanics with the most significant association at rs7444 ($P_{combined}=1.25 \times 10^{-14}$). These results establish that variants within *TNIP1* and *UBE2L3* contribute to differential risk of SLE in multiple populations.

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Evaluation of muscle contraction genes role in isolated clubfoot. *K.S. Weymouth[1,2], S.H. Blanton[3], M.B. Dobbs[4], C.A. Gurnett[4], L.E. Mitchell[5], J.T. Hecht[1,2].* 1) Dept Pediatrics, Univ Texas Medical Sch, Houston, TX; 2) Graduate School of Biomedical Sciences, Houston, TX; 3) University of Miami Miller School of Medicine, Miami, FL; 4) Washington School of Medicine, St. Louis, MO; 5) University of Texas School at Public Health, Houston, TX.

Clubfoot is a common birth defect that affects 4000 newborns in the US each year. Isolated clubfoot is characterized by an inward turning of the foot towards the midline of the body that is held in a rigid, downward position. Calf muscles in the affected leg(s) are underdeveloped and remain small even after corrective treatment. Syndromes with a complex phenotype have been used to identify candidate genes for isolated conditions. Distal Arthrogryposis (DA) is a rare genetic disorder that is characterized by congenital contractures with clubfoot associated with some DA subtypes. Mutations in five different genes encoding proteins that play key roles in muscle contraction cause four types of DA. Interrogation of fifteen muscle contraction genes in our discovery clubfoot population identified positive associations with twelve genes. Six SNPs in TNNC2 were out of Hardy Weinberg Equilibrium (HWE) in our NHW discovery population with only the mothers deviating from HWE, suggesting a maternal effect. Relative risk and likelihood ratio tests showed evidence for a maternal genotype effect with two SNPs, rs4629 and rs383112. The strongest associations were with TPM1, MYH13 and TNNT3. Single SNP, 2-SNP haplotypes and gene interactions found evidence of an association with potential regulatory SNPs located near fourteen of the fifteen muscle contraction genes. This suggests that perturbation of gene expression in muscle contraction genes contributes to clubfoot and is being examined in functional assays. To confirm these findings, validation studies were performed in case-control and second clubfoot family-based (144 NHW) populations. Positive associations were again identified for TPM1 in both populations (rs12148828/p=0.04 and rs1972041/p=0.000074, respectively). In addition, the association with rs2734495 in TNNT3, was also validated (p=0.04). Four TPM1 2-SNP haplotypes, all containing rs1972041 (p<0.001) were overtransmitted in the validation population. In complimentary studies, we have found association with apoptotic genes. Apoptosis and muscle contraction are two key components in limb development. Gene interactions between apoptotic and muscle contraction genes are being evaluated. Altogether, these results further suggest that perturbation of muscle contraction genes play a role in clubfoot and define an etiologic pathway for clubfoot.

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RUNX2 Polymorphisms in Patients with Nonsyndromic Sagittal Craniosynostosis. *G. Yagnik[1], H. Drissi[2], C. Stevens[1], S. Boyadjiev Boyd[1].* 1) University of California, Davis, Davis, CA; 2) Department of Orthopedics, University of Connecticut, Health Center.

Craniosynostosis (CS), the premature fusion of one or more cranial sutures, is a common malformation occurring in 3 to 5 per 10,000 live births. In 85% of the cases, it occurs as an isolated (i.e. nonsyndromic) anomaly. RUNX2 is a transcriptional activator of osteoblast differentiation and thus a potential candidate gene for nonsyndromic CS. Our work to date has shown that polymorphisms within RUNX2 may lead to gain-of-function (GOF) of the protein, as demonstrated by dual-luciferase analysis of the RUNX2 R237C polymorphic variant. Based on this work, we hypothesize that GOF variants in RUNX2 may contribute to the development of nonsyndromic sagittal CS. We are currently screening a large cohort of patients for additional polymorphisms to support this, as well as interrogating a previously described RUNX2 isoform with an 18 bp deletion leading to a truncated Q/A domain.

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Allelic Expression Imbalance Analysis under the Chromosome 1q21-24 Linkage Peak for Type2 Diabetes Shows Cis- Regulatory SNPs. *AK. Mondal, SK. Das, NK. Sharma, KA. Langberg, SC. Elbein.* Int Med,Endocrinology, Wake Forest University Health Sciences, Winston-Salem, NC.

Linkage of the chromosome 1q21-24 region to T2D has been demonstrated in multiple ethnic groups, but fine-mapping failed to identify common variants contributing to the linkage. We hypothesized that the linkage signal resulted from multiple common, noncoding regulatory variants, many of which act in cis to regulate adjacent transcripts. We designed a screening strategy based on allelic expression imbalance (AEI), which examines relative levels of maternal and paternal alleles in transcribed single nucleotide polymorphism (cSNP) from heterozygous individuals. We compared AEI for heterozygous individuals in cDNA derived from transformed lymphoblastoid (TL) cell line RNA to allelic imbalance calculated from genomic DNA (gDNA) using pyrosequencing. We identified 176 genes under the 22 Mb linkage peak that were both expressed in TL and had a known cSNP with minor allele frequency over 5%. From these, we have tested 69 cSNPs for 69 genes in 95 European American (EA), and in 95 African American (AA) TLs. We considered significance based on comparison of cDNA to gDNA in at least 4 heterozygous individuals using the nonparametric Wilcoxan sign rank test at nominal p£ 0.05. We observed significant AEI for 33 cSNPs in EA of which 20 cSNPs also showed AEI in AA. An additional 13 cSNPs showed AEI only in AA cell lines. Among the genes showing AEI in both populations were *SLAMF7, THEM4, ADAR, RIT1, EFNA1, ATF6, UBAP2L, SCYL3, SNAPIN, SELL, MEF2D, SLC25A44, SLC39A1, CD84, DAP3, ARHGAP30, PKLR/HCN3 , SYT11* and *PMVK*, with EA p values of 0.05 to 7x10-8. Additional genes seen only in EA included *USF1, RFX5, CD48, CD244, EFNA3, POGK, CRLA, SNX27, ADAM15, MPZL1, PBXIP1, SLAMF8* and *GATAD2B*. Genes showing highly significant (p <2 x10-4) allelic imbalance only in AA population were *IL6R, CRTC2, ZBTB7B, CRTC2, ISG20L2* and *IQGAP3*. Using a conservative test for AEI (Bonferroni corrected p£0.05), we find evidence for cis-acting variants in approximately 23% of the EA SNPs, with replication in two populations for 7/69 SNPs with more testing ongoing. These results support our hypothesis that cis-acting regulatory variants under linkage peaks are common, and include known genes with metabolic effects (*ATF6, USF1, THEM4, SLC39A1*, and others). Studies of remaining genes are in progress. Further functional analysis and fine mapping studies are needed to identify functional SNPs for type 2 diabetes.

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Association of RNASEH1 SNPs with Type 1 Diabetes in Colombian families. *N. Pineda-Trujillo[1], A. Rodriguez-Acevedo[1], J. Gutierrez-Achury[1], G. Bedoya[2], F. Uribe[3], A. Ruiz-Linares[2, 4], V. Balthazar[1], J-M. Alfaro[1].* 1) Mapeo Genetico, Department of Pediatrics, University of Antioquia, Medellin- Colombia; 2) GENMOL, University of Antioquia. Medellin-Colombia; 3) Endocrinologia y Metabolismo, Medicine Faculty, University of Antioquia. Medellin-Colombia; 4) Department of Biology, University College London, UK.

Type 1 diabetes (T1D) is a complex trait in which genetic and environmental factors are involved. We have previously found linkage and association of T1D to 2p25. Our purpose was to analyze the role of the gene RNASEH1 in the susceptibility to T1D in one hundred Colombian families. RNASEH1 locates at 2p25. One hundred familial trios with T1D from Antioquia-Colombia were analyzed. In patients sera autoantibodies against anti-GAD, anti-IA2 and anti-TPO were tested. We began typing the RNASEH1 intragenic SNPs rs10186193 and rs7563960. According to the first results we then typed four extra SNPs in RNASEH1. These SNPs were rs11538545, rs760788, rs 6730126 and rs55981318. SNPs typing was done by PCR-RFLP and Tetraprimer-ARMS methods. Genetic association analysis was done by the transmission disequillibrium test (TDT). Positive autoantibodies were found in 73%, 41% and 17% for anti-IA2, anti-GAD and anti-TPO, respectively. Together, 86% of the affected individuals were positive for at least one auto-antibody. SNP rs10186193 showed significant association with T1D (p=5.3x10-3). Further SNPs typing in RNASEH1 showed that SNP rs760788 was also associated (p value= 9,6x10-4). Even though the significance decreased, a stratified analysis by autoimmune and idiopathic cases was still significant. Autoimmunity was found in similar proportions to previous reports. It was also found that two out of six SNPs tested at RNASEH1 gene are associated with T1D in the sample analized. Haplotype analysis is currently being done. Our results indicate a causative participation of the gene RNASEH1 in T1D. Evaluation of these three SNPs in an independent sample, from the same region in Colombia, is underway. It is interesting that this region has not been shown in previous GWAS. This study was funded by COLCIENCIAS grants # 111534319156 and 111540820532.

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Association testing of 50,000 SNPs in candidate genes with type 2 diabetes: The CARe study. *R. Saxena[1,2], J.S. Pankow[3], W.H.L. Kao[4], C. Fox[5], J.M. Zmuda[6], M.W. Steffes[7], S.K. Musani[8], S.R. Patel[9], S.F.A. Grant[10,11], E. Crook[12], D.W. Bowden[13], S.S. Rich[14], S.J. Bielinski[15], J. Rotter[16], J.B. Meigs[17], D. Siskovick[18], B. Keating[10].* 1) Center for Human Genetic Research & DACCPM, Massachusetts General Hospital, Boston, MA; 2) Medical and Population Genetics, Broad Institute of Harvard and MIT, Cambridge, MA; 3) Division of Epidemiology and Community Health, University of Minnesota, Minneapolis, MN; 4) Department of Epidemiology, Johns Hopkins School of Medicine, Baltimore, MD; 5) NHLBI's Framingham Heart Study, NIH, Boston, MA; 6) Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; 7) Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN; 8) University of Mississippi Medical Center, Jackson MS; 9) Center for Clinical Investigation, Case Western Reserve University, Cleveland, OH; 10) Center for Applied Genomics, Division of Human Genetics, Children's Hospital of Philadelphia, Philadelphia, PA; 11) Dept. of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia PA; 12) Department of Internal Medicine, University of South Alabama College of Medicine, Mobile, AL; 13) Center for Human Genomics, Wake Forest University School of Medicine, Winston-Salem, NC; 14) Center for Public Health Genomics, University of Virginia, Charlottesville, VA; 15) Division of Epidemiology, Mayo Clinic College of Medicine, Rochester, MN; 16) Medical Genetics Institute and Division of Medical Genetics, Cedars-Sinai Medical Center, Los Angeles, CA; 17) Department of Medicine, Harvard Medical School, General Medicine Division, Massachusetts General Hospital, Boston MA; 18) Cardiovascular Health Research Unit, Departments of Medicine and Epidemiology, University of Washington, Seattle, WA.

Type 2 diabetes (T2D) poses a large public health burden. Although candidate gene and genome-wide association analyses (GWAS) have found >30 T2D loci primarily in European populations, these variants explain <10% of the genetic contribution to inter-individual risk. Examination of candidate genes in multi-ethnic populations may identify additional variants and pathways dysregulated in T2D. Through a collaborative effort, the Candidate Gene Association Resource (CARe), we combined results for T2D across nine cohort studies genotyped for ~50,000 SNPs in 2016 candidate genes. Forty-nine candidate genes (~5000 SNPs) were specifically selected based on prior evidence in Mendelian diabetes, diabetes patho-physiology, linkage studies and meta-analyses of GWAS. Component studies included ARIC, CARDIA, CFS, CHS, FHS, JHS and MESA comprising 1794 cases and 7485 controls of African-American ancestry and 2636 cases and 21,944 controls of European-American ancestry. An additive genetic model with age, sex and 10 principal components to adjust for ancestry was used to test for genetic association, and results were combined by a fixed-effects inverse variance meta-analysis. In African-Americans, study-wide significant ($P<2x10^{-6}$) association after Bonferroni correction was observed for SNPs at *TCF7L2* ($P=3x10^{-11}$) and *HMGA2* (rs9668162 $P=6x10^{-8}$). *HMGA2* is a transcription factor that regulates a known diabetes gene, *IGF2BP2*, and a T2D signal near *HMGA2* ($r^2=0.005$ in CEU, $r^2=0.35$ in YRI) has recently been identified in Europeans. Rare SNPs (<5% MAF) in *EDN2*, *KCNQ1* and *ARNT* showed suggestive ($P<1x10^{-4}$) evidence of association with T2D in African-Americans. In European-Americans, we confirmed known loci, with SNPs at *TCF7L2*, *GCKR*, *IGF2BP2*, *FTO*, chr11p12 and *CDKAL1* exceeding study-wide significance, and found novel SNPs at *MSRA*, *HSF1* and *KCNJ1* with suggestive evidence. Little overlap of signals was found between the ethnicities, even though the African-American sample had ~80% power to detect European risk alleles on the array at α=0.05. Meta-analyses with additional T2D case control studies in samples of European and African-American ancestry are ongoing and should increase power to discriminate true T2D genes from false positives. In summary, meta-analysis of IBC chip data from nine CARe cohorts identified several putative novel associations in African- and European-Americans that should provide additional biological clues about the pathogenesis of type 2 diabetes.

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Genotype score of common susceptible genotypes for prediction of type 2 diabetes in Japanese: the J-SHIPP study. *y. tabara, h. osawa, r. kawamoto, h. onuma, k. kohara, h. makino, t. miki.* Ehime University Graduate School of Medicine, Toon, Japan.

BACKGROUNDS: Recent genome wide association studies (GWAS) have successfully identified several genotypes susceptible for type 2 diabetes (T2DM). These associations have been replicated in various populations including East-Asians. In contrast to the accumulated evidence for a cross-sectional association, however, only a few studies have investigated whether these variations confer a risk of the future development of T2DM. Here, to clarify the prognostic significance of these T2DM-associated variants, we conducted a longitudinal genetic epidemiological study in a community-dwelling general population.

RESULTS: Among 21 recently identified candidate SNPs, significant association was observed in the KCNQ1, TCFL72, CDKAL1, HHEX, IGF2BP2, CDKNN2B, SLC30A8, KCNJ11, PPARG and GCKR in replication analysis in the 506 T2DM case and 402 non-diabetic control subjects. To clarify the prognostic significance of the T2DM SNPs in the development of diabetes, we retrospectively analyzed the association between genetic risk score calculated by adding the risk allele number of these 10 SNPs and the development of T2DM with 9.4 years follow-up. Among the 1,824 subjects who were not T2DM at baseline, 95 cases of T2DM were newly diagnosed during the follow-up period. These subjects were slightly older (58±9 vs. 56±10 years, p=0.091) and more frequent in male (50.5%; vs. 39.6%;, p= 0.035). The mean risk score in these subjects was significantly higher than that in the 1,729 subjects who remained non-diabetes (9.5±1.8 vs. 9.1±2.0, p=0.042). Although the initial mean BMI (24.7±3.2 vs. 23.0±2.8, p<0.001) and initial glucose (106±18 vs. 90±13 p<0.001) were also significantly higher in those subjects who developed T2DM, the genetic risk score remained an independent determinant of the future development of T2DM after adjustment for these possible confounding factors. Per-allele odds ratio for the development of T2DM was 1.12 (95% C.I. 1.00-1.25), p=0.049. Although none of 10 SNPs showed significant correlation with plasma levels of glucose, as well as insulin levels, HOMA-IR and HOMA-β, the genetic risk score showed stepwise association with plasma glucose levels ($1.2*10^{-6}$) and HOMA-β (p=0.006) after adjustment for possible covariates.

CONCLUSION: A genotype score based on 10 risk alleles predicted new cases of diabetes in the community but provided only a slightly better prediction of risk than knowledge of common risk factors alone.

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Epistatic effects between variants of the high affinity IgE receptor (*FCER1A*) confers to eczema risk. *H. Baurecht[1,2], E. Rodríguez[1], A. Naumann[1,3], S. Wagenpfeil[2,3], N. Klopp[4], H.-E. Wichmann[4], K. Van Steen[5], S. Weidinger[1,6].* 1) Department of Dermatology and Allergy , Technische Universität München, Munich, Germany; 2) Graduate School of Information Science in Health (GSISH), Technische Universität München, Munich, Germany; 3) Institute for Medical Statistics and Epidemiology IMSE, Technische Universität München, Munich, Germany; 4) Institute of Epidemiology, Helmholtz Zentrum München, Neuherberg, Germany; 5) Systems and Modeling Unit, Department of Electrical Engineering and Computer Science, University of Liège, Liège, Belgium; 6) Department of Dermatology, University Hospital Schleswig-Holstein, Campus Kiel, Germany.

Atopic diseases (eczema, asthma, rhinitis) are often driven by high levels of total and allergen-specific IgE levels. The high-affinity receptor for IgE, which is composed of one alpha (*FCER1A*), one beta (*FCER1B*), and two gamma (*FCER1G*) subunits, represents the central receptor of IgE-induced reactions. Recently, a genome-wide association analysis identified associations between functional *FCER1A* variants and total serum IgE levels. For the *FCER1B* gene linkage and association with total IgE and atopic traits has been reported previously. So far the *FCER1G* subunit has not been examined on the genetic level with regard to atopic traits. Filaggrin (*FLG*) is the strongest known risk gene for eczema, in particular the allergic subtype of eczema.

We investigated the association of the *FCER1*, *FCER1B* and *FCER1G* variants with total IgE levels in a large population-based cohort and in a large eczema case-control sample. We used the model-based multifactor dimensionality reduction method (MBMDR) to test for epistatic effects. For further exploration, variable importance measures were computed by means of the random forest method.

We could not confirm associations for *FCER1B*, neither did we detect associations with *FCER1G*. However, three strongly correlated *FCER1A* polymorphisms were significantly associated with total and specific IgE levels. After adjustment for *FLG*, we found epistatic effects of the *FCER1A* variants rs10489854 and rs2511211 on eczema risk. In the random forest approach both variants showed positive variable importance values. These results suggest that *FCER1A* variants by themselves and in combination influence IgE levels and act synergistically to influence eczema risk.

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Analysis of Genetic Variation in genes associated with Liver Disease in the Mexican Population. *D. Lau-Corona[1,2], J.C Fernandez-Lopez[2], G. Gutierrez-Reyes[1], G. Jimenez-Sanchez[2], D. Kershenobich[1], I. Silva-Zolezzi[2].* 1) Experimental Medicine, UNAM-Hospital General de México, México DF, Mexico; 2) National Institute of Genomic Medicine, Mexico DF, Mexico.

Two recent GWAS found SNPs in 8 genetic regions (*CPN1, CHUK, PNPLA3, SAMM50, HNF1A, GGT1, GPLD1, ABO*) associated to plasma levels of liver enzymes, additionally rs738409[G] variant in *PNPLA3* has been associated with susceptibility to nonalcoholic fatty liver disease and fibrosis progression. In a stratified analysis of these SNPs, US Hispanics had the highest frequency of the risk allele. In Mexico, chronic liver disease (CLD) is the third cause of death in the general population and the first in Amerindian men; in the US it is the twelfth death cause in the general population, sixth in Hispanics and fifth in Native Americans. Our study aims to explore the potential impact of these genetic variants as liver disease risk factors in Mexican Mestizos, as well as their relationship to the Amerindian contribution in this group. We have analyzed the frequency distribution of 12 SNPs associated to liver traits in the Mexican population. We determined their allele frequencies in 243 non-related Mexican Mestizos and 182 Mexican Amerindians of 3 different ethnic groups (Mayas, Zapotecos, Tepehuanes) from the Mexican Genome Diversity Project (MGDP) and included data of the CEU HapMap group. Genotypes were obtained from results of the MGDP with the Affy 500K, and using allelic discrimination assays. We estimated the contribution of the 12 SNPs to individual risk by calculating the number of accumulated risk- or protection-associated alleles. “Risk” was defined as the association either to a positive β coefficient in the analysis of liver enzyme levels, or to a higher liver fat content, while “protection” was defined as a negative β coefficient or lower liver fat content. We observed differences in 10 of 12 risk and protection-associated alleles between Mexican Amerindians and CEU (p£2.8E-7), and in 9 of 12 between Mestizos and CEU (p£1.7E-2). The proportion of individuals with 10 or more risk-associated alleles was, 32% in Amerindians 5% in Mestizos and 0 in CEU, in contrast the proportion of individuals with more than 4 protection-associated alleles was 2% in Amerindians, 8% in Mestizos and 18% in CEU. These results suggest an important contribution of genetic factors with ethnic related differences in CLD. Further analysis will require a comprehensive assessment in Latin American populations of the contribution of variants related to Amerindian ancestry on liver enzyme levels and clinical outcomes, as well as its potential relationship to natural selection.

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High density genomewide linkage analysis of exceptional human longevity identifies multiple novel loci. *S. Boyden[1,2], L. Kunkel[1,2,3].* 1) Dept Genomics, Children’s Hosp, Boston, MA; 2) Dept Genetics, Harvard Medical School, Boston, MA; 3) Howard Hughes Medical Institute, Children’s Hosp, Boston, MA.

Human lifespan is approximately 25% heritable, and genetic factors may be particularly important for achieving exceptional longevity. Accordingly, siblings of centenarians have a dramatically higher probability of reaching extreme old age than the general population. To map the loci conferring this survival advantage, we performed the second genomewide linkage scan on human longevity and the first using a high density marker panel of single nucleotide polymorphisms. By systematically testing a range of minimum age cutoffs in 279 families with multiple long-lived siblings, we identified a locus on chromosome 3p24-22 with a genomewide significant allele-sharing LOD score of 4.02 (empirical P = 0.037) and a locus on chromosome 9q31-34 with a highly suggestive LOD score of 3.89 (empirical P = 0.054). The empirical P value for the combined result was 0.002. A third novel locus with a LOD score of 4.05 on chromosome 12q24 was detected in a subset of the data, and we also obtained modest evidence for a previously reported interval on chromosome 4q22-25. These linkage peaks should facilitate the discovery of both common and rare variants that determine genetic variability in lifespan.

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Evidence of genetic linkage of the Class III Malocclusion phenotype with human chromosome 7 in 35 South American families. *G. Falcao Alencor[1,2,3], L. Otero[4], R.M. Cruz[3], T.M. Foroud[5], L. Dongbing[5], D. Koller[5], L.A. Morford[1,2], I. Ferrari[3], S.F. Oliveira[3], J.K. Hartsfield[2,5,6].* 1) Dept of Oral Health Practice, Univ Kentucky College of Dentistry, Lexington, KY; 2) Hereditary Genomics Laboratory, Center for Oral Health Research, Univ Kentucky College of Dentistry, Lexington, KY; 3) Dept of Genetics and Morphology, Universidade de Brasília - Brazil; 4) Dept of Orthodontics, Pontificia Universidad Javeriana, Bogotá - Colombia; 5) Dept of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN; 6) Dept Oral Health Science, Division of Orthodontics, Univ Kentucky College of Dentistry, Lexington, KY.

Introduction: Class III Malocclusion is when the sagittal position of the permanent mandibular first molars is more anterior than usual, typically with the mandibular incisors beyond the maxillary incisors. This dental relationship is usually caused by overgrowth of the mandible, undergrowth of the maxilla or a combination of both. Recent studies have indicated genetic linkage of Class III Malocclusion in humans with chromosomes 1p, 3q, 11q, and 12q in four Colombian families, and chromosomes 1p, 6q and 19p in Japanese and Korean brothers. **Aim:** Identify the genetic loci that contribute to Class III Malocclusion in 35 Colombian and Brazilian families using linkage analysis. **Methods:** The project was approved by: the National Committee on Research Ethics, Brazil; the Research and Ethics Committee of Pontificia Universidad Javeriana, Colombia; and the University of Kentucky IRB. Blood and saliva from 156 individuals belonging to 21 unique families in Colombia, and 77 individuals belonging to 14 unique families in Brazil, were collected for DNA isolation. Each family contained at least two Class III-affected members within the pedigree. DNA from 40 individuals in the most informative family (#9 from Colombia) was analyzed on the *Illumina® Infinium HumanLinkage-12* SNP-based array panel and suggested linkage of a region of chromosome 7 to the Class III phenotype. Based on the array results, three SNPs on chromosome 7 (rs1044701, rs1299548, and rs1882600) that showed evidence of linkage within Family 9, and five additional (rs1294611, rs9640034, rs9640038, rs11526212, and rs7800782), were genotyped in the remaining 34 Colombian and Brazilian families using TaqMan-based single point genotyping and were statistically analyzed for linkage to the Class III phenotype. **Results:** Suggestion of linkage (LOD= 2.36) was found at SNP rs1882600 for all the families. rs1882600 is an intergenic SNP, residing between the *C1GALT1* and *COL28A1* genes on chromosome 7, specifically 7p21.3. The *Sp8* gene is also located in this region, which may play a role in chondrogenic and osteoblastic differentiation. **Conclusion:** The Class III phenotype is a heterogeneous autosomal dominant trait with variable expressivity and incomplete penetrance. This study demonstrates the novel finding that a region of human chromosome 7 near SNP rs1882600 may contribute to this phenotype. Studies are underway to fine map this region of chromosome 7 within the 35 families described in this study.

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Analysis of SNPs with an effect on gene expression identifies UBE2L3 and BCL3 as risk genes for Crohn's disease. *K. Fransen[1,2], M.C. Visschedijk[2,3], S. van Sommeren[1,2], J.Y. Fu[1], L. Franke[1], E.A.M. Festen[1,2], P.C.F. Stokkers[4], A.A. van Bodegraven[5], J.B.A. Crusius[6], D.W. Hommes[7], P. Zanen[8], D.J. de Jong[9], C. Wijmenga[1], C.C. van Diemen[1], R.K. Weersma[2].* 1) Department of Genetics, University Medical Centre Groningen, Groningen, Groningen, Netherlands; 2) Department of Gastroenterology and Hepatology, University Medical Centre Groningen, University of Groningen, Groningen, the Netherlands; 3) Department of Gastroenterology, Isala Klinieken, Zwolle, Overijssel, the Netherlands; 4) Department of Gastroenterology and Hepatology, Academic Medical Centre, Amsterdam, the Netherlands; 5) Department of Gastroenterology and Hepatology, VU University Medical Centre, Amsterdam, the Netherlands; 6) Laboratory of Immunogenetics, Department of PathologyVU University Medical Centre, Amsterdam, the Netherlands; 7) Department of Gastroenterology and Hepatology, Leiden University Medical Centre, Leiden, the Netherlands; 8) Department of Pulmonology, University Medical Center Utrecht, Utrecht, the Netherlands; 9) Department of Gastroenterology and Hepatology, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands.

Background and Aims: Genome-wide association studies (GWAS) for Crohn's disease (CD) have identified loci explaining ~20% of the total genetic risk of CD. Part of the other genetic risk loci is probably partly hidden among signals discarded by the multiple testing correction needed in the analysis of GWAS data. Strategies for finding these hidden loci require large replication cohorts and are costly to perform. We adopted a strategy of selecting SNPs for follow-up that showed a correlation to gene expression (cis-eQTLs) since these have been shown more likely to be trait associated. Materials and methods: First we show that there is an overrepresentation of cis-eQTLs in the known CD-associated loci. Then SNPs were selected for follow-up by screening the top-500 SNP hits from a CD GWAS dataset. We identified 10 cis-eQTL SNPs. These 10 SNPs were tested for association to CD in two independent cohorts of Dutch CD patients (1539) and healthy controls (2648). Results: Three SNPs were significantly associated to CD in the initial replicaton phase, these were selected for follow-up. In a combined analysis two of these cis-eQTL SNPs were associated to CD rs2298428 in UBE2L3 ($p=5{,}22 \times 10^{-05}$) and rs2927488 in BCL3 ($p=2{,}94 \times 10^{-04}$) After adding additional publicly available data from previously reported meta-analysis the association with rs2298428 almost reached genome wide significance with a p-value of 2.40×10^{-07} and the association with rs2927488 was corroborated($p=6.46 \times 10^{-04}$) Discussion: We have identified UBE2L3 and BCL3 as novel risk genes for CD. UBE2L3 is also associated to other immune-mediated diseases. These results show that eQTL-based pre-selection for follow-up is a useful approach for identifying risk loci from a moderately sized GWAS.

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Evidence of a novel candidate gene at 7q11 regions in Intracranial aneurysm based on positional screening in patients from South India. *M. Banerjee[1], L.V. Koshy[1], H.V. Easwer[2], S. Premkumar[3], J.P. Allapatt[3], R.N. Bhattacharya[2].* 1) Dept Human Molecular Genetics, Rajiv Gandhi Center Biotechnology, Thiruvananthapuram Kerala, India; 2) Department of Neurosurgery, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram, Kerala; 3) Dept. Of Neurosurgery, Calicut Medical College, Calicut.

Intracranial aneurysm is a fairly common condition that is often asymptomatic until the time of rupture, resulting in Aneurysmal subarachnoid hemorrhage (aSAH), ensuing significant morbidity and mortality. The reported prevalence rate of intracranial aneurysms in India varies from 0.75% to 10.3 % as determined in angiographic and autopsy studies. The pathogenesis of intracranial aneurysms has a multifactorial origin, where the essential defect of the arterial wall may, at least in part be genetically determined. Till date, 13 different susceptibility loci have been identified for intracranial aneurysm in different populations, which suggests its complex nature as well as the likelihood of clinical and genetic heterogeneity among populations. In, the present study we tested for association based on positional screening of 7q11 region using 12 STR markers spanning 33.67 cM in south Indian patient population. The study design was a case-control based association study comprising of 150 patients having angiographically documented saccular aneurysms with SAH and 175 controls selected from ethnically and geographically matched subjects. A positive association was detected for D7S2421 microsatellite marker positioned in intron 21 of the MAGI2 gene. Additionally 13 SNPs spanning 1.1 Kb surrounding the D7S2421 marker were further genotyped to identify risk conferring alleles in MAGI2 gene, which may be in LD with this microsatellite marker. rs12539225 and rs12531571 revealed significant association with IA both at an allelic and genotypic level (p=0.006 and 0.005, respectively). Haplotype analysis also revealed evidence of association with with two locus and three locus combinations of rs12539225 and rs12531571. The rs12539225 though present in the intron, has been found to function as an intronic enhancer, which alters the binding site of a transcription factor in the intronic region. This novel association of MAGI2 gene could open up new avenues for investigation. MAGI2 has been reported to be involved in the tight junction pathway (KEGG: hsa04530). Down-regulation of tight junction proteins has been suggested to trigger and promote cerebral aneurysm formation in rats. Studies have also shown that MAGI2 associates with the tumor suppressor PTEN and enhance PTEN-dependent inactivation of AKT, thus favoring apoptosis. Apoptosis has also been suggested to play a role in the development and rupture of intracranial aneurysms.

978/W

Copy Number Variations in Shortsightedness (Myopia). *M. Schache, M. Dirani, A.J. Richardson, P.N. Baird.* Centre for Eye Research Australia, University of Melbourne, Royal Victorian Eye and Ear Hospital, Melbourne, Australia.

Myopia is a complex disease influenced by genetic and environmental factors. Many genome-wide linkage and candidate gene association studies have been undertaken for myopia but these have had little impact on our understanding of its causative genetic variants. We hypothesize that copy number variations (CNVs) may play a role in myopia and to date the role of CNVs in myopia is relatively unexplored. To test this hypothesis we undertook a pilot study using a subset of twins from the Genes in Myopia (GEM) study. For the pilot study, 87 unrelated individuals including 51 myopic cases and 36 controls were genotyped using the Illumina Human610-Quad BeadChips. Output data was analyzed using the PennCNV software package for CNV calling. Frequencies of CNVs were compared between cases and controls using the Fisher Exact test. The quality of data from the pilot study was high with the average oligonucleotide call rate for each sample being 99.84%±0.07. A total of 3,250 CNVs were identified. Of these, 14 CNVs showed a statistically significant difference ($p > 0.05$) between myopic cases and controls including 8 deletions and 6 duplications. These CNVs were located in 11 chromosomal regions including 1p13, 2p21, 2q33, 3q26, 4p16, 4q32, 4q32, 6p21, 6p21, 14q11, 15q11. One of these CNVs appears to be novel whilst the remaining 13 have been previously reported in the Database of Genomic Variants. In conclusion, this pilot study suggests that CNVs do play a role in the development of myopia.

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Age-varying association of a SNP at 2p16 with primary open angle glaucoma. *L. Chen[1], P.O.S. Tam[1], D.Y.L. Leung[1,2], S.W.Y. Chiang[1], Y.Q. Zheng[3], M.Z. Zhang[3], C.P. Pang[1].* 1) Department of Ophthalmology & Visual Sciences, the Chinese University of Hong Kong, Hong Kong, China; 2) Hong Kong Eye Hospital, Hospital Authority, Hong Kong S.A.R, China; 3) Joint Shantou International Eye Center of Shantou University and the Chinese University of Hong Kong, Shantou, China.

Purpose: Recently, whole-genome linkage analysis and regional fine-mapping have revealed strong association of primary open-angle glaucoma (POAG), a leading cause of irreversible blindness, with two SNPs at the 2p16.3 region in the Afro-Caribbean population of Barbados. In this study, we evaluated these two SNPs (rs1533428 and rs12994401) in Chinese. Methods: Totally 1,245 unrelated Chinese subjects in 3 cohorts were involved: a Hong Kong sample of 184 patients with high-tension POAG (HTG), 206 with normal-tension POAG (NTG) and 230 controls, a Shantou sample of 102 HTG patients and 147 controls, and a Beijing sample of 176 HTG patients and 200 controls. The SNPs were genotyped using TaqMan technology. Results: No significant association was observed between rs12994401 and POAG. In contrast, rs1533428 showed consistent association patterns in all three cohorts. Moreover, an age-varying association was detected for rs1533428 with both HTG and NTG. In the pooled sample, the higher risk genotype (TT+CT) conferred a 2.28- and 1.74-fold of increased risk to late-onset HTG (P=5.03×10-5) and NTG (P=0.011) for patients with age at diagnosis >60 years, whilst the genotypes were not associated with juvenile-onset POAG (aged at diagnosis <35 years). Conclusions: We have identified discrepant association patterns from the initial study and confirmed rs1533428 to be a common genetic marker for POAG. Our results revealed for the first time that a common SNP can predict the risk of late-onset POAG.

980/W

Interaction between rs2476601 SNP of PTPN22 gene and rs10818488 of TRAF1 gene in causing risk for rheumatoid arthritis. *S. Ahmadloo[1], H. Shomali[1], A. Salimzadeh[2], M. Akhyani[3], M. Keramatipour[1].* 1) Department of Medical Genetics, School of Medicine,Tehran University of Medical Sciences, Tehran 14176-13151, Iran; 2) Rheumatology Department, Sina Hospital, Tehran University of Medical Sciences, Emam Khomeini St., Tehran, Iran; 3) Rheumatology Department, Alborz Hospital, Baghestan e gharbi St., Karaj, Iran.

Rheumatoid arthritis (RA) is a common autoimmune disease with a complex etiology affecting 1% of the world population. It appears that RA requires the complex interaction of genetic and environmental factors with the immune system, and ultimately in the synovial tissues. The role of genetic elements in determining both the risk of developing RA and the severity of the disease had already been acknowledged. Association studies in various populations have reported a number of genetic variations affecting the individual susceptibility to RA. The strongest association has been reported from genes within the HLA region, particularly the HLA-DRB1 gene. The rs2476601 SNP of PTPN22 (protein tyrosine phosphatase nonreceptor 22) gene can be named as the second polymorphism that has been repeatedly reported to be associated with RA. The association of another SNP, rs10818488 located near TRAF1 gene, has been recently picked up by genome wide association studies. So far there is no report investigating the possible association of rs2476601 and rs10818488 SNPs with RA in Iranian population. Therefore the aim of this study is to determine the possible association of these SNPs with RA in Iranian population using family-based (parents-child trios) as well as population-based case-control studies. In addition to testing for association, analysis of interaction between the two SNPs will be performed on obtained data. Currently the genotyping of samples are being carried out. The results will be presented at the meeting.

981/W

Polymorphisms of the *HLA-DP* locus are associated with hepatitis B virus infection and clearance in Han Chinese. *P. An[1], Z. Zeng[3], L. Guan[1], S.J. O'Brien[2], C.A. Winkler[1], the HBV study consortium.* 1) Lab Genomic Diversity, SAIC-Frederick Inc, Frederick, MD; 2) Lab of Genomic Diversity, National Cancer Institute-Frederick, Frederick,MD; 3) Department of Infectious Diseases, Peking University First Hospital, Beijing, P.R. China.

Background. Hepatitis B Virus (HBV) infection infects 10% of Asian populations, and is the leading cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC). The impact of host genetic control of HBV infection is largely unknown. Several single nucleotide polymorphisms (SNPs) in the *HLA-DP* locus were recently shown by a genome-wide association study (GWAS) to be associated with chronic HBV infection in Japanese and Thai (Kamatani et al., 2009). This study sought to assess the role of *HLA-DP* SNPs in modulating HBV outcomes in a Han Chinese population. Methods. We replicated two HLA-DP) in a Chinese cohort consisting of all major HBV outcomes: viral clearance, uninfected hypernormals, chronic diseases, cirrhosis and HCC (N=1700). Results. We extended the *HLA-DPA1* rs3077 association to Han Chinese for decreased risk of chronic HBV infection (OR=0.62, P=0.001), consistent with the previous report, and showed for the first time that rs3077 is a predictor for HBV clearance (OR=2.41. P= $4.7x10^{-10}$). The *HLA-DP* SNPs were not associated with development of cirrhosis or HCC. In Han Chinese, the *HLA-DPA1* rs3077 accounts for 36% of the population fraction for HBV infection and 56% for people who fail to clear HBV infection. Among global populations, the frequency of the risk allele tends to be higher in African and Asian populations (20-90%) with high HBV prevalence, but is less frequent in European populations (<20%) with low HBV prevalence, suggesting this allele may in part account for differential geographic distributions of HBV in the world. Conclusion. *HLA-DP* variation is a major genetic determinant of HBV infection and clearance in Asian populations. [Funded by NCI Contract HHSN261200800001E].

982/W

Fine-mapping linkage and family-based association of idiopathic scoliosis and chromosome 1. *D. Behneman[1,2], C. Justice[1], T. Beaty[2], K.Y. Liang[2], N.H. Miller[3], A.F. Wilson[1].* 1) Genometrics Section, IDRB/NHGRI/NIH, Baltimore, MD; 2) Johns Hopkins University, Bloomberg School of Public Health, Baltimore, MD; 3) University of Colorado Health Science Center at Aurora, Aurora, CO.

Idiopathic scoliosis is a lateral curvature of the spine with unknown etiology. Previous studies have reported linkage to several regions across the genome, as well as linkage and association with several candidate genes. In this study, we tested for association in two regions on chromosome 1 that were previously identified and replicated with genome-wide linkage analysis. Region 1 is 8 Mb on the p arm; Region 2 is 19 Mb on the q arm. Linkage analysis and family-based tests of association were performed in two samples: 95 families most-likely to have autosomal-dominant mode of inheritance (Sample 1) and 187 families selected for parent-child trios (Sample 2). Scoliosis was defined as two qualitative traits (lateral curvature ≥10° and ≥30°) and as a quantitative trait (the degree of lateral curvature). Model-independent sib-pair linkage analysis, as well as allelic, genotypic and haplotypic tests of association, were performed on 110 SNPs in Sample 1 and 18 SNPs in Sample 2 within Region 1, and among 202 SNPs in Sample 1 and 214 SNPs in Sample 2 from Region 2.

For Region 1, 13 SNPs showed evidence of linkage and another 9 SNPs showed evidence of association in Sample 1, and in Sample 2 linkage was found with 2 SNPs and association was found to another 5 SNPs, for at least one scoliosis phenotype. At the 30° threshold, linkage and association were observed for rs4512614 and rs4654549 in Sample 1 and rs10915548 in Sample 2.

For Region 2, 16 SNPs showed evidence of linkage and another 13 SNPs showed evidence of association in Sample 1, and in Sample 2 linkage was found with 45 SNPs and association was found to another 20 SNPs, for at least one scoliosis phenotype. In Sample 2, linkage and association were observed for rs11586173, rs7519036, rs12117045 and rs6428417 at the 10° threshold. However, in Sample 1 none of the SNPs were significant for both linkage and association.

Candidate genes were identified within 1 Mb of these linked SNPs and within 0.1 Mb of associated SNPs then prioritized by statistical evidence (p-values) and biologic plausibility (gene function). These findings suggest gene(s) in these regions may contribute to scoliosis, an important step in determining genetic etiology for this disorder.

983/W

Leptin and its receptor are not associated with the co-occurrence of mood and BMI in adolescent females. *S. Louise[1], L. Briollias[2], P.A. McCaskie[1], E. Mattes[3], L.J. Beilin[4], C.E. Pennell[5], L.J. Palmer[1].* 1) The Centre of Genetic Epidemiolgy and Biostatistics, The University of Western Australia, Perth, Australia; 2) Samuel Lunenfeld Research Institute, Mount Sinai Hospital, University of Toronto, Canada; 3) Telethon Institute for Child Health Research, Centre for Child Health Research, The University of Western Australia, Perth, Australia; 4) School of Medicine and Pharmacology, Royal Perth Hospital Unit, The University of Western Australia, Perth, Australia; 5) School of Women's and Infants' Health, The University of Western Australia, Perth, Australia.

Purpose: Negative moods, such as depression, have been associated with obesity in multiple child and adolescent studies. Common genetic factors have been hypothesised as an explanation for this co-occurrence. While the co-occurrence of obesity and depression has been observed many times, the potential genetic basis of this co-occurrence has not yet been thoroughly investigated. This study therefore aims to investigate the genetic basis, specifically whether leptin (LEP) and leptin receptor (LEPR), underlie the co-occurrence of depressive and/or anxious mood and Body Mass Index (BMI)in adolescent females. Leptin is a key regulator of body weight and has been separately associated with mood in both animal and human studies. **Methods:** This study uses data on female participants from the Western Australian Pregnancy Cohort (Raine) Study at the 14 year follow-up (n= 545). Tagged SNPs for LEP and LEPR were identified from HapMap Phase II (CEU) data using a pairwise approach (minor allele frequency(MAF)>0.05%; and r^2>0.8). Regions analysed include the entire gene, plus additional sequences 10 kb upstream and downstream of the gene. Adolescent weight and height were assessed by trained assessors. BMI was then calculated. Depressive and/or anxious mood scores were available from the Youth Self Report (YSR) and Beck Youth Depression Inventory (BDI-Y). A linear regression model was used to examine cross sectional associations between BMI (outcome) and tagged SNPs within LEP and LEPR. Mixed distribution models were used to account for the extra zero counts in our data and examine associations between depressive and/or anxious mood scores (outcome) and tagged SNPs within LEP and LEPR. **Results:** SNPs within LEP and LEPR were not associated with both BMI and depressive and/or anxious mood (p>0.05). SNPs within LEPR (RS10493380, RS1171267, RS7602, RS9436740, RS1171271,RS17127618, RS3762274, RS4655537 and RS9436301)were associated with depressive and/or anxious mood. These associations however did not persist after correcting for multiple testing. **Conclusion:** Tagged SNPs within LEP and its receptor (LEPR) did not underlie the co-occurrence of depressive and/or anxious mood and BMI in adolescence females within the Raine study. However larger cohorts may be needed to explore this possible association. LEPR may be associated solely with depressive and/or anxious mood.

984/W

Intergenerational allelic interaction: Excess maternal-fetal allele sharing in the HLA genomic region is associated with preterm birth. *J.R. Shaffer[1], E. Feingold[1], K. Ryckman[2], F. Begum[1], B. Feenstra[3], F. Geller[3], H.A. Boyd[3], D.E. Weeks[1], M.M. Barmada[1], C. Laurie[4], Q. Zhang[4], K. Doheny[5], E. Pugh[5], M.L. Marazita[1], M. Melbye[3], J.C. Murray[2].* 1) University of Pittsburgh, Pittsburgh, PA; 2) University of Iowa, Iowa City, IA; 3) Statens Serum Institut, Copenhagen, Denmark; 4) University of Washington, Seattle, WA; 5) Johns Hopkins, Baltimore, MD.

MOTIVATION: The incidence of preterm birth (PTB; gestation <37 weeks), which is the leading cause of mortality and adverse health complications among newborns, has increased in the US in recent decades. Previous studies have suggested that excess maternal-fetal sharing at loci in the HLA genomic region is associated with poorer pregnancy outcomes, which supports the hypothesis that maternal response to paternally-derived fetal HLAs may be beneficial to implantation and/or maintenance of pregnancy. However, little is known about the effect of maternal-fetal HLA sharing on PTB as a pregnancy outcome. We examined HLA sharing among 642 PTB case and 943 control mother-child pairs enrolled in the Danish National Birth Cohort Study as part of the inter-NIH sponsored GENEVA consortium. METHODS: Subjects were genotyped by CIDR on the Illumina 660W platform, which included 1,334 SNPs meeting genotyping quality criteria in the HLA genomic region (i.e. all SNPs between HLA-F and HLA-DPB1 on chromosome 6). Maternal-fetal genotype combinations at each SNP were dichotomized as to whether or not the fetus carried a paternally-derived allele absent in the mother. This binary measure of allele sharing was tested against PTB case/control status. RESULTS: Allele sharing at the HLA region as a whole, which contains blocks of strong linkage disequilibrium, showed excess association at the alpha=0.01 significance level (i.e. p<0.01 for 26 of 1334 SNPs compared to 13 SNPs expected by chance; lambda=1.164). This is in marked contrast to the whole genome which did not show excess association between allele sharing and PTB (514,200 autosomal SNPs; lambda=0.979). Moreover, for 23 of the 26 nominally significant (p<0.01) SNPs, greater allele sharing was a risk for PTB. Further exploration of all possible maternal-fetal genotype combinations in the three "top hits" (i.e. p<0.001; within or near the ABCF1 gene) showed that all like genotype combinations (e.g. mom=AA, fetus=AA) were risk factors for PTB, whereas unlike genotype combinations (e.g. mom=AA, fetus=AB; or mom=AB, fetus= AA) were protective. CONCLUSIONS: Our interpretation of these findings is that excess maternal-fetal allele sharing in the HLA region is a risk factor for PTB; this result supports the hypothesis that maternal response to foreign HLA alleles may be favorable for successful pregnancy. NIH grants: U01-HG004423, U01-HG004438, U01-HG004446.

985/W

Direct and Comprehensive Genotyping of Classical HLA Loci Reveals a Distinct Spectrum of Alleles Associated with Graves' Disease in Asians. *P. Chen[1,2], C. Fann[3,4], C. Chu[5], C. Chang[3], S. Chang[3], H. Hsieh[3], W. Yang[2,6,7], T. Chang[2,7].* 1) Department of Medical Genetics, National Taiwan University Hospital, Taipei, Taiwan, Taiwan; 2) Division of Endocrinology and Metabolism, Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan; 3) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 4) Institute of Public Health, National Yang-Ming University, Taipei, Taiwan; 5) Transfusion Medicine Laboratory, Medical Research Department, Mackay Memorial Hospital, Taipei, Taiwan; 6) Graduate Institute of Clinical Medicine, College of Medicine, National Taiwan University; 7) Department of Medicine, College of Medicine, National Taiwan University.

Graves' disease (GD) is the leading cause of hyperthyroidism and thyroid eye disease inherited as a complex trait. There has been no conclusion concerning which human leukocyte antigen (HLA) loci or alleles are associated with GD in populations other than Caucasians. We conducted a case-control association study (499 unrelated GD cases and 504 controls) and a replication in an independent family sample (419 GD individuals and their 282 relatives in 165 families). To minimize genetic and phenotypic heterogeneity, we included only ethnic Chinese Han population in Taiwan and excluded subjects with family history of hypothyroidism. We performed direct and comprehensive genotyping of six classical HLA loci (*HLA-A*, *-B*, *-C*, *-DPB1*, *-DQB1* and *-DRB1*) to 4-digit resolution. Combining the data of two sample populations, we found that *B*46:01* (odds ratio under dominant model [OR] = 1.33, combined *P* [*Pc*] = 3.4×10^{-4}), *DPB1*05:01* (OR = 2.34, *Pc* = 7.6×10^{-12}), *DQB1*03:02* (OR = 0.62, *Pc* = 5.8×10^{-4}), *DRB1*12:02* (OR = 0.51, *Pc* = 3.4×10^{-3}), *DRB1*15:01* (OR = 1.68, *Pc* = 3.6×10^{-4}) and *DRB1*16:02* (OR = 2.63, *Pc* = 4.3×10^{-7}) were associated with GD. *HLA-DPB1*05:01* is the major gene of GD in our population and singly accounts for 48.4% of population-attributable risk. These alleles, with considerable supporting evidence from previous studies in Asians, are distinct from the known associated alleles in Caucasians. Identification of population-specific association alleles is the critical first step for individualized medicine. Furthermore, comparison between different alleles across populations provides a great opportunity for better understanding of GD pathogenesis.

986/W

Candidate genes associated with non-diabetic end stage renal disease in African Americans: analysis of a pooled DNA-based genome wide association study. *M. Bostrom[1, 4, 5], L. Lu[2], J. Chou[2], P. Hicks[1], J. Xu[5], C. Langefeld[2], D. Bowden[1,4,5], B. Freedman[3].* 1) Dept Biochem, Wake Forest Univ, Winston-Salem, NC; 2) Biostatistical Sciences, Wake Forest Univ, Winston-Salem, NC; 3) Internal Medicine/Nephrology, Wake Forest Univ, Winston-Salem, NC; 4) Center for Human Genomics, Wake Forest Univ, Winston-Salem, NC; 5) Center for Diabetes Research, Wake Forest Univ, Winston-Salem, NC.

African Americans (AA) have increased susceptibility to non-diabetic (non-DM) etiologies of end-stage renal disease (ESRD), particularly attributed to hypertension and glomerular disease. Studies have shown that this susceptibility has a genetic component. To identify genes associated with non-diabetic ESRD in African Americans, we performed a genome wide association study (GWAS) on 1000 African American non-diabetic ESRD cases and controls. DNA from 500 case and 500 control samples was quantified using gel electrophoresis and spectrophotometric analysis. Samples were pooled to create 10 case pools and 10 control pools with each pool containing 50 samples. DNA pools were genotyped in duplicate on the Illumina HumanHap550-Duo BeadChip. Top scoring SNPs from the GWAS were genotyped on 966 African American non-DM ESRD cases and 953 non-nephropathy controls using the Illumina 1536 GoldenGate custom genotyping chip at the Center for Inherited Disease Research. 1420 SNPs were successfully genotyped on 962 African American non-DM ESRD cases and 947 non-nephropathy controls. SNPs were tested for association with non-DM ESRD with covariate adjustment for age, gender, logBMI, and percentage of African Ancestry (as determined by 70 admixture informative markers). The top six most associated SNPs were located on chromosome 22 in the region of the *MYH9* gene, which has been previously associated with non-DM ESRD (p = 4.87E-20 - 6.15E-5, odds ratio (OR) = 0.47 - 0.74; additive model). Other top SNPs included rs379489, in the *CFH* gene (p = 1.02E-4, OR = 0.72, confidence interval (CI) = 0.61-0.85; additive model) and rs3798696, located in *UGT2A3* (p = 8.04E-4, OR = 0.64, CI = 0.49 - 0.83; additive model). Case-only interaction analysis for risk status resulted in association at rs9808028 in the *ERBB4* gene (p = 5.04E-4, OR = 1.58, CI = 1.22 - 2.04, additive model), rs9687184 in an intergenic region (p = 6.07E-4, OR = 0.56, CI = 0.40 - 0.78, dominant model), and rs4682417 in the *CCDC80* gene (p = 6.19E-4, OR = 1.6, CI = 1.22 - 2.10, dominant model). We have performed the first genome-wide association study for non-DM ESRD in African Americans and have replicated association in the *MYH9* region of chromosome 22 and identified several additional candidate loci for follow-up studies.

987/W

IRF6 and 8q24 SNPs contribution to nonsyndromic cleft lip with or without cleft palate predisposition varies according to the geographic region in Brazil. *L.A. Brito[1,2], C.B.F. Silva[1,2], K.M. Rocha[2], L.A. Cruz[1,2], D. Meyer[1], D. Schlesinger[1,2], L.B. Kobayashi[1,2], M. Aguena[1,2], D. Bertola[3], D.F. Bueno[1,2], N. Alonso[3], M.R.S. Passos-Bueno[1,2].* 1) Department of Genetics, Institute of Biosciences, University of São Paulo, São Paulo - SP, Brazil; 2) Human Genome Research Center, University of São Paulo, São Paulo-SP, Brazil; 3) School of Medicine, University of São Paulo, São Paulo-SP, Brazil.

Nonsyndromic cleft lip with or without cleft palate (NSCL/P), the most common craniofacial birth defect, is a complex disease with multifactorial inheritance, and its prevalence varies among populations (1:1000 in Europeans (EU), 0.3:1000 in Africans (AF) and 3.6:1000 in Amerindians (AI)). IRF6 (1q32) SNPs, including one within an enhancer (rs642961), has been the locus of more replicated associations with NSCL/P. Studies also have pointed 8q24.21 as a candidate region to harbor a susceptibility locus to NSCL/P, and the SNP rs987252 in that region has been associated to the disease in some studies. As Brazilians constitute an admixed population mainly from EU, AF and AI, approaches such as admixture mapping and structured association are more recommended in order to avoid false correlations. In this study, the SNPs rs642961 and rs987252 were tested for association NSCL/P in our population. We performed the genotyping of the SNPs and of 40 biallelic insertion-deletion ancestry informative markers in 611 patients and 284 controls. Patients were from 5 different regions of Brazil (Rio de Janeiro, n=116; Maceio, n=107; Barbalha, n=63; Fortaleza, n=234 and Santarem, n=91). Ancestry contributions and association tests were performed with Admixmap, Structure 2.3.3 and STRAT softwares. Frequency of rs642961 risk allele was estimated at 0.181 (cases) and 0.131 (controls), whereas frequency of rs987252 risk allele was 0.357 (cases) and 0.330 (controls). Overall ancestry contributions were 58% EU, 29% AF and 13% AI for cases and 66% EU, 23% AF and 11% AI for controls. Individual admixture were used to stratify the association analysis and correct for effects of admixture in case-control comparison, resulting in significant association only for rs642961 in overall sample (P=0.013). Analyzing each region separately, rs642961 did not reach significance level in Rio de Janeiro and Maceio; in contrast, rs987252 showed significant association in Barbalha and Santarem (P<0.01). In summary, we corroborated the association of IRF6 and NSCLP in Brazilian population, and the 8q24.21 at risk alleles showed an important contribution only in some groups. Different results among the regions indicate that susceptibility to NSCLP is heterogeneous in Brazilian population. In order to further validate these results, admixture mapping is being conducted. FAPESP, CNPq.

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The Signal vs. Noise Balance: Exploring Gene Summary Methods. *A.E. Hendricks[1], J. Dupuis[1], M. Gupta[1], E.J. Benjamin[3], R.H. Myers[2], K.L. Lunetta[1].* 1) Biostatistics, Boston University School of Public Health, Boston, MA; 2) Neurology, Boston University School of Medicine, Boston, MA; 3) Cardiology and Preventive Medicine Sections, Boston University School of Medicine, Boston, Massachusetts.

As Genome-Wide Association Studies have become common, many research groups want to explore the full potential of their data by conducting complex analyses such as gene-gene interaction or gene pathway analysis. Due to the large number of SNPs in most gene regions, multi-gene analyses share a common obstacle of how to represent or summarize each gene region at the subject level. Creating a summarized value for each subject, rather than one value or statistic for each gene over all subjects, retains variation at the subject level enabling the summary values to be used in established analyses such as gene pathway analysis. A common per subject gene-region summary method uses the genotypes of the SNP with the lowest association p-value. However, this method may not be effective when a gene region has more than one trait associated variant and other summary methods exist that may better capture this type of variation. Most summary methods can be reduced to a weighted sum of SNP genotypes with the goal to assign the true risk SNPs (signals) the highest weights and to give all other SNPs (noise) weights of zero. Some methods, such as choosing the top associated SNP, put weight on a very small proportion of SNPs in the region and thus likely eliminate many false signals, but may also leave out SNPs with true effects. Other methods assign a non-zero weight to a much larger proportion of markers, which likely keeps more truly associated SNPs, but also includes more noise SNPs. Here, we use a simulation study to compare several summary methods: a) single SNP, b) haplotype, c) principal component, d) LASSO regression, e) BIC model selection, and f) Bayesian model averaging. We compare type I error and power of the methods over many simple and complex risk scenarios and simulate our replicates using a method that retains the LD structure of the gene region. We also modify the LD structure of the gene region by changing the SNPs available for analysis (for example, only SNPs on the Affy 6.0 chip or on the Illumina Omni-Quad chip). We find that in most simple risk scenarios and many complex risk scenarios choosing the top associated SNP has the highest power, with haplotype, LASSO regression, and BIC selection also performing well. Future research includes looking at the performance of these gene summary methods in gene x gene interaction analysis with the goal to scan the genome to identify SNPs that interact with the summarized gene region of interest.

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Genotype-phenotype relationships of susceptibility loci from genome-wide association studies in intracranial aneurysm. *H. Miura, H. Nakaoka, K. Akiyama, T. Cui, A. Tajima, I. Inoue.* Division of Molecular Life Science, School of Medicine, Tokai University, Isehara, Kanagawa, Japan.

Recently, a genome-wide association study identified associations between single nucleotide polymorphisms (SNPs) on chromosome 8q11, 9p21, 10q24, 13q13 and 18q11 and risk of harboring intracranial aneurysm (IA). Aneurysm characteristics or subphenotypes of IAs such as history of subarachnoid hemorrhage (SAH), presence of multiple IAs and location of IAs are clinically important but the genotype-phenotype relationships have not been approached yet. We investigated whether the association between variation in these loci and risk of IA varied among these subphenotypes.

We conducted a case-control study of 981 cases and 699 controls in Japanese. SNPs tagging these five risk loci were genotyped. The odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using logistic regression analyses. To estimate subphenotype-specific ORs, the frequency of the 9p21 variant in each case-subgroup (e.g. patients with ruptured or unruptured IA) was compared with the frequency in controls using a polytomous logistic regression.

Among SNPs on the five loci, a SNP on 9p21 (rs1333040) showed the strongest evidence of association with IA (P=1.5x10-6; per allele OR=1.43; 95% CI: 1.24-1.66). None of the patient characteristics (sex, age, smoking and hypertension) was a significant confounder or effect modifier of the association. Subgroup analyses of IA subphenotypes showed that among the most common sites of IAs, the association was strongest for IAs of the posterior communicating artery (PcomA) (OR=1.69; 95% CI, 1.26-2.26) and not significant for IAs in the anterior communicating artery (OR=1.22; 95% CI, 0.96-1.57). When dichotomizing IA sites, the association was stronger for IAs of the posterior circulation-PcomA group (OR=1.73; 95% CI, 1.32-2.26) versus the anterior circulation group (OR=1.28; 95% CI: 1.07-1.53). The associations did not vary when stratifying neither by history of SAH nor by multiplicity of IA. A similar approach would be undertaken for other loci.

Our results suggest that genetic influence on formation may vary between IA subphenotypes.

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Experimental follow-up of the *TCF7L2* association with type 2 diabetes. *D. Savic[1], H. Ye[2], S.Y. Park[2], G.I. Bell[2], M.A. Nobrega[1].* 1) Department of Human Genetics, The University of Chicago, Chicago, IL; 2) Department of Medicine, The University of Chicago, Chicago, IL.

Several genome wide association studies (GWAS) have identified a 92.1-kb block of strong linkage disequilibrium (LD) within the *TCF7L2* locus associated with type 2 diabetes (T2D). How *TCF7L2* affects risk and which tissues are involved are areas of active investigation by many laboratories. Coding variations within *TCF7L2* were not identified, raising the possibility that regulatory variations in distant enhancers may underlie the disease risk. Using a combination of bioinformatics, comparative genomics and mouse genetic engineering, we have identified a variety of tissue-specific enhancers within a region spanning the diabetes-associated interval that recapitulate the endogenous pattern of *Tcf7l2* expression. Several of these *TCF7L2* enhancers exhibit expression in tissues with established roles in maintaining glucose homeostasis, including brain, pancreas, stomach, bones and intestines, strengthening the hypothesis that sequence variants within these enhancers might underlie the association signal seen in the GWAS. In order to functionally establish *TCF7L2* as a T2D gene, we have developed *Tcf7l2* knockout mice using zinc finger nuclease technology. Preliminary analyses have shown that *Tcf7l2* heterozygous knockout mice display lower body weight, bone mineral density and fat mass but increased basal metabolic rate compared to wild-type littermates. Our study supports the notion that distant *cis*-regulatory elements are often present in regions associated with common diseases and suggests a role for *TCF7L2* in metabolism.

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Possible roles of macrophage migration inhibitory factor and mannose-binding lectin gene polymorphisms in Turkish children with acute rheumatic fever. *N. Col Araz[1], S. Pehlivan[2], O. Pinarbasi[3], T. Sever[2], S. Oguzkan Balci[2], A. Balat[4].* 1) University of Gaziantep, Faculty of Medicine, Department of Pediatrics, Gaziantep, Turkey; 2) University of Gaziantep, Faculty of Medicine, Department of Medical Biology and Genetics, Gaziantep, Turkey; 3) University of Gaziantep, Faculty of Medicine, Department of Pediatric Cardiology, Gaziantep, Turkey; 4) University of Gaziantep, Faculty of Medicine, Department of Pediatric Nephrology, Gaziantep, Turkey.

Objectives of Study: Acute rheumatic fever (ARF) is the most important immune-mediated sequela of upper respiratory tract infection with group A, β-hemolytic streptococci, and the immune mechanisms that provoke concomitant inflammation of synovial joints and cardiac valves remain poorly defined. Macrophage migration inhibitory factor (MIF) is recognized as a soluble factor produced by sensitized T lymphocytes and inhibits the random migration of macrophages. Mannose-binding lectin (MBL) is a central component of the innate immune response. They play important roles in the pathogenesis of several acute and chronic inflammatory / autoimmune disorders. Our aim was to investigate any possible association between MBL and MIF gene polymorphisms and ARF in a group of Turkish children. **Methods:** The study was approved by the local ethical committee and informed consents were obtained from the patients. Genomic DNA was extracted from peripheral blood using the salting out procedure. Thirty-eight children with ARF and 40 age-and sex-matched healthy controls were analyzed for codon 54 A/B polymorphism in MBL gene and -173 G/C polymorphism in MIF gene by using PCR-RFLP methods. **Results:** The distribution of AA, AB, and BB genotypes for MBL codon 54 were 55.3 %, 38.9 % and 35.2 % in ARF compared with 65%, 35 % and 0 % in the controls (p= 0.372). BB genotype was higher than AA and AA+AB genotypes in patient group (p=0.033, p=0.035, respectively). The allele frequency of A/B in MBL was 72.4 %, 27.6 % in ARF compared with 82.5 %, 17.5 % in the controls. (p=0.129). The distribution of GG, GC, and CC genotypes for MIF (-173) were 47.4 % , 31.6 %, and 21 % in ARF compared with 57.5 %, 40 % and 2.5 % in the controls (p=0.047). CC genotype was found higher than GG and GG+GC genotypes in patient group (p=0.014, p=0.010, respectively). The allele frequency of G/C in MBL was 63.2 %, 36.8 % in ARF compared with 77.5 %, 22.5 % in the controls. (p=0.049). The observed genotype counts were not deviated significantly from those expected according to the Hardy- Weinberg Equilibrium for MBL and MIF gene polymorphisms (P>0.05). **Conclusions:** The present study is the first to investigate the MBL and MIF gene polymorphisms in children with ARF. We conclude that BB genotype of MBL (codon 54) and CC genotype of MIF (-173) may have a role in the immunoinflammatory process of ARF. However, further studies in a larger population are needed to confirm the results.

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Deleterious allele of olfactory receptor gene *OR51J1* is associated with specific inability to smell the musty odor of 3-methyl-2-hexenoic acid. *A. Knaapila[1], Y. Hasin[2], D. Reed[1], F. Duke[1], J. Louie[1], D. Lancet[2], C. Wysocki[1].* 1) Monell Chemical Senses Center, Philadelphia, PA; 2) Dept. of Molecular Genetics, the Weizmann Institute of Science, Rehovot, Israel.

Olfaction, the sense of smell, is important for detecting dangers (fire, spoiled food) and for enjoying pleasant odors from food, flowers, and perfumes. The lowest concentration at which an odorant can be perceived (detection threshold) can vary more than 10000-fold among human subjects. In an extreme case, an individual whose sense of smell is generally normal is unable to detect a specific odorant even at a very high concentration (specific anosmia). The gene family encoding olfactory receptors (ORs) is one of the largest in the genome with more than 400 potentially functional OR genes, but how polymorphism in the OR genes relates to the phenotypic variation in odor perception remains largely unknown. The aim of the study was to explore associations between 62 selected OR coding region polymorphisms, that segregate between intact and deleterious alleles (segregating pseudogenes), and specific anosmia, detection threshold, and perceived pleasantness of 9 odorants (androstenone, benzyl salicylate, Galaxolide™, isovaleric acid, isoamyl acetate, Jeger's ketal, 3-methyl-2-hexenoic acid [3M2H], muscone, and pentadecalactone). The analyses were based on data from 413 individuals (aged 18 to 62 years, mean age 27 years; 64.4% women). The statistical software Plink was used to screen the data for significant associations. Bonferroni correction for multiple testing was applied to adjust the criterion for statistical significance to $\alpha = 3.0e\text{-}5$. Specific anosmia to 3M2H (musty/sweaty odor) was associated with SNP *rs1909261* (nucleotide change G/A corresponding to a strong missense amino acid change C105Y) within gene *OR51J1* (unadjusted p = 6.0e-6). The frequency of 3M2H anosmia was significantly higher in subjects who were either homozygous for the allele or heterozygous (AA/GA; n = 67, of whom 38.8% were anosmic) as compared to individuals homozygous for the intact allele (GG; n = 301, of whom 14.6% were anosmic) (unadjusted Pearson Chi-squared p = 5.1e-6). *OR51J1* is located on chromosome 11p15.4 in the middle of a large OR gene cluster, that includes 74 OR genes. Thus, it is possible that *OR51J1* and/or another OR gene in linkage disequilibrium with *OR51J1* encode receptor(s) that respond to 3M2H, as implied by the theory of combinatorial receptor codes for odors. Although the functionality of these receptors remains to be demonstrated, the results of the present study suggest that the product of *OR51J1* is involved in detection of 3M2H.

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Epistatic Interactions Among Genes for Athleticism. *E. McRae[1], J.R. Ruiz[2], F. Gómez-Gallego[3], C. Santiago[3], A. Buxens[4], A. Lucia[3], M. Ruvolo[1,5].* 1) Dept. of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA; 2) Dept. of Biosciences and Nutrition, Karolinska Institute, Stockholm, Sweden; 3) Universidad Europea de Madrid, Madrid, Spain; 4) Progenika Biopharma, Zamudio, Spain; 5) Dept. of Human Evolutionary Biology, Harvard University, Cambridge, MA.

Human athleticism is a complex trait, influenced by many genes as well as the environment. Hundreds of alleles have been identified as being associated with athletic performance and fitness, and the number continues to grow. For the majority of the history of this field, such work has focused on the influence of individual genes. While such studies inform us about what genes could be involved in athleticism, they do not help us understand the complex nature of the trait. The goal of this study was to look for genes that interact epistatically in determining elite athlete status. We examined 37 health-related genes, 13 of which had previously been associated with athleticism, in 253 males of Spanish ancestry - 100 elite endurance athletes, 53 elite power athletes, and 100 sedentary controls. We used several methods to look for these interactions, including multifactor dimensionality reduction (MDR), generalized MDR (GMDR), and entropy-based methods. We found a significant interaction between the Met235Thr SNP of angiotensinogen (AGT) and the C-786T SNP of the endothelial nitric oxide synthase 3 (NOS3) gene that strongly distinguishes endurance athletes from power athletes. We found that an interaction between these same SNPs also distinguishes elite athletes as a group from sedentary controls, along with a second interaction between myostatin (GDF8) and the Ile105Val polymorphism of glutathione S-transferase π1 (GSTP1). We also found a suggestive four-way interaction between AGT, GSTP1 (105), NOS3 (-786), and superoxide dismutase 2 (SOD2). We found no significant interactions distinguishing endurance athletes and controls, only main effects from α-actinin 3 (ACTN3) and the β3 adrenergic receptor (ADRB3) gene. Finally, in examining power athletes and controls, we found a significant two-way interaction between the Cys112Arg SNP of apolipoprotein E (APOE) and the Glu298Asp polymorphism of NOS3. This comparison also yielded a suggestive four-way interaction among angiotensin I-converting enzyme (ACE), the Arg268Lys SNP of N-acetyltransferase 2 (NAT2), interleukin-6, and NOS3 (-786). This study allows us, for the first time, to look at specific gene interactions that are associated with elite athletic performance. Most of the polymorphisms identified have previously been associated with athleticism, but others had not, and should therefore be studied more closely.

994/W

Application of the *Gentrepid* candidate gene prediction system to Genome-Wide Association Studies. *S. Ballouz[1,2], J.Y. Liu[1], M. Oti[1], B. Gaeta[2], D. Fatkin[1,3], M. Bahlo[4], M.A. Wouters[1,3].* 1) The Victor Chang Cardiac Research Institute, Sydney, NSW, Australia; 2) School of Computer Science and Engineering, University of New South Wales, Sydney, NSW, Australia; 3) School of Medical Sciences, University of New South Wales, Sydney, NSW, Australia; 4) The Walter and Eliza Hall Institute, Melbourne, VIC, Australia.

Genome-wide association studies (GWAS) aim to identify the genetic architecture of complex diseases by using genotyping arrays to test a large number of SNP markers for disease correlation. However the data is very noisy and identifying the relevant disease gene is not straightforward. Here we analysed data from the Wellcome Trust Case Control Consortium on seven disease phenotypes: bipolar disorder, coronary artery disease, Crohn's disease, hypertension, rheumatoid arthritis, and type I and type II diabetes. We used the *Gentrepid* candidate gene prediction system to select likely gene candidates associated with SNPs of less stringent statistical thresholds than applied in the original analysis, using multiple SNP to gene mapping assumptions. Mapping assumptions resulted in search spaces ranging in size from 2 to 4431 genes for specific phenotypes. Under the common nearest neighbour SNP mapping assumption, only 76% of characterized genes are associated with a SNP on the Affymetrix 500K chip set. Gene coverage increases to 99% using other mapping assumptions tested. However, even when the entire genome is considered, only 57% of characterized genes have *Gentrepid* annotations and are thus potentially predictable as candidates. Predictions were made using protein domains and pathway information of known disease genes or by searching for enrichment of these features within the search space. *Gentrepid* was able to extract known disease genes and predict novel plausible disease genes in known and novel, WTCCC-implicated loci for the seven phenotypes. The results show the approach is feasible and may be a valuable way to analyse other GWAS data.

995/W

Genetic and molecular basis of QTL of alcohol preference in mice: genes and polymorphisms. *W. Gu[1], Y. Cao[2], Q. Xiong[3], Y. Jiao[1], B. Bennett[4], Y. Wang[5].* 1) Dept Orthopedic Surgery, Univ. of Tennessee Health Sci Ctr., Memphis, TN; 2) 2 Institute of Kaschin-Beck Disease, Center for Endemic Disease Control, Centers for Disease Control and Prevention, Harbin Medical University, Harbin, 150081, PR China; 3) Institute for Genome Science and Policy, Duke University, Durham, NC 27708, USA; 4) Department of Pharmacology, Universitty of Colorado Denver, Aurora, CO 80045-0508, USA; 5) Department of Neurology, Beijing Tiantan Hospital, Capital University of Medical Sciences, Beijing, 100050, PR China.

Alcoholism is a complex disease that is affected by multiple genetic and environmental factors. To understand the genetic and molecular basis of alcoholism, a large number of quantitative trait loci (QTL) that regulate experimental alcoholic preference have been identified using various mouse models for alcoholism. However, identifying the particular responsible genes within these QTL remains a major challenge. Using currently available genomic data and gene annotation information, we systematically examined alcoholic preference-associated genes and polymorphisms within and outside previously-reported QTL over the whole mouse genome. We found significantly more alcoholism-associated genes in QTL regions as contrasted with non-QTL regions. Rapid development of high throughput screening technology and genomic resource in the near future will determine whether these known alcoholism-associated genes or polymorphisms are genetic components causing the QTL effect.

996/W

Behçet's disease (BD) and the mitochondrial genome. *A. Rosa[1], J.M. Xavier[2,3], N.M. Shafiee[4], F. Ghaderi[4], B.S. Abdollahi[4], F. Shahram[4], F. Davatchi[4], S.A. Oliveira[2,3].* 1) Unidade de Ciências Médicas, Centro de Competências das Ciências da Vida, Universidade da Madeira, Funchal, Portugal; 2) Instituto de Medicina Molecular, Lisbon, Portugal; 3) Instituto Gulbenkian de Ciência, Oeiras, Portugal; 4) Rheumatology Research Center, Tehran University of Medical Sciences, Tehran, Iran.

BD is a chronic inflammatory disorder, classified within the group of vasculitides, that may involve several organs, such as skin, mucocutaneous membranes (oral and genital aphtae), eyes, joints, lungs, gastrointestinal and central nervous systems. Only nuclear genes have been investigated thus far for BD risk, while the role of the mitochondrial DNA (mtDNA) has been completely neglected. In our expression profiling study of BD patients, we found an over-representation of nuclear genes linked to oxidative stress among those differentially expressed. Mitochondrial normal functioning is required for cellular respiration and vascular cell growth and function. mtDNA coding region polymorphisms (encoding for 13 oxidative phosphorilation polypeptides, 22 tRNAs and 2 rRNAs) may enhance oxidative stress by increased production of mitochondrial reactive oxygen species (ROS), which cause damage to DNA, proteins, and lipids. Since its dysfunction may underlie a multitude of clinical features in multifactorial and multisystemic diseases, we assessed whether mtDNA SNPs and haplogroups confer susceptibility to BD. Patients were selected as consecutive patients, according to ICBD criteria. 22 mtDNA tagging-SNPs sufficient for classifying our Iranian samples into the most prevalent regional haplogroups (West Eurasian H, pre-R0/R0, J, T, U, K, N1(I) and W; Eastern Eurasian M (xD), N (xN1, I, W and X) and R (xJT and UK); African L, and Asian D haplogroups) were genotyped in 550 BD patients and 436 controls, and their association with BD tested individually or in combination into haplogroups, adjusting for gender. mtDNA SNP 709G/A in the 12S rRNA was found to have a modest association with BD (adjusted p=0.052, and unadjusted p=0.03). None of the haplogroups tested were associated with BD. Since 12S RNA molecules participate in assemble of amino acids into functioning proteins that carry out oxidative phosphorylation inside the mitochondria, this modest association suggests a potential role of oxidative stress in BD pathogenesis. mtDNA SNP 709G/A association with BD requires further validation and investigation.

997/W

Association of the matrix metalloproteinase 13 gene with child-onset asthma. *Y. Suzuki[1], Y. Mashimo[1], S. Hattori[1], K. Takeuchi[1], N. Shimojo[2], T. Hirota[3], K. Fujita[4], A. Miyatake[5], S. Doi[6], Y. Okamoto[7], Y. Kohno[2], M. Tamari[3], A. Hata[1].* 1) Department of Public Health, Chiba University Graduate School of Medicine, Chiba, Japan; 2) Department of Pediatrics, Chiba University Graduate School of Medicine, Chiba, Japan; 3) Laboratory for Respiratory Diseases, Center for Genomic Medicine, RIKEN, Tsurumi, Japan; 4) School of Human Nursing, The University of Shiga Prefecture, Shiga, Japan; 5) Miyatake Asthma Clinic, Osaka, Japan; 6) Department of Pediatric Allergy, Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, Osaka, Japan; 7) Department of Otolaryngology, Graduate School of Medicine, Chiba University, Chiba, Japan.

Background: Matrix metalloproteinases (MMP) are a family of zinc- and calcium- dependent endopeptidases that are involved in various physiological processes. Several MMP genes such as MMP9 and MMP12 have been shown to be involved in pathogenesis of asthma. However, little is known about roles of MMP13.

Methods: We recruited 1039 Japanese atopic asthma patients (age mean: 23.2 years; range: 3 to 83 years), 333 child (6 to 12 years) controls, and 628 adult (20 to75 years) controls. Onset of 64% of the patients was younger than 15 years of age. In this study, these patients were defined as child-onset asthma (COA). Patients with onset of 16 years or older were defined as adult-onset asthma (AOA). We investigated linkage disequilibrium (LD) status of 38 polymorphisms in the MMP13 gene region. Eight tag SNPs were genotyped in the entire samples. The promoter activity of the gene region was determined with a luciferase assay. Expression of MMP13 mRNA and protein in the small airway epithelial cells (SAEC) was measured before and after the addition of pathogen associated molecular patterns, interferons (IFN), or cytokines.

Results: Out of 8 tag SNPs, 3 SNPs were in moderate to strong LD (LD group 1) and the other five were in weak LD. None of the SNPs showed different genotype frequencies between the child control and adult control; thereby we compared genotypes of AOA or COA group with the combined control group. The SNPs of LD group1 were significantly associated with COA group. P values of association test in dominant model of -2976G/A (SNP2), -290G/A (SNP5), and 16385C/A (SNP7) were 0.000055, 0.000065, and 0.000011, respectively. The odds ratio and its 95% confidence interval of SNP5 were 1.90 and 1.37-2.55, respectively. SNP2 and SNP5 were located in the promoter region, while SNP7 were in the 3' downstream of the last exon. Only SNP5 significantly affected the promoter activity. Lung and bone marrow express MMP13 mRNA much higher than any other normal tissues. IFN beta and poly(I:C) were potent inducers of MMP13 in SAEC.

Conclusion: The MMP13 gene may be involved in defense mechanisms to viral infection in airway epithelial cells and play a role in pathogenesis of child-onset asthma.

998/W

Meta-analysis of the major histocompatibility complex in systemic lupus erythematosus. *K.E. Taylor[1], D. Morris[2], M.M. Fernando[2], J. Nititham[1], R.R. Graham[3], J.B. Harley[4,5], B.P. Tsao[4,6], J.D. Rioux[7,8], L. Hammarström[7,9], C.O. Jacob[4,10], R.P. Kimberly[4,11], M.J. Daly[7,12], L. Klareskog[7,9], P.K. Gregersen[7,13], J.R. Oksenberg[7,14], S.L. Hauser[7,14], C.D. Langefeld[4,15], M.F. Seldin[16], M.E. Alarcón-Riquelme[4,17], K.L. Moser[4,17], P.M. Gaffney[4,17], T.W. Behrens[3], L.F. Barcellos[18], T.L. Vyse[2,4,7], L.A. Criswell[1,4].* 1) Rosalind Russell Medical Research Center for Arthritis, University of California San Francisco, San Francisco, CA; 2) Imperial College London, London, UK; 3) Genentech, Inc., South San Francisco, CA; 4) The International SLE Genetics Consortium (SLEGEN); 5) Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 6) University of California Los Angeles, Los Angeles, CA; 7) The International MHC and Autoimmunity Genetics Network (IMAGEN); 8) Research Center, the Montreal Heart Institute Montréal, Canada; 9) Karolinska Institutet, , Stockholm, Sweden; 10) University of Southern California, Los Angeles, CA; 11) University of Alabama Birmingham, Birmingham, AL; 12) The Broad Institute of MIT and Harvard, Boston, MA; 13) Feinstein Institute for Medical Research, Manhasset, NY; 14) Department of Neurology, University of California San Francisco, San Francisco, CA; 15) Wake Forest University Health Sciences, Winston-Salem, NC; 16) University of California Davis, Davis, CA; 17) Oklahoma Medical Research Foundation, Oklahoma City, OK; 18) University of California Berkeley, Berkeley, CA.

Systemic lupus erythematosus (SLE) is a genetically complex disease with heterogeneous clinical manifestations. Alleles of the *HLA-DRB1* locus in the major histocompatibility complex (MHC) are known to increase risk of SLE; previous studies have found additional but inconsistent risk signals in other areas of the MHC, and extensive linkage disequilibrium (LD) in the region makes it difficult to identify true risk loci. In order to more precisely understand the role of the MHC region in SLE etiology, we have collected data for the most comprehensive study to date on the MHC and SLE. We have acquired MHC single-nucleotide polymorphism (SNP) genotypes for 3,812 cases from four whole-genome scans and two recent MHC studies. We have also obtained genotype data on 12,817 controls, from those six studies and from additional publicly-available controls. In addition to the MHC we have collected available whole-genome data or ancestry-informative markers (AIMs) for detection of unknown duplication or relatedness and ancestry analysis. We identified duplicates and first-degree relatives by identifier and identity-by-state similarity; one subject of each relative pair or set was retained; for duplicates, genotypes were merged by consensus call. Quality-control filters were applied before merging; loci were removed for Hardy-Weinberg disequilibrium in controls by study source, removing those resulting in a false-discovery rate of greater than 0.05. Ancestry outliers (i.e. likely non-European ancestry, n=58) were removed using a series of principal component analyses performed using each type of available whole-genome or AIM data. As the six parent studies were performed on six different platforms without a high degree of overlap, we are imputing up to a common set of SNPs for analysis. Due to overlapping subjects and different genotyping platforms, each study will be re-analyzed after removal of duplicates and with adjustment for ancestry, and per-study results combined via Mantel-Haenszel statistics. We anticipate that multiple signals, some due to LD, obtained in the initial association screen will be tested for independence with each other and with *HLA-DRB1* alleles using multiple conditional analysis methods such as multivariate logistic regression and log-ratio testing of haplotypes. In sum, we are using a series of rigorous methods to investigate SLE in the largest collection of MHC data ever assembled for SLE.

999/W

Multiple independent major histocompatibility complex associations with nephritis and autoantibody production in systemic lupus erythematosus (SLE). *S.A. Chung[1], K.E. Taylor[1], S.L. May[2], P.P. Ramsay[2], H.L. Quach[2], J.A. Lane[3], J. Nititham[1], J.A. Noble[3], D.L. Quach[2], J.A. Kelly[4], K.L. Moser[4], T.W. Behrens[5], M.F. Seldin[6], J.B. Harley[7], P.M. Gaffney[4], L.F. Barcellos[2], L.A. Criswell[1].* 1) Rosalind Russell Medical Research Center for Arthritis, University of California, San Francisco, CA; 2) University of California, Berkeley, CA; 3) Children's Hospital Oakland Research Institute, Oakland, CA; 4) Oklahoma Medical Research Foundation, Oklahoma City, OK; 5) Genentech, Inc., South San Francisco, CA; 6) University of California, Davis, CA; 7) Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

The major histocompatibility complex (MHC) on chromosome 6p21 is the most significantly associated genomic region with systemic lupus erythematosus (SLE). Prior attempts to identify MHC associations independent of *HLA-DRB1* (the most consistently associated locus) have been limited, in part, by the region's extensive linkage disequilibrium. In addition, MHC associations with specific SLE manifestations such as lupus nephritis, anti-double-stranded DNA (anti-dsDNA), and anti-Ro/La (SSA/SSB) autoantibody production have not been fully explored. To identify independent MHC associations with these SLE manifestations, we genotyped 1,610 SLE cases of European descent for 2,360 MHC single nucleotide polymorphisms (SNPs), *HLA-DRB1* alleles, and 384 ancestry informative markers. Lupus nephritis and autoantibody status were obtained from medical record review. After applying stringent quality control criteria (including removal of SLE cases with <90% northern European ancestry), we analyzed 1,974 SNPs in 1,125 SLE cases. Thirty-two percent (32%) had lupus nephritis, 50% had anti-dsDNA autoantibodies, and 27% had anti-Ro/La (SSA/SSB) autoantibodies. Relative predispositional effects analysis showed that different *HLA-DRB1* alleles were associated with lupus nephritis as compared to anti-Ro/La (SSA/SSB) autoantibody production (**1501* and **1302* for nephritis vs. **0301*, **0401*, **0404*, **1301*, and **0101* for anti-Ro/La). No *HLA-DRB1* alleles were significantly associated with anti-dsDNA autoantibody production (global χ^2 p>0.05). Forward selection with conditional logistic regression based on haplotypes identified associations independent of *HLA-DRB1* in the *HLA-DOB*, *HLA-A/HCG9*, and *LEMD2* regions with lupus nephritis (OR for the most associated haplotype [OR_{hs}] 3.04, haplotype-specific p [p_{hs}]= 3.1E-04). *HLA-DRB1*-independent associations were observed in the *HLA-DPA1/HLA-DOA*, *C6orf205*, and *OR2H2* regions with anti-Ro/La (SSA/SSB) autoantibody production (OR_{hs} 3.23, p_{hs}=1.6E-08). SNPs in or near the *TAP2*, *C6orf10*, and *TRIM40/TRIM15* regions were associated with anti-dsDNA autoantibody production (OR_{hs} 2.82, p_{hs}=1.5E-08). In conclusion, we have identified several significant MHC associations with lupus nephritis and autoantibody production that are independent of *HLA-DRB1*. Of interest, different genes/genetic regions were associated with these specific SLE manifestations. These results indicate that MHC genetic variation contributes significantly to disease heterogeneity in SLE.

1000/W

Fine-Mapping the 13q21 Specific Language Impairment Locus in Extended Pedigrees. *T.R. Simmons[1], J.F. Flax[2,3], A.S. Bassett[4], P. Tallal[2], L.M. Brzustowicz[3], C.W. Bartlett[1].* 1) Battelle Center for Mathematical Medicine, The Research Institute at Nationwide Children's Hospital & Department of Pediatrics, The Ohio State University, Columbus, OH 43203, USA; 2) Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102, USA; 3) Department of Genetics, Rutgers University, Piscataway, NJ 08854; 4) Department of Psychiatry, University of Toronto, Ontario M5T 1R8 and, Clinical Genetics Research Program, Centre for Addiction and Mental Health, Toronto, Ontario M5S 2S1 Canada.

Specific language impairment (SLI) is defined by the failure to develop language at the expected rate in the absence of cognitive, neurological, or psychological explanations. SLI is characterized by significant problems of comprehension and/or expression of spoken language though it is commonly comorbid with reading difficulties. Our previous linkage studies in 4 multiplex pedigrees implicated 13q21 as a critical region for an SLI susceptibility allele in those pedigrees selected for SLI and including family members with a significant reading impairment. To further refine localization in these pedigrees, dense SNP array genotyping was performed on one of two Illumina BeadArray systems. A total of 1865 SNPs with minor allele frequency > 5% in 13q21 were analyzed with the same reading impairment phenotype that was originally associated with the 13q21 locus. PPL linkage analysis of individual SNPs is in agreement with overall multiple linkage analysis with a peak posterior probability of 81%. Ongoing gene-gene interaction analysis using a functional coding SNP in the brain-derived neurotrophic factor (BDNF) gene increases the peak PPLs in the region to 99.9% in a narrowly defined region. These largest gene-gene interaction linkage signals span approximately 2 cM, thus narrowing down the critical region approximately 1.5 Mb. This region encompasses one known gene, protocadherin 17 (PCDH17), and ~1Mb upstream of the documented transcription start site. As the largest BDNF gene-gene interaction PPL results are upstream of PCDH17, rather than within the gene, it is possible that the SLI susceptibility allele will be present within the tissue-specific regulatory elements of PCDH17.

1001/W

A Genome-Wide Linkage Search for Genetic Loci Controlling Resistance to Tuberculosis. *E. Hoal[1], A. Cobat[2,3], C. Gallant[4], L. Simkin[4], G. Black[1], K. Stanley[1], J. Hughes[5], M. Doherty[6], W. Hanekom[5], B. Eley[7], N. Beyers[8], J. Jaïs[3], A. Boland-Auge[10], P. van Helden[1], J-L. Casanova[2,3,9], L. Abel[2,3,9], A. Alcaïs[2,3,9], E. Schurr[4].* 1) Molecular Biology and Human Genetics, Stellenbosch University, Tygerberg, South Africa; 2) Laboratory of Human Genetics of Infectious Diseases, Necker Branch, Institut National de la Santé et de la Recherche Medicale, Paris, France; 3) University Paris Descartes, Necker Medical School, Paris, France; 4) McGill Centre for the Study of Host Resistance & Departments of Human Genetics and Medicine, McGill University, Montreal, Canada; 5) South African Vaccine Initiative, Institute of Infectious Diseases and Molecular Medicine, Health Sciences Faculty, University of Cape Town; 6) Statens Serum Institute, Copenhagen, Denmark; 7) Paediatric Infectious Diseases Unit, Red Cross Children's Hospital, School of Child and Adolescent Health, University of Cape Town, South Africa; 8) Desmond Tutu TB Centre, Department of Paediatrics and Child Health, Faculty of Health Sciences, Stellenbosch University, Tygerberg; 9) Laboratory of Human Genetics of Infectious Diseases, Rockefeller Branch, Rockefeller University, New York, NY 10065; 10) Centre National de Génotypage, Institut de Génomique, Commissariat à l'energie atomique, Cedex Evry 91057, France.

Two-thirds of the global population is infected with Mycobacterium tuberculosis, yet tuberculosis (TB) disease manifests in less than 10% of infected individuals. TB is highly endemic in the Western Cape, South Africa, and this population is therefore very suitable to study genetic susceptibility. We found high heritability (>50%) for in vitro secretion of tumor necrosis factor a and interferon γ (IFNγ), and the frequency of antigen-specific IFNγ+CD4+ and IFNγ+CD8+ cells in the response of whole blood to mycobacterial challenge. In principal component analysis, the first 3 components explain 78% of the overall variance consistent with the effect of pleiotropic regulatory genes of human antimycobacterial immunity. We then set out to find genetic variants controlling susceptibility to infection by studying about 400 children and young adults in 128 families by means of a genome-wide linkage search. Most of the subjects tested were likely to have been exposed to TB, but about 20% did not show delayed type hypersensitivity (DTH) in a skin antigen test, appearing to be naturally resistant to infection by M. tuberculosis. This strong resistance mapped to a 6-Mbp chromosome region, 11p14 ($p = 1.5x10-5$) called TST1. The existence of this locus suggests that we could one day manipulate cellular mechanisms to achieve TB prevention - an important goal given the lack of an effective vaccine and the rise in drug-resistant strains. The second locus, in the 2.9-Mbp 5p15 region ($p < 10-5$) called TST2, segregated with differing extents of TB skin test response or the intensity of T-cell-mediated DTH to tuberculin. These genetic factors might contribute to whether an infected individual keeps the bacterium dormant or develops the disease. The measurement of TST did not correlate with IFNγ release, indicating that other pathways could be more important than this well-known cytokine. Fine mapping of the 5p15 region identified SLC6A3 as a promising candidate gene. This gene is a solute carrier (SLC) family member, which could influence granuloma responses to mycobacteria, and loss of the mouse SLC6A3 protein reduces DTH response to ovalbumin. We therefore identified one major locus that determines innate resistance to M. tuberculosis infection in endemic areas and a second that controls the extent of that response via critical regulators of T-cell dependent DTH. These results indicate that one major genetic locus may control resistance to the pathogen in humans.

1002/W

Variants in the neuregulin 1 gene are associated with nicotine dependence. *K. Jackson, J. Chen, K. Kendler, X. Chen.* Virginia Commonwealth University 800 E. Leigh St, Suite 390A Richmond, VA 23298.

Neuregulin 1 (NRG1), located on chromosome 8p12, encodes a protein that acts as a signaling molecule involved in cell-cell interactions and glutamatergic neurotransmission. NRG1 is a leading candidate gene in schizophrenia, and has been implicated in bipolar disorder. While smoking is highly comorbid with many mental disorders, including schizophrenia and bipolar disorder, the role of NRG1 in smoking and nicotine dependence (ND) is unclear. Thus, the goal of this study was to determine if variants in the NRG1 gene are associated with ND. We first tested the association of SNP markers with ND, the Fagerström Test for ND (FTND) and the number of cigarettes smoked per day (numCIG) in the control subjects of the study of Molecular Genetics of Schizophrenia supported by the Genetic Association Information Network (MGS-GAIN) and MGS-nonGAIN European-American samples. Four representative SNPs were selected to be replicated in selected subjects from the Virginia Adult Twin Study of Psychiatric and Substance Use Disorder (VA-Twins). Of the 4 markers tested, 2 markers (rs2976514 and rs16879814) were significantly associated with ND and FTND score. After correction for multiple testing, one marker (rs2976514) reached experiment wide significance for both phenotypes. The markers were also tested for association in MGS-GAIN African-American subjects. Two markers were found to be significant for ND or FTND, and one marker was marginally significant after correction for multiple testing for both phenotypes. These results suggest that variants in NRG1 are associated with ND.

1003/W

Identifying The Mitochondrial Genetic Contribution To Metabolic Syndrome. *P. Bonnen[1], I. Pe'er[2], M. Stoffel[3], J. Friedman[3], J. Breslow[3], D. Murdock[1].* 1) Baylor College Med, Houston, TX; 2) Columbia University, New York, NY; 3) Rockefeller University, New York, NY.

Metabolic Syndrome (MetS) is the clustering of obesity, diabetes, hypertension, and dyslipidemia in one individual and is thought to affect over 50 million people in the United States. Mitochondrial function is central to energy metabolism. Rare mutations in the mitochondrial genome cause diabetes with deafness and other metabolic phenotypes. We are conducting family-based studies aimed at determining the mitochondrial genetic contribution to MetS and its component disorders. The Micronesian island of Kosrae is a rare genetic isolate that offers significant advantages for genetic studies of human disease. Kosrae suffers from one of the highest rates of MetS (41%), obesity (52%), and diabetes (17%) globally and has a homogeneous environment making this an excellent population in which to study these significant health problems. We have generated mitochondrial genotype and sequence data on over 3,000 individuals and are conducting family-based analyses aimed at identifying specific mitochondrial variants that contribute to obesity and other co-morbid conditions.

1004/W

A mitochondrial polymorphism 12361A>G is associated with non-alcoholic fatty liver disease. *M. Lu[1], J. Huang[1], Y. Liao[1], R. Bai[2], R. Trieu[2], W. Chuang[1], M. Yu[1], S. Juo[1], L. Wong[2].* 1) Kaohsiung Medical University, Kaohsiung, Taiwan; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, USA.

Background Non-alcoholic fatty liver disease (NAFLD) can range from simple hepatic steatosis, nonalcoholic steatohepatitis (NASH) to cirrhosis. Insulin resistance and oxidative stress are responsible for the pathogenesis of NAFLD. Mitochondrial dysfunction is one of the causes of insulin resistance and impairment of oxidative phosphorylation. We hypothesized that mtDNA polymorphisms are associated with the development of NAFLD. **Methods** We conducted a two stage case-control study with 1281 participants to investigate the association between mtDNA variants and NAFLD in a Chinese population. The diagnosis of NAFLD was based on the presence of fatty liver by ultrasound sonography, and exclusion of alcohol abuse and other secondary factors. We initially screened 79 selected mitochondrial single nucleotide polymorphisms (mtSNPs) in 279 subjects (NAFLD n=160; control n=119). The most significant SNP (mt12361A>G) was validated in 1002 additional subjects (NAFLD n=477, control n=525). **Results** In the screening dataset, two mtDNA variants (mt12361A>G and mt709A>G), haplogroups D4 and M8 were found to be associated with the predisposition to NAFLD. In the follow-up dataset, the individuals carrying the mt12361G variant had an odds ratio (OR) of 8.61 for the severe NAFLD group (95% confidence interval [CI] = 2.85~26.05, adjusted p-value=1.4x10-4) and an OR of 4.13 for the moderate NAFLD group (95% CI =1.64~10.38, adjusted p-value=2.6x10-3) compared with those carrying the mt12361A variant. Moreover, the mt12361 G carriers had a 4.19-fold risk for NASH compared to mt12361 A carriers (95% CI=1.12~12.95, p-value=0.031). **Conclusions** Subjects carrying the mt12361G variant had higher risk to develop moderate/severe NAFLD and NASH.

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GENETIC POLYMORPHISMS OF WNT INHIBITORS ARE ASSOCIATED WITH BONE MASS: A GENE-WIDE STUDY. *J.A. Riamcho[1], C. Valero[1], B. Pineda[2], J.L. Hernandez[1], J.M. Olmos[1], A. Cano[2], M.A. Garcia[2], M.T. Zarrabeitia[1].* 1) Hospital U.M.Valdecilla, Santander, Spain; 2) Hospitales Clinico and Pesset, Univ. Valencia, Valencia, Spain.

Wnt agonists stimulate bone formation and have an anabolic effect on bone. Their activity is modulated by a number of extracellular inhibitors, including SFRP3 and sclerostin, the products of FRZB and SOST genes. The objective of this study was to explore the relationship of common allelic variants of these genes with bone mineral density (BMD). Twelve tagging SNPs of the FRZB gene and 7 of the SOST gene were analyzed in 1043 Spanish postmenopausal women over 50 years of age (mean 66±8). Women with present or past diseases or treatments known to influence skeletal homeostasis were excluded. BMD was measured by DXA. Several FRZB polymorphisms and haplotypes were associated with BMD in the discovery cohort, particularly at the spine (p=0.002), but the results were not confirmed in the replication cohort. On the other hand, several polymorphisms located in the 5' region of the SOST gene, within 1.1 kb of the translation start site, were associated with BMD in both the discovery and replication cohorts, in the single locus and the haplotypic analyses. The strongest association was found with the G/C rs851056 polymorphism. The combined standardized weighted mean difference between women homozygotes for the minor allele and those with other genotypes was 0.3 standard deviations (95% confidence interval 0.2-0.5; p<0.0001). Bioinformatic tools suggested that a putative c-Myc binding site was lost in G alleles. In summary, this study confirms that polymorphisms in the 5' region of the SOST gene are associated with BMD in postmenopausal women. We also found an association of some FRZB polymorphisms with BMD, but it could not be replicated in an independent cohort. Therefore, its actual biological relevance is uncertain.

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The genetic polymorphisms of Apelin are associated with obesity phenotypes in Chinese women but not men. *Y.T. Wang[1], Y.C. Liao[2,3], Y.N. Li[1], S.H.H. Juo[1,4].* 1) Department of Medical Genetics, Kaohsiung Medical University, Kaohsiung, Taiwan; 2) Graduate Institute of Medicine, Kaohsiung Medical University, Taiwan; 3) Section of Neurology, Taichung Veterans General Hospital, Taichung, Taiwan; 4) Department of Medical Research, Kaohsiung Medical University Hospital.

Background: Obesity is a risk factor for cardiovascular related diseases and cancers. Adipocytes play an important role in obesity. Recent research on adipocyte biology has revealed that adipocytes produce and secrete a variety of bioactive substances, named ''adipokines''. Apelin is a newly found adipokine and apelin serum levels have been reported to be associated with obesity. We aimed to test whether genetic polymorphisms at the apelin gene is associated with obesity and waist circumference. Methods: We selected four common (MAF > 10%;) tagging SNPs (rs3115757, rs2235310, rs3761581 and rs2235307) and used the TaqMan assay for genotyping. A total of 1041 women and 790 men were recruited in this study. Two obese phenotypes were used: waist circumference (less than 80 cm in women, less than 90 cm in men as Asian criteria in metabolic syndrome), and BMI (BMI < 24 normal, 24-27 overweight, > 27 obesity according to the guideline by the Taiwan government). Results: The mean age is 51.7 (13.0) for women and 53.5 (13.9) for men. Forty-four percent (n=460) of women and 44.6% (n=352) of men had over waist circumference. For women, 51.5% (n=467), 28.0% (n=262) and 19.6% (n=178) were in normal, over-weighted and obese categories, respectively. For men, 36.5% (n=261), 34.1% (n=244) and 29.4% (n=210) were in normal, over-weighted and obese categories, respectively. Both waist circumference and obesity are associated with SNP rs3115757 (p=0.002 and 0.009, respectively, after adjusting for age, DM, hypertension and hyperlipidemia) in women. However, this SNP was not significant in men (p > 0.1). SNP rs2235310 was associated with waist circumference (p=0.007) but not obesity in women. Again, this SNP is not significant in men (p>0.7). Conclusion: Apelin genetic variants can predict the obesity phenotypes in women but not in men. Since this gene is located on X chromosome, we are investigating whether there is a chromosome dose effect.

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Polymorphisms in Heat Shock 70kDa Protein 4 Gene Are Associated with Severity of Sepsis and Acute Lung Injury in African Americans. *N.M. Rafaels[1], L. Gao[1], T. Murray[1,2], R.A. Mathias[1], J.P. Maloney[4], M. Moss[4], G. Martin[5], C. Shanholtz[6], J.G.N. Garcia[7], J. Sevransky[3], T.H. Beaty[2], R. Brower[3], P.H. Hassoun[3], K.C. Barnes[1].* 1) Division of Allergy and Clinical Immunology, Department of Medicine, Johns Hopkins, Baltimore, MD; 2) Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins, Baltimore, MD; 3) Division of Pulmonary and Critical Care Medicine, Department of Medicine, Johns Hopkins, Baltimore, MD; 4) University of Colorado Health Sciences Center, Denver, CO; 5) Emory University School of Medicine, Atlanta, GA; 6) University of Maryland School of Medicine Baltimore, MD; 7) Department of Medicine, Pritzker School of Medicine, University of Chicago, Chicago, IL.

Rationale: Acute lung injury (ALI) and sepsis are significant causes of morbidity and mortality worldwide. Severe sepsis (and sepsis-associated ALI) is characterized by increased inflammation throughout the entire body and an elevated white blood cell count. Short term highly intense physiological stress has been shown to increase levels of heat shock proteins in leukocytes. In this study, we tested the hypothesis that single nucleotide polymorphisms (SNPs) in Heat Shock70kDa Protein, *HSPA4*, are associated with severity (characterized by APACHE II score) in a population of 976 subjects.

Methods: We genotyped 9 *HSPA4* SNPs using the Illumina GoldenGate platform of 1536 SNPs in a primary population of 925 European American patients with severe sepsis (N=326), sepsis-associated ALI (N=297) and 302 control subjects. An independent population of 759 African American subjects (195 severe sepsis patients, 158 ALI patients, and 406 controls) served as the replication group. SNP associations were tested under a logistic regression model using PLINK adaptive permutations model.

Results: Analysis of APACHE II score using 1536 SNPs revealed four *HSPA4* intronic SNPs (rs4705990, rs10075878, rs4367292, and rs4574536) were associated with higher APACHE II scores in African Americans (P= 0.0008, 0.0011, 0.0011, and 0.0097, respectively). When comparing severe sepsis patients to controls, these same 4 SNPs were associated with greater risk of sepsis but the risk allele for APACHE II score was protective against sepsis in African Americans (0.021, 0.037, 0.035, and 0.010, respectively). No associations were found in European Americans, however, allele frequencies between European American and African American controls were quite different: for rs4705990 (AA: 0.29 (C), EA: 0.74 (C)), rs10075878 (AA: 0.28 (A), EA: 0.48 (A)), and rs4367292 (AA: 0.41 (C), EA: 0.72 (C)).

Conclusions: Our findings suggest *HSPA4* is a putative candidate gene for severity of sepsis and sepsis-associated ALI in African Americans but the different frequencies and linkage disequilibrium patterns in the *HSPA4* gene between European American and African American groups warrant further investigation.

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ADHD genes are associated with being born small for gestational age: Results from the Auckland Birthweight Collaborative study. *A.R. Morgan[1,2], J.M.D. Thompson[3], K.E Waldie[4], C.M. Cornforth[5], D. Turic[5], E.J.S. Sonuga-Barke[5], W.J. Lam[1,2], L.R. Ferguson[1,2], E.A. Mitchell[1,2].* 1) Discipline of Nutrition, FMHS, The University of Auckland, New Zealand; 2) Nutrigenomics New Zealand; 3) Department of Paediatrics, FMHS, The University of Auckland, New Zealand; 4) Department of Psychology, The University of Auckland, New Zealand; 5) Institute for Disorders of Impulsivity and Attention, School of Psychology, The University of Southampton.

Children born small for gestational age (SGA) are at increased risk for developing Attention-Deficit Hyperactivity Disorder (ADHD), symptoms of which include inattention, overactivity and impulsivity. Altered neurotransmitter activation in frontal brain structures, accompanied by an imbalance of activity in frontostriatal regions, is thought to characterise this genetically complex disorder. Here we present data from the Auckland Birthweight Collaborative (ABC) Study in which we investigated possible association between ADHD susceptibility genes involved in neurotransmission and being born SGA. After controlling for gestational age, gender, socio-economic status, maternal education, marital status, antenatal class attendance, primiparity, maternal tobacco / marijuana smoking during pregnancy and maternal hypertension, the COMT, HTR2A, SLC1A3, and SLC6A1 genes continued to show significant associations with SGA. Although these have been linked to ADHD in earlier research, this is the first report to date to reveal associations for these genes with SGA.

1009/W

A functional variant within the ubiquitin-associated domain-containing protein 2 gene (UBAC2) is associated with increased risk of Behçet's disease. *A. Sawalha[1], T. Hughes[1], A. Nadig[1], V. Yilmaz[2], K. Aksu[3], G. Keser[3], A. Cefle[4], A. Yazici[4], A. Ergen[5], H. Direskeneli[6], G. Saruhan-Direskeneli[2].* 1) Dept Rheumatology & Arthritis and Immunilogy, OUHSC/VAMC/OMRF, Oklahoma City, OK; 2) Department of Physiology, Istanbul University, Istanbul School of Medicine, Istanbul, Turkey; 3) Department of Rheumatology, Ege University, School of Medicine, Izmir, Turkey; 4) Department of Rheumatology, Kocaeli University, School of Medicine, Kocaeli, Turkey; 5) Ophthalmology Clinic, Okmeydani Research and Education Hospital, Istanbul, Turkey; 6) Department of Rheumatology, Marmara University, School of Medicine, Istanbul, Turkey.

Objectives: Using a genome-wide association scan and DNA pooling, we previously identified 5 novel genetic susceptibility loci for Behçet's disease. Herein, we fine-map the genetic effect within the UBAC2 gene, replicate this genetic association in an independent cohort of Behçet's disease patients and controls, and identify a functional polymorphism in this locus. Methods: Two independent cohorts of Behçet's disease patients and controls from Turkey were studied. The discovery and replication cohorts included 152 patients and 172 controls, and 376 patients and 369 controls, respectively. Genotyping of 14 SNPs within and around UBAC2 was performed using TaqMan SNP genotyping assays. Results: The genetic association between Behçet's disease and UBAC2 was established and confirmed in two independent cohorts of patients and controls (Meta-analysis OR= 2.05, meta-analysis P= 1.75X10-7). Haplotype analysis identified both a disease risk and a protective haplotype (P= 0.00014 and 0.0075, respectively). Using conditional haplotype analysis we identified that the SNP rs7999348 (A/G) within UBAC2 is the most likely SNP to explain the genetic effect in this locus. Indeed, we demonstrate that rs7999348 is a functional SNP that results in increased mRNA expression of a UBAC2 transcript variant in PBMCs of individuals homozygous for the Behçet's disease-associated "G" allele. Conclusion: We establish and confirm the genetic association between UBAC2 and Behçet's disease in two independent cohorts of patients and controls. Fine mapping of this genetic effect and conditional analysis followed by functional studies identified the minor allele in rs7999348 as a disease-risk allele that alters UBAC2 expression.

1010/W

Localization of psoriasis candidate gene product CCHCR1 at centrosomes. *M.H. Tervaniemi[1,2], C. Söderhäll[3], H.A. Siitonen[1,2], L. Samuelsson[5], S. Suomela[4], U. Saarialho-Kere[4], J. Kere[1,2,3], O. Elomaa[1,2].* 1) Department of Medical Genetics, Haartman Institute, University of Helsinki, Helsinki, Finland; 2) Folkhalsan Institute of Genetics, Biomedicum Helsinki, Helsinki, Finland; 3) Department of Biosciences and Nutrition, Karolinska Institutet, Stockholm, Sweden; 4) Department of Dermatology, Helsinki University Central Hospital and University of Helsinki, Helsinki, Finland; 5) Department of Clinical Genetics, Sahlgrenska University Hospital, Gothenburg, Sweden.

Psoriasis is a common dermatological disorder, affecting 2-3 percent in Caucasians. It is characterized by abnormal proliferation, differentiation of keratinocytes and infiltration of inflammatory cells. This multifactorial disease is caused by both genetic and environmental factors. Several candidate gene loci for psoriasis (PSORS1-9) have been identified. *CCHCR1* (coiled-coil alpha-helical rod protein 1) is a plausible candidate gene in the PSORS1 locus (6p21.3) whose role as a psoriasis effector gene is unsettled although it resides in the region of strongest associations even in GWA studies. CCHCR1 has different expression pattern in psoriatic vs. healthy skin and is suggested to have a role in cell proliferation. We have recently cloned a novel variant of CCHCR1 (isoform 1) in which the N-terminal domain is 89 amino acid residues longer than in the previously studied form (isoform 3). In the present study we have genotyped the SNP (rs3130453) that controls the formation of the alternative isoforms using samples from 435 Finnish and Swedish psoriasis families. The allele for the variant 3 shows preferential transmission from heterozygous parents to affected offspring ($p<10^{-5}$). Here we show by immunofluorescent staining that both CCHCR1 isoforms have a centrosomal localization. They co-localize with a centrosome marker γ-tubulin but not with microtubule proteins α- and β-tubulin. However, the localization of CCHCR1 is partially dependent of microtubules indicated by Nocodazole treatment that disrupts the microtubules. After the treatment CCHCR1 can still be observed in association with centrosomes but also as granules in the cytoplasm. Stable over-expression of CCHCR1 in HEK293 cells results in nuclear aberrations, suggesting abnormalities in cell cycle control. As centrosomes are cell organelles that have a role in organization of the microtubules, cell cycle and division, centrosomal localization of CCHCR1 provides a connection to the abnormal proliferation. This novel hypothesis offers a link to the cellular pathways that are altered in psoriasis.

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Association of Calpain 10 (CAPN10) gene polymorphisms with Type 2 Diabetes (T2D) in the Population of Punjab. *K. Matharoo, I.S. Sidhu, R. Sharma, A.J.S. Bhanwer.* Department of Human Genetics, Guru Nanak Dev University, Amritsar, Punjab, India.

The polymorphisms of Calpain 10 (CAPN 10)gene has been associated with T2D. Calpain10 is a member of a large family of intracellular proteases and resides in the 2q37.3 region. Variation in the calcium-activated neutral protease 10 gene (CAPN 10) was identified as a possible T2D susceptibility gene through positional cloning in Mexican-Americans. However, large differences were shown between ethnic groups at risk alleles and haplotypes as well as in their attributable risk. There is dearth of data pertaining to association of Calpain10 risk alleles with T2D in the North Indian Punjabi population, which is experiencing a considerable increase in the prevalence of T2D due to economic shift. Thus, it is important to determine the role of calpain 10 in the North Indian Punjabi population. We examined three SNPs in the Calpain10 gene (-43, -19, and -63) in case control cohort comprising 200 T2D cases and 200 age and sex matched controls from Punjabi population. Genomic DNA was extracted from peripheral blood (obtained with informed consent) by phenol-chloroform method. Genotyping for SNP19 (insertion/deletion polymorphism) was done PCR followed by detection on agarose gel while SNP43 and 63 were genotyped by PCR-RFLP method. The genotype and allele frequency distribution did not reveal any statistically significant difference for SNP19 (p=0.653, 0.357, respectively) and SNP 43 (p=0.507, 0.301). For SNP63, a significant association of genotypes was observed (p=0.009). However, no significant association could be documented after correction for age, sex and BMI using linear regression in CC vs CT&TT [p=0.295; 1.45(0.72-2.93)] and comparison of CT with CC&TT [p= 0.11; 0.59(0.31-1.12)]. Further analysis in males and females revealed a significant association in genotype distribution between cases and controls in males for SNP63 (p=0.000). After correction for age, BMI and WHR a protective association of CT vs. CC+TT was observed against T2D in males [p=0.002; 0.22(0.08-0.57)]. However, no association was observed in females. Haplotype analysis revealed highest frequency of 112/112 (0.229) followed by 111/111(0.217). Conversely, none of the haplotype combinations were found to be associated with T2D. The results of the present study suggest a sex specific protective association of SNP63 genotypes with T2D. However, analysis on a larger number of samples is required to determine the association of Haplotype at the CAPN 10 locus.

1012/W

Variants in Dynamin Binding Protein Increase Risk of Glaucoma. *M. Hauser[1], Y. Liu[1], X.-J. Qin[1], M. Ulmer[1], B.-J. Fan[3], J.-D. Ding[2], L. Pasquale[3], C. Bowes Rickman[2], J. Wiggs[3], E. Oh[4], N. Katsanis[4], S. Schmidt[1], R. Rand Allingham[2].* 1) Ctr Human Gen, Duke Univ Med Ctr, Durham, NC; 2) Department of Ophthalmology, Duke Univ Med Ctr, Durham, NC; 3) Department of Ophthalmology, Harvard Med Sch, Boston MA; 4) Department of Cell Biology, Duke Univ Med Ctr, Durham, NC.

Genes identified to date account for only a small proportion of primary open angle glaucoma (POAG). We report here the identification of a C1413W coding variant of dynamin binding protein (DNMBP) that confers risk of POAG (OR=1.26; 95%CI=1.07-1.47). Evidence for association increases in a subset of patients characterized by onset of disease prior to age 53 (OR= 1.61; 95%CI=1.28-2.02), and increased intraocular pressure as compared to other cases (p=0.0002). DNMBP is a multifunctional docking protein that could modulate glaucoma risk by affecting vesicle transport, cell signaling, axon and dendrite growth and cell motility in the neural retina and the trabecular meshwork. The C1413W variant conveys a population attributable risk percentage of 17%, the largest genetic effect in POAG described to date. A knockdown of DNMBP in primary cultures of murine neonatal cortical neurons induces statistically significant neurite extension and arborization phenotypes, making it likely that allele-specific disruptions of DNMBP activity could be resonsible for glaucoma-related neuropathology.

1013/W

Type 2 Diabetes (T2D) Associated SNPs Regulate Expression of Adjacent Transcripts in Subcutaneous Adipose Tissue and Muscle from Caucasian and African American Subjects. *S.K. Das, N.K. Sharma, K.A. Langberg, A.K. Mondal, S.C. Elbein.* Internal Medicine/Endocrinology, Wake Forest University Health Sciences, Winston-Salem, NC.

Published genome wide association scans have identified regions with single nucleotide polymorphisms (SNPs) that increase T2D susceptibility, alter glucose homeostasis, or both. Because most variants are noncoding and of unknown function, we hypothesized that these SNPs act by modulating the transcription of nearby genes in adipose and muscle. To address this hypothesis, we tested the association of these SNPs with the expression of their nearest transcripts in subcutaneous (sc) adipose and muscle from individuals who spanned a broad range of insulin sensitivity (SI) and body mass index (BMI). We successfully tested association of 22 SNPs with 21 transcripts in sc. adipose from 153 individuals [13 T2D, 140 nondiabetic; 106 Caucasian (C), 47 African American (AA)] and 17 SNPs with 15 transcripts in 168 individuals [13 T2D, 155 nondiabetic; 122 C, 46 AA]. Expression values from qRT-PCR were normalized with 18S control and log transformed for analysis. Association was tested with or without adjustment for age, sex, BMI and ethnicity. *SYN2* expression in adipose was associated with rs17036101 in nondiabetic C, with lower expression among homozygous carriers of the risk allele (p_{adj}= 0.001). *DNAJC11* expression in adipose was similarly reduced in C in rare homozygotes for rs1043681 (p< 0.05), as was *TSPAN8* with rs7961581 (p< 0.05). *NOTCH4* expression was strongly associated in sc adipose from all individuals and C, with lowest expression in the rare allele homozygotes (p= 0.0003, p_{adj}= 0.001). Nominal associations were also seen for rs906216 and *HK1* expression (p<0.05). No association was observed with nearby SNPs for genes for *ADAMTS9, CAMK1D, CDC123, CDKAL1, FTO, HHEX, IDE, JAZF1, NOTCH2, PPARG, SMARCAD1, TCF7L2, THADA, VEGFA*, or *WFS1* in adipose. *PKN2* transcript levels were associated with rs6698181 in both adipose (p= 0.009, p_{adj}= 0.004) in C and muscle (p= 0.00004, p_{adj}= 0.001) in all subjects with reduced expression in risk allele homozygotes. Nominal associations of SNPs with muscle transcript levels were also seen for rs864745 and *JAZF1* in all subjects (p= 0.02); for rs7578597 and *THADA* in C (p= 0.05); and rs10010131 and *WFS1* in C (p= 0.05). We show that a subset of T2D associated SNPs likely increase T2D susceptibility by directly or indirectly modulating expression of neighboring transcripts. Our studies in diabetes-relevant tissues provide the basis for mapping the functional variants and determining the role of these genes in adipose and muscle.

1014/W

Sex Hormone Binding Globulin (*SHBG*) $(TAAAA)_n$ polymorphism is associated with SHBG levels and measures of fetal size in a Hispanic cohort. *C. Ackerman[1], M.G. Hayes[1], L.P. Lowe[2], H. Lee[1], A.R. Dyer[2], B.E. Metzger[1], W.L. Lowe[1], M. Urbanek[1], HAPO Study Cooperative Research Group.* 1) Division of Endocrinology, Dept of Medicine, Northwestern University, Chicago, IL; 2) Dept of Preventive Medicine, Northwestern University, Chicago, IL.

Sex hormone-binding globulin (SHBG) is a glycoprotein that binds sex hormones and regulates their biological activity. Low circulating levels of SHBG are associated with altered glucose levels and an increased risk of type 2 diabetes (T2D). Given the known impact of maternal glycemia and fetal insulin production on fetal size at birth, we hypothesized that genetic variation in *SHBG* may impact circulating SHBG levels and subsequent size at birth.

The $(TAAAA)_n$ repeat in the *SHBG* promoter affects transcription of the gene and serum SHBG levels. To determine whether the $(TAAAA)_n$ repeat is associated with fetal size, we genotyped 3422 Caucasian babies, 2066 Thai babies, 1203 Afro-Caribbean babies, and 768 Hispanic babies at the *SHBG* $(TAAAA)_n$ repeat in the multi-ethnic Hyperglycemia and Adverse Pregnancy Outcome (HAPO) cohort, and tested for associations between *SHBG* $(TAAAA)_n$ genotype and fetal traits (birth weight, birth length, head circumference, sum of skinfolds, fat mass, percent bodyfat, cord C-peptide, and neonatal 2-hr plasma glucose). Associations were assessed through linear regressions with single outcomes under an additive model and adjusted for covariates. We found evidence for associations between *SHBG* allele 7 (A7, 7 TAAAA repeats) and measures of fetal growth and adiposity in the Hispanic population. Specifically in the Hispanic male babies, after adjusting for covariates, *SHBG* A7 was negatively associated with birth weight (β= -72.23g per allele, *P*=0.02), head circumference (β= -0.22cm, *P*=0.02), log-transformed sum of skinfolds (3.92% decrease in sum of skinfolds per allele, *P*=0.01), fat mass (β= -27.1g, *P*=0.03), and percent body fat (β= -0.57%, *P*=0.001). Trends in the same direction were also observed in the Hispanic females and Caucasian males, but did not reach significance.

To determine if *SHBG* $(TAAAA)_n$ genotype had an impact on SHBG levels, we assayed 255 Hispanic babies for serum SHBG. We tested for associations between *SHBG* $(TAAAA)_n$ repeat length and SHBG levels. We found significant negative associations between average *SHBG* $(TAAAA)_n$ repeat length and log-transformed SHBG levels (4.88% decrease in SHBG level per repeat, *P*=0.003) in male babies, and similar but nonsignificant trends in the females. Our results indicate that genetic variation at the *SHBG* promoter $(TAAAA)_n$ repeat is inversely correlated with circulating SHBG levels and fetal size at birth.

1015/W

Genetic basis of Common Human Disease: Insight into the role of non-synonymous SNPs from Genome Wide Association Studies. *L.R. Pal[1], J. Moult[1,2].* 1) Institute for Bioscience and Biotechnology Research, University System of Maryland, Rockville, MD; 2) Department of Cell Biology and molecular Genetics, University of Maryland at College Park.

Recent genome wide association studies have led to the reliable identification of multiple common SNPs associated with increased incidence of a number of common human diseases. These results offer the tantalizing prospect of major new insights into disease mechanisms. A variety of mechanisms may link the presence of a SNP to altered in vivo gene product function and hence contribute to disease risk. We have explored the role of one of these mechanisms, non-synonymous SNPs in proteins, for associations found in the Wellcome Trust Case Control Consortium (WTCCC) seven disease study and follow-up work. Using Hapmap data, we first identified all non-synonymous SNPs in linkage disequilibrium with those associated with increased disease risk. We then applied two computational methods to determine which of these SNPs has a significant deleterious effect on in vivo protein function (SNPs3D, http://www.snps3d.org). A number of these disease loci are found to be linked to one or more deleterious non-synonymous SNPs. In some cases, these SNPs are in well known proteins, such as HLAs. In others, they are in less well established disease genes (for example, MST1 for Crohn's disease) and in still others, they are in so far little investigated proteins (for example gasdermin B, also for Crohn's disease). Together, these data suggest a very significant role for this class of SNPs in common human disease susceptibility.

1016/W

A 3'UTR Variant Is Associated with Impaired Expression of CD226 in T and NK T Cells and Susceptibility to Systemic Lupus Erythematosus. *S.E. Löfgren[1], A.M. Delgado-Vega[1], C.J. Gallant[1], E. Sanchez[2], J. Frostegård[3], L. Truedsson[4], E. de Ramón[5], J.M. Sabio[6], M.F. González-Escribano[7], B.A. Pons-Estel[8], S. D'Alfonso[9], T. Witte[10], B.R. Lauwerys[11], E. Endreffy[12], L. Kovács[13], B.M. da Silva[15], C. Vasconcelos[14], J. Martin[2], M.E. Alarcón-Riquelme[1,2,16,17], S.V. Kozyrev[1].* 1) Inst Genetics and Pathology, Uppsala University, Uppsala, Sweden; 2) Instituto de Biomedicina y Parasitología López-Neyra, CSIC, 18100, Granada, Spain; 3) Department of Medicine, Karolinska University Hospital, Huddinge, 14183, Stockholm, Sweden; 4) Department of Laboratory Medicine, section of M.I.G., Lund University, 221 00, Lund, Sweden; 5) Hospital Carlos Haya, 29009, Málaga, Spain; 6) Hospital Virgen de las Nieves, 18001, Granada, Spain; 7) Hospital Virgen del Rocío, 41013, Sevilla, Spain; 8) Department of Rheumatology, Sanatorio Parque, Rosario, 2000, Argentina; 9) Department of Medical Sciences and IRCAD, University of Eastern Piedmont, 28100, Novara, Italy;; 10) Hannover Medical School, 30625, Hannover, Germany; 11) Cliniques Universitaires Saint-Luc, Université catholique de Louvain, B-1200, Bruxells, Belgium; 12) Department of Pediatrics and Health Center,University of Szeged, H-6721, Szeged, Hungary; 13) Department of Rheumatology, Albert Szent-Györgyi Clinical Centre, University of Szeged, H-6725, Szeged, Hungary; 14) Hospital Santo Antonio and ICBAS, 4099, Porto, Portugal; 15) UMIB/ICBAS, Immunogenetics laboratory, Universidado do Porto, 4099-003, Porto, Portugal; 16) Arthritis and Immunology Program, Oklahoma Medical Research Foudation, Oklahoma, 73104, Oklahoma City, OK, USA; 17) Andalucian Center for Genomics and Oncological Research Pfizer-University of Granada-Junta de Andalucia, 18100, Armilla, Granada, Spain.

Objective. CD226 has recently been associated with a number of autoimmune diseases with the SNP rs763361 been proposed as the putative causative variant. We here report a fine mapping of the gene locus and the genetic association of several SNPs with systemic lupus erythematosus in Europeans, together with functional analyses to give a better understanding on the mechanisms behind the gene association and potential contribution of these variants. Methods. Genetic association of 12 SNPs in the CD226 gene was conducted using 1194 SLE patients and 1454 healthy controls from a European multicenter collection. Genotyping was performed using a GoldenGate Custom Genotyping Assay and a BeadXpress Reader from Illumina or Taqman assay and the statistical association analyzed with PLINK v1.07. Gene expression was analyzed by quantitative real-time PCR and SYBR Green for signal detection, using total RNA purified from PBMCs from healthy donors. Surface detection of the protein was performed by a three-color flow cytometry using leukocytes isolated from healthy donors, where total lymphocytes, CD3+CD4+, CD3+CD8+, CD3-CD56+, CD3-CD19+ and CD3+CD56+ cells were analyzed. Expression analysis of reporter plasmids including different alleles of the risk haplotypes of the CD226 3'-UTR region was assessed by transfection of HEK293 cells and dual luciferase assay. Results. A risk haplotype ($P = 3.96 \times 10^{-6}$) was detected in the 3'-UTR region of the gene, and revealed rs727088 as the strongest associated variant ($P_{CMH\text{-}adjusted} = 0.0098$). Expression analysis showed that the risk haplotype correlated with decreased levels of CD226 transcripts and protein levels in the surface of T helper cells (CD4+), Cytotoxic T cells (CD8+) and NK T cells (CD3+CD56+), but not NK cells (CD3-CD56+) or B cells (CD19+). Luciferase assays suggest that rs727088 is the main polymorphism responsible for altered gene expression. Conclusion. Our data does not support Gly307Ser as main functional variant within CD226 gene and indicates rs727088 located in the 3'UTR region as the potential causative SNP, by a mechanism that alters protein expression in T cells, potentially involving mRNA processing and/or stability.

1017/W

The evaluation of a loss-of-function GBA variant found in patients with Parkinson disease. *N. Tayebi, Y. Blech-Hermoni, JH. Choi, W. Westbroek, BK. Stubblefield, E. Sidransky.* Section on Molecular Neurogenetics, Medical Genetics Branch/NHGRI, NIH, Bethesda, MD.

Recent studies demonstrate an increased frequency of mutations in the gene encoding glucocerebrosidase (GBA), the enzyme deficient in Gaucher disease, among patients with Parkinson disease. One particular GBA mutation, c.84dupG, a founder mutation in the Ashkenazi Jewish population, was found with a frequency of 2.1% among Ashkenazi patients with Parkinson disease as compared to 0.27% in controls. In this mutant allele, the insertion of a G introduces a frameshift in the signal peptide sequence of glucocerebrosidase, causing a complete deficiency of the enzyme and generating a STOP codon ,TAA, 25 amino acids downstream from the insertion. The mutation has never been encountered in the homozygosus state and is presumed to be lethal. We evaluated the consequences and potential toxicity resulting from this alteration. RNA was extracted from fibroblasts from patients heterozygous for c.84dupG and controls. An RNA protection assay, RT-PCR using different primer sets, and subcloning followed by sequencing of each allele were performed, excluding an alternative exonic splice site. In vitro translation assays indicated no translation of the predicted 25 amino acid peptide. The small peptide was synthesized and an antibody was generated. Using the conjugated peptide as a positive control, the small peptide as not detected in Western blots of fibroblasts from patients with the c.84dupG allele. Moreover, the peptide was not seen in these fibroblasts on confocal microscopy. No morphological changes were observed in Cos-7 and CHO cell lines transfected with a pcDNA3.1 GBA construct expressing c.84dupG, excluding a toxic effect of the peptide. These studies indicate that c.84dupG is a loss-of- function mutation, implicating the deficiency of enzymatic activity in Parkinsonian patients carrying this mutation.

1018/W

A functional polymorphism at 3' UTR of the PAX6 gene confers risk for extreme myopia in Chinese. *E. Hsi[1,2], C. Liang[3], K. Chen[4], Y. Pan[4], Y. Wang[4], S. Juo[2,4].* 1) Dept Med, Kaohsiung Med Univ, Kaohsiung, Taiwan; 2) Dept Med Research, Kaohsiung Med Univ, Kaohsiung, Taiwan; 3) Bright-Eyes Clinic, Kaohsiung, Taiwan; 4) Dept Med Genetics, Kaohsiung Med Univ, Kaohsiung, Taiwan.

Purpose: The *paired box 6* (*PAX6*) is involved in eye development and associated with several ocular diseases. Conflicting results were reported regarding the association between *PAX6* polymorphism and myopia. We conducted a case-control study and functional assay, and identified a functional risk polymorphism for myopia. **Methods:** We enrolled 1083 cases (£ -6.0 D) and 1096 controls (≥ -1.5 D) from a Chinese population residing in Taiwan. Four common tagging single nucleotide polymorphisms (SNPs) and a SNP at the 3' untranslated region (UTR) were selected. The chi-square test was performed to assess the genetic effect and permutation was used to adjust for multiple testing. Luciferase reporter assay was conducted for the 3' UTR SNP to assess the allelic effect on gene expression. **Results:** SNPs rs644xxx and rs622xxx had marginal significance and further analyses showed that these SNPs were associated with extreme myopia (£ -11 D). The OR for extreme myopia was 2.1 (nominal p=0.012; empirical p=0.008) for the CC genotype at SNP rs662xxx at 3' UTR. Functional assay for SNP rs662xxx demonstrated that the C allele had a significantly lower expression level than the T allele (P=0.0001). SNP rs662xxx was predicted to be located in the microRNA-328 binding site and this mechanism may explain the differential allelic effect on gene expression. **Conclusion:** We conducted a large study to identify a functional SNP located at the 3' UTR influencing the risk for extreme myopia. The functional assay suggested the risk allele can reduce *PAX6* protein level leading to a high risk for myopia.

1019/W

Inhibition of the Angiotensin Pathway Increases Bone Mass by Reducing Osteoclastogenesis. *S. Chen, T. Sibai, T. Yang, J. Tao, D. Napierala, B. Dawson, J. Black, Y. Chen, M. Jiang, B. Lee.* Howard Hughes Medical Institute and Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Hypertension and osteoporosis are common diseases affecting an increasingly aging population. The angiotensin pathway plays a central role in controlling blood pressure and is an important target for anti-hypertension drugs. Interestingly, through epidemiological studies in human as well as animal experiments, it has been found that the blockage of this pathway is associated with reduced risk of fractures, while activation of this signaling leads to bone loss. However, the mechanistic basis is largely unknown. Our aim in this study is to understand the effects of inhibiting the angiotensin pathway on bone homeostasis using the mouse model. We administered Losartan, an angiotensin II type I receptor antagonist in both wild-type (WT) and the ovariectomized (OVX) mice. After 6 weeks, Losartan-treated WT mice showed a significant increase in femoral cortical thickness, increased in trabecular bone volume/total bone volume (BV/TV), trabecular thickness, and trabecular number, and corresponding decrease of trabecular separation. Interestingly, we observed that osteoclast numbers were significantly decreased whereas osteoblast numbers did not change. Similarly, OVX mice treated with Losartan showed a significant improvement in trabecular BV/TV, trabecular thickness and bone mineral density. In conclusion, the inhibition of Angiotensin signaling by Losartan shows beneficial effects on bone mass via reduction of bone resportion secondary to a decrease in osteoclast numbers. These data suggest that use of angiotensin receptor antagonists may have added beneficial effects beyond treatment of hypertension.

1020/W

Association study of MMP 13 gene polymorphisms and Kawasaki disease in Taiwanese children. *H. Chi[1,5,6], LY. Chang[2], FY. Huang[1,4], MR. Chen[1,6], NC. Chiu[1,6], HC. Lee[1,4], SP. Lin[1,3,6,7], WF. Chen[3], CL. Lin[3], HW. Chang[3], HF. Liu[3], LM. Huang[2,5], YJ. Lee[1,3,4].* 1) Pediatrics, Mackay Memorial Hosp, Taipei, Taiwan; 2) Pediatrics, National Taiwan University Hospital, Taipei, Taiwan; 3) Medical Research, Mackay Memorial Hospital, Tamshui, Taipei, Taiwan; 4) Pediatrics, Taipei Medical University, Taipei, Taiwan; 5) Graduate Institute of Clinical Medicine, National Taiwan University College of Medicine, Taipei, Taiwan; 6) Mackay Medicine, Nursing and Management College, Taipei, Taiwan; 7) Department of Infant and Child Care, National Taipei College of Nursing, Taipei, Taiwan.

Introduction Kawasaki disease (KD) is a systemic vasculitis. Coronary artery lesions (CALs) complicate 15%-25% of cases of untreated KD. Matrix metalloproteinases (MMPs) have been considered to play pathophysiologic roles of CALs and the MMP-13 gene appears to be a susceptibility gene to the development of CALs in patients with KD in Japanese children. The aim of this study was to investigate if the single-nucleotide polymorphism (SNP) rs2252070 (A>G) and rs675392 (C>G) of the MMP 13 promoter gene is associated with susceptibility to KD or with CALs in Taiwanese children. Materials and Methods All patients with KD were from the Department of Pediatrics at Mackay Memorial Hospital and National Taiwan University Hospital, Taipei, Taiwan. A total of 170 unrelated Taiwanese children (101 boys and 69 girls) with KD were included, 66 of whom had CALs. Mean age at diagnosis was 1.8 ± 1.6 (0.1 - 7.4) years. rs2252070 and rs675392 were genotyped in children with KD and 344 ethnically matched healthy controls using the TaqMan Allelic Discrimination Assay. Results The genotype frequencies of the controls and patients were in Hardy-Weinberg equilibrium (P>0.05). The frequencies of the AA, AG, and GG genotypes for the rs2252070 were 24.1%;, 48.2%; and 27.6%; in the KD group, while they were 25.3%;, 46.5%; and 28.2%; in the control subjects, respectively. No significant differences in genotype, allele, and carrier frequencies of the SNP rs2252070 were found between healthy controls and children with KD (P=0.93, P=0.92 and P=0.95, respectively) or those with CALs (P=0.63, P= 0.64 and P=0.78, respectively). The frequencies of the CC, CG, and GG genotypes for the rs675392 were 40.0%;, 48.8%; and 11.2%; in the KD group, while they were 40.4%;, 43.3%; and 16.3%; in the control subjects, respectively. No significant differences in genotype, allele, and carrier frequencies of the SNP rs675392 were found between healthy controls and children with KD (P=0.25, P=0.46 and P=0.74, respectively) or those with CALs (P=0.72, P=0.99 and P=0.96, respectively). Three haplotypes with frequencies of more than 0.10 were estimated and accounted for about 99% of all haplotypes in both KD patients and controls. Discussion Despite the association of MMP13 gene polymorphism and KD with CALs in Japanese children, we did not find a similar association in our series. A more extensive array of SNPs may need to be assessed to find such an association.

1021/W

NXF/ARNT2/SIM2, RET expression regulation and specific HSCR associated DNA variants. *Y. Sribudiani[1], M. Metzger[2, 3], J. Osinga[1], A. Rey[1], A.J. Burns[2], N. Thapar[2], R.M.W. Hofstra[1].* 1) Genetics, University Medical Center Groningen, Groningen, Groningen, Netherlands; 2) Gastroenterology and Neural Development Units, Institute of Child Health, University College London, London, United Kingdom; 3) Translational Centre for Regenerative Medicine, University of Leipzig, Philipp-Rosenthal-Strasse 55, 04103 Leipzig, Germany.

Two non-coding RET variations, the T allele of SNP rs2435357 (Enh1-T) and the A allele of SNP rs2506004 (Enh2-A), are strongly associated with Hirschsprung (HSCR) susceptibility. These SNPs are in strong linkage disequilibrium and located in an enhancer element in intron 1 of the RET gene. For Enh1 it has been shown that the disease-associated T allele results in reduced expression in Luciferase experiments, via reduced SOX10 binding, compared to the non-disease associated C allele. We aimed to determine whether Enh2-A is also a functional variant by generating reporter constructs containing both alleles, separately or in combinations, coupled to the Luciferase gene. Luciferase assays showed that not only the Enh1-T allele but also the Enh2-A allele decreased Luciferase expression, thus both SNPs can contribute independently to HSCR development. Our results show that more than one SNP on an associated haplotype might influence disease development. MatsInspector software identified the sequences of Enh2-C (non-disease associated variant) and its surroundings sequences (-ACGTG-) as a potential binding site for the heterodimer transcription activator NXF/ARNT2, and the heterodimer transcription repressors SIM2/ARNT2. The binding affinity of NXF/ARNT2 to Enh2-C was confirmed by Electrophoresis Mobility Shift Assays and Supershift. Transfections of NXF/ARNT2 or SIM2/ARNT2 into neuroblastoma cell lines increase and decrease RET expression, respectively. Therefore, these data strongly indicated an involvement of NXF, ARNT2 and SIM2 in the development of HSCR through RET expression regulation. Furthermore, as SIM2 is located on chromosome 21, SIM2 overrepresentation might explain the high occurrence of HSCR in Down syndrome patients.

1022/W

Functional screening of genes at loci associated with type 2 diabetes using glucose-stimulated insulin secretion assays. *J.R. Kulzer, M.P. Fogarty, K.L. Mohlke.* Department of Genetics, UNC Chapel Hill, Chapel Hill, NC.

Genome-wide association (GWA) studies have identified at least 39 genetic loci associated with type 2 diabetes (T2D), but for almost all of them, the biological mechanisms relating the DNA variants to T2D remain to be determined. We hypothesize that some of these loci contain genes that influence glucose homeostasis by altering glucose-stimulated insulin secretion (GSIS) in pancreatic beta cells. To identify these genes, we are screening plausible positional candidates at GWA loci by decreasing gene expression in cultured cells and evaluating the effect on GSIS. Positional candidate genes were selected for study based on evidence of SNP association with T2D or fasting glucose levels, relative level of expression in pancreas or islets, biological function based on literature review, and gene density at a locus. We used siRNA to reduce gene expression in the rat insulinoma-derived cell line 832/13, incubated the cells in basal (3 mM) or high (15 mM) levels of glucose, and measured the amount of insulin secreted after two hours. Insulin levels were quantified by ELISA and normalized to total protein, and transcript levels were measured by quantitative real time PCR (qPCR). We tested positional candidate genes *ARAP1* and *PDE2A* with this approach. Both genes are located at the same human T2D association locus. Biologically, ARAP1 associates with the Golgi apparatus and plays a role in cell receptor trafficking and signaling. PDE2A is a phosphodiesterase that removes phosphate groups from cAMP and cGMP to degrade these signaling molecules within the cell. Transfection with siRNAs targeting *Arap1* and *Pde2a* reduced gene expression by 38% and 65%, respectively. Insulin response to high glucose after siRNA knockdown was compared to the 4.4-fold response observed with a control siRNA that does not target any known genes. Preliminary results showed that decreased *Arap1* gene expression reduced insulin response to 1.6-fold (P=0.07), while decreased *Pde2a* gene expression reduced insulin response to 3.2-fold (P=0.34). If these results are verified, we may be able to conclude that differential *Arap1* expression influences GSIS, suggesting *ARAP1* as the more likely T2D susceptibility gene at this locus. Identification of additional genes that affect GSIS will improve our understanding of glucose metabolism and regulation, and may lead to the discovery of novel biochemical pathways that can be targeted for the development of new T2D therapies.

1023/W

A Functional Haplotype in *EIF2AK3*, an ER Stress Sensor, is Associated with Lower Bone Mineral Density. *J. Liu, N. Hoppman-Chaney, J.C. McLenithan, D.J. McBride, J.R. O'Connell, H. Wang, E.A. Streeten, B.D. Mitchell, A.R. Shuldiner.* Division of Endocrinology, Diabetes and Nutrition, University of Maryland School of Medicine, Baltimore, MD.

EIF2AK3 is a type I transmembrane protein that functions as an endoplasmic reticulum (ER) stress sensor to regulate global protein synthesis. Rare mutations in *EIF2AK3* cause Wolcott-Rallison syndrome (OMIM 226980), an autosomal recessive disorder characterized by diabetes, epiphyseal dysplasia, osteoporosis, and growth retardation. To investigate the role of common genetic variation in *EIF2AK3* as a determinant of bone mineral density (BMD) and osteoporosis, we genotyped 6 common SNPs in this gene in 997 Amish subjects and performed association analysis. We found that the minor allele A of nonsynonymous SNP rs13045 was associated with decreased forearm BMD (P=0.036 and β=-0.007) in the Amish. This association was replicated in 887 Mexican American subjects (P=0.031 and β=-0.008). Rs13045 was in high linkage disequilibrium (r^2 > 0.98) with three other potentially functional SNPs, including one promoter SNP (rs6547787) and two nonsynonymous SNPs (rs867529 and rs1805165), forming two haplotypes (denoted low-BMD associated haplotype A (MAF = 0.319) and common haplotype B). Allele-specific expression of mRNA from lymphoblastoid cell lines did not detect any evidence for differential expression between the two haplotypes. However, lymphoblastoid cell lines from individuals with haplotype A showed increased sensitivity to ER stress induced by thapsigargin treatment (P=0.014), compared to cell lines with haplotype B. Our results suggest that common nonsynonymous sequence variants in *EIF2AK3* have a modest effect on ER stress response and may contribute to risk for low BMD through this mechanism.

1024/W

High temperature requirement factor A1 is a potential disease-causing gene for age-related macular degeneration: evidences from the protective effect of a variant. *TszKin. NG, ChiPui. PANG.* Department of Opthalmology & Visual Sciences, The Chinese University of Hong Kong, Hong Kong, Hong Kong, Hong Kong.

Age-related macular degeneration (AMD) is a leading cause of severe visual impairment and blindness in most developed countries, affecting about 50 million elderly worldwide. Chromosome 10q26 has long been suggested to be an AMD-associated locus; however, the disease-causing gene (*LOC387715* or *HTRA1*) has yet to be identified. A recent study suggested that the *LOC387715* gene might not be a potential disease-causing gene. In this study, the genotyping of a previously described *HTRA1* insertion-deletion (indel) variant (34delCinsTCCT) was replicated, and the characteristics of the HtrA1 variant were also investigated. It was confirmed that the prevalence of 34delCinsTCCT indel allele was higher in control subjects than in exudative AMD patients (p = 0.002), indicating a protective effect. This protective effect was validated by the differential haplotypes of the 34delCinsTCCT indel allele with the risk A allele of rs11200638. This variant was predicted to have a serine amino acid insertion in and influence the recognition of signal peptide. Compared to recombinant wildtype HtrA1, the variant protein was more ER localized and its secretion was deterred. Moreover, the cell viability was higher and the cell apoptosis was lower in ARPE-19 cells expressing HtrA1 variant than wildtype protein. This confirmed the protective effect of HtrA1 variant and suggests that *HTRA1* is a potential disease-causing gene for AMD in the 10q26 region.

1025/T

Genome-wide association study for age-related hearing impairment. *R.A. Friedman[1], J. Ohmen[1], C. White[1], J. Corneveaux[2], A. Allen[2], S. Bonneux[3], E. Fransen[3], G. VanCamp[3], M. Huentelman[2], European Age-related Hearing Impairment (ARHI) project.* 1) Cell Biology and Genetics, House Ear Institute 2100 West Third Street Los Angeles, CA 90057; 2) Neurogenomics Division The Translational Genomics Research Institute (TGen), 445 N 5th Street, Suite 600 Phoenix, AZ 85004; 3) Medical Genetics Centre Dept. of Biomedical Sciences University of Antwerp Universiteitsplein 1 2610 Antwerp Belgium.

Age-Related Hearing Impairment (ARHI), or presbycusis, is the most prevalent sensory impairment in the elderly. ARHI is a complex disease caused by an interaction between environmental and genetic factors. While the environmental factors conferring altered risk for ARHI have been extensively studied, investigations into the genetic risk factors have only recently been initiated. Here we describe the results of the first whole genome association study for ARHI using individual samples. Our previous GWAS for this disease with pooled DNA samples resulted in the identification of a highly significant and replicated SNP located in GRM7, the gene encoding the metabotropic glutamate receptor type 7 (mGluR7) protein. The current study used a two phase study design. Phase 1 consists of a cohort with over 1100 samples assayed by whole-genome genotyping. No single SNP was associated with ARHI after correcting for multiple comparisons. There were several genes that are attractive candidates that await confirmation in phase II. The results of phase I and current progress will be presented.

1026/T

Functional Linear Model in Inferring the Relationship between a set of Variants and Quantitative Phenotype. *Y. Zhu, L. Luo, M. Xiong.* University of Texas School of Public Health, Houston, TX.

To better understand the path from genomic information through intermediate traits, such as lipid levels, to the disease outcome, we need to identify DNA variants which predict variation in the intermediate quantitative traits. The traditional quantitative genetic analysis has mainly focused on investigation of a single variant one at a time. While the genetic effect for each single variant may be too small to be detected, we seek to find a method for testing a region of DNA variants. The multiple linear regression model might have higher power to detect a quantitative traits than simple linear regression model since it jointly uses multiple marker information. However, as the number of marker increases, the degree of freedom of the test statistics will also increase. To reduce the degrees of freedom in the model and variances of the estimators due to presence of rare variants in the model, we developed a functional linear model for a quantitative trait. Its type 1 error rates were not appreciably different from the nominal levels by simulation studies. The proposed statistic was applied to Framingham Heart Study (FHS). We observed that 20% of the genes associated with glucose and 15% of the genes significantly associated with TG contain no single significant SNPs by conventional methods. For example, the P-values of the best associated with glucose in the genes TMEM16C, TULP4, SMPD3 and CNTLN, and the P-values of the best SNPs associated with TG in the genes RUOS, GRK4, and PRAGMIN were 1.52×10-3, 4.57×10-4, 5.80×10-5, 2.76×10-5, 5.38×10-3, 4.29×10-3 and 3.08×10-4, respectively, but the overall P-values of these genes were 1.20×10-10,1.17×10-12, 5.62×10-9, 6.66×10-16, <1.00×10-17, <1.00×10-17 and 2.37×10-7. These examples demonstrate that Functional Linear Model is powerful in inferring the relationship between a set of SNPs and quantitative phenotype.

1027/T

Mutation screen of significant GWAS signal implicates *ARHGAP29* in nonsyndromic cleft lip and/or palate. *E.J. Leslie[1], M.A. Mansilla[1], M.L. Marazita[2], T.H. Beaty[3], J.C. Murray[1].* 1) Department of Pediatrics, University of Iowa, Iowa City, IA; 2) University of Pittsburgh, School of Dental Medicine, Pittsburgh, PA; 3) Johns Hopkins University, School of Public Health, Baltimore, MD.

Nonsyndromic cleft lip and/or cleft palate (CL/P) is a common birth defect with complex etiology due to multiple genetic and environmental factors. Multiple genome wide association studies have been successful in identifying novel loci associated with CL/P including a locus on 1p22.1. The association signal resides in the *ABCA4* gene, which is associated with retinal disease, however neither expression analysis nor mutation screening support a role for this gene in the etiology of CL/P. We have investigated an adjacent gene, *ARHGAP29*, which encodes a Rho GTPase activating protein. This protein has been shown to have activity towards Rho and is an effector of Rap2. Rho (and ARHGAP29, by association) can be placed in many signaling pathways related to cell shape, movement, cell-cell interactions, and cell proliferation. The *ARHGAP29* gene is adjacent to a linkage disequilibrium (LD) block containing the most significant SNPs for this association but the region surrounding this gene has little LD with the associated block, making it difficult to identify associated SNPs. Sequencing of this gene in individuals from the Philippines with CL/P revealed a nonsense mutation and several missense mutations. The nonsense mutation, K326X, was identified in one individual with bilateral cleft lip with cleft palate. The mutation was also found in his unaffected mother and grandfather. A missense mutation, R616H, was found in cases and controls with a minor allele frequency of approximately 5%. This amino acid change is predicted to be “possibly damaging” by Polyphen. The identification of a rare, damaging mutation, such as the K326X nonsense mutation, provides evidence to implicate this gene in cleft lip and palate. Additional sequence, genotyping and expression analysis will allow us to determine if there is additional support for its etiologic role. Studies of model systems will allow us to understand the role of this gene in the development of the face.

1028/T

Genetic association analysis highlights new loci that modulate hematological trait variation in Caucasians and African Americans. *G. Lettre[1,17], K.S. Lo[1], J.G. Wilson[2], L.A. Lange[3], A.R. Folsom[4], G. Galarneau[1], S.K. Ganesh[5], S.F.A. Grant[6,7], B.J. Keating[6], S.A. McCarroll[8,9], E.R. Mohler III[10], C.J. O'Donnell[11,12,13], W. Palmas[14], W. Tang[4], R.P. Tracy[15], A.P. Reiner[16], CARe Project.* 1) Montreal Heart Institute, Montréal, Québec, H1T 1C8, Canada; 2) Department of Medicine, G.V. (Sonny) Montgomery V.A. Medical Center and the University of Mississipi Medical Center, Jackson, MS 39216, USA; 3) Department of Genetics, University of North Carolina, 5112 Genetic Medicine Building, Chapel Hill, NC 27599-7264, USA; 4) Division of Epidemiology and Community Health, University of Minnesota, Minneapolis, MN 55454, USA; 5) Division of Cardiovascular Medicine, Department of Internal Medicine, the University of Michigan. Ann Arbor, MI 48109, USA; 6) Center for Applied Genomics, Division of Human Genetics, Children's Hospital of Philadelphia Research Institute, Philadelphia, Pennsylvania 19104, USA; 7) Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104, USA; 8) Department of Genetics, Harvard Medical School, Boston, MA 02115, USA; 9) Broad Institute, Seven Cambridge Center, Cambridge, MA 02142, USA; 10) Department of Medicine, Cardiovascular Division, Vascular Medicine Section, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA; 11) National Heart, Lung, and Blood Institute (NHLBI), Division of Intramural Research, Bethesda, MD 20892, USA; 12) NHLBI's Framingham Heart Study, Framingham, MA 01702, USA; 13) Cardiology Division, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA; 14) Department of Medicine, Columbia University, 622 West 168th Street, Ph 9 East, 107, New York, NY 10032, USA; 15) Departments of Pathology and Biochemistry, University of Vermont, 208 S. Park Drive, suite 2, Colchester, VT 05446, USA; 16) Department of Epidemiology, University of Washington, Box 357236, Seattle, WA 98195, USA; 17) Département de Médecine, Université de Montréal, C.P. 6128, succursale Centre-ville, Montréal, Québec, H3C 3J7, Canada.

Red blood cell, white blood cell, and platelet measures, including their count, sub-type and volume, are important diagnostic and prognostic clinical parameters for several human diseases. To identify novel loci associated with hematological traits, and compare the architecture of these phenotypes between ethnic groups, the CARe Project genotyped 49,094 single nucleotide polymorphisms (SNPs) that capture variation in ~2,100 candidate genes in DNA of 23,439 Caucasians and 7,112 African Americans from five population-based cohorts. We found strong novel associations between erythrocyte phenotypes and the glucose-6 phosphate dehydrogenase (*G6PD*) A- allele in African Americans (rs1050828, $P<2x10^{-13}$), and between platelet count and a SNP at the tropomyosin-4 (*TPM4*) locus (rs8109288, $P=1.2x10^{-7}$ in Caucasians; $P=2.5x10^{-7}$ in African Americans). We strongly replicated many genetic associations to blood cell phenotypes previously established in Caucasians. We also specifically explored the role in hematological trait variation of known rare functional SNPs that had been included on the array (nonsense, missense, and splice site markers with minor allele frequency £1%). Our results show similarities but also differences in the genetic regulation of hematological traits in European- and African-derived populations, and highlight the role of natural selection in shaping these differences.

1029/T

The perception of quinine taste intensity is associated with common genetic variants in a bitter receptor cluster on chromosome 12. *D. Reed[1], G. Zhu[2], P. Breslin[1,3], F. Duke[1], A. Henders[2], M. Campbell[2], G. Montgomery[2], S. Medland[2], N. Martin[2], M. Wright[2].* 1) Monell Chem Senses Ctr, Philadelphia, PA; 2) Queensland Institute of Medical Research, Brisbane, Australia; 3) Rutgers University, New Brunswick, NJ,.

The perceived taste intensities of quinine HCl, caffeine, sucrose octaacetate (SOA), and propylthiouracil (PROP) solutions were examined in 1,457 twins and their siblings. Previous heritability modeling of these bitter stimuli indicated a common genetic factor for quinine, caffeine, and SOA (22-28%), as well as separate specific genetic factors for PROP (72%) and quinine (15%). To identify the genes involved, we performed a genome-wide association study with the same sample as the modeling analysis, genotyped for ~610,000 single nucleotide polymorphisms (SNPs). For caffeine and SOA, no SNP association reached a genome-wide statistical criterion. For PROP, the peak association was within *TAS2R38* (*rs10246939*, I296V, P=1.1x10-101), which accounted for 45.9% of the trait variance. For quinine, the peak association was centered in a region that contains bitter receptor as well as salivary protein genes and explained 5.8% of the trait variance (*TAS2R19*, *rs10772420*, R299C, P=1.8x10-15). We confirmed this association in a replication sample of twins of similar ancestry [p=0.00001]. The specific genetic factor for the perceived intensity of PROP was identified as the gene previously implicated in this trait (*TAS2R38*). For quinine, one or more bitter receptor or salivary proline rich protein genes on chromosome 12 have alleles which affect its perception but tight linkage among very similar genes precludes the identification of a single causal genetic variant.

1030/T

Identification of a Novel Obesity-Related QTL Using a Genome-Wide Association Approach in Mexican American Families. *C. Bellis[1], MA. Carless[1], E. Drigalenko[1], MP. Johnson[1], HHH. Goring[1], TD. Dyer[1], LA. Almasy[1], MC. Mahaney[1], JB. Jowett[2], EK. Moses[1], AG. Comuzzie[1], J. Blangero[1], JE. Curran[1].* 1) Dept Gen, SFBR, San Antonio, TX; 2) Baker IDI Heart and Diabetes Institute, Melbourne, Australia.

Obesity, like most other common complex human diseases possesses a genetic signature that has largely eluded identification of specific causal genes and their underlying polymorphisms. A complex disease inheritance pattern, elaborate interactions with other metabolic disorders such as diabetes, heart disease and hypertension, and a host of environmental factors all contribute to variation in obesity measures. We are currently entering a generation where demand on the health budget has never been more critical, mainly due to escalating rates of cardiovascular diseases and related traits including obesity. Therefore, identification of genes influencing risk of obesity will be useful to enhance pharmacological target development. In this study we performed a genome-wide association scan to search for obesity-related QTLs on 1,189 Mexican Americans from approximately 40 extended pedigrees. Associations were evaluated using a variance component-based measured genotype approach implemented in SOLAR. Using 550,000 SNPs genotyped on the Illumina HumanHap550 BeadChip, we localized a QTL at chromosome 8q12 near the *SDCBP* (syntenin) gene, a gene thought to influence critical cell adhesion and cell signalling processes. Multiple obesity-related phenotypes exhibited signal at an intronic SNP (rs7833413) in the *SDCBP* gene. Obesity measures showing genome-wide significant or genome-wide suggestive associations included waist ($p=4.0 \times 10^{-8}$) and hip ($p=9.1 \times 10^{-8}$) circumferences, percentage body fat as measured by bioimpedance ($p=3.6 \times 10^{-7}$), and BMI ($p=6.0 \times 10^{-7}$). The minor allele frequency in this population was 0.06 and corresponded with an increased level of obesity in all of these traits. Genome-wide gene expression, obtained from lymphocytes from these same individuals, revealed that the rarer SNP allele is associated with a decrease in *SDCBP* gene expression ($p=0.0089$), directly supporting *SDCBP* as a causal player in the observed QTL. These novel results suggest a role for syntenin in obesity-related phenotypes. Our current sequencing effort, aimed at identifying the *SDCBP* causal variant/s through interrogation of all coding and potentially functional gene regions, will further elucidate the role for this novel gene in obesity.

1031/T

A genome wide association study of body mass index in African Americans. *M.C.Y. Ng[1,2], J.M. Hester[1,2,3], M.R. Wing[1,2,3], J. Li[1,2], N.D. Palmer[1,2,4], P.J. Hicks[1,2,4], B.H. Roh[1,2], L. Lu[5,6], L.E. Wagenknecht[6], J. Divers[5,6], C.D. Langefeld[5,6], B.I. Freedman[7], D.W. Bowden[1,2,4,7].* 1) Center for Diabetes Research; 2) Center for Genomics and Personalized Medicine Research; 3) Program in Molecular Genetics and Genomics; 4) Department of Biochemistry; 5) Department of Biostatistical Sciences; 6) Division of Public Health Sciences; 7) Department of Internal Medicine, Wake Forest University School of Medicine, Winston-Salem, NC.

Obesity is a major public health problem in the US, exhibiting racial differences in obesity prevalence of 44% and 32% in African Americans (AA) and European Americans, respectively. We have completed a genome-wide association study (GWAS) on body mass index (BMI) in 1715 unrelated AA (S1: 816 healthy subjects; S2: 899 subjects with type 2 diabetes and nephropathy) using the Affymetrix 6.0 platform. Single SNP association analyses were conducted on 746,626 good quality autosomal SNPs with adjustment for age, gender and first principal component reflecting admixture proportion. Meta-analysis revealed 75 SNPs showing nominal significance ($4.4X10^{-9} < P_{ADD} < 1X10^{-4}$) with the same direction of association in S1 and S2 samples.

We further genotyped these SNPs in 3224 AA subjects (S3: 611 healthy subjects and S4: 1490 diabetic subjects from Wake Forest University, S5: 286 subjects from Diabetes Heart Study (DHS) and AA DHS, S6: 837 subjects from Insulin Resistance Atherosclerosis Study (IRAS) study and IRAS family study) for replication. The analysis was conducted in each of the four replication samples adjusted for age, gender, admixture and/or disease status using a variance component approach. Five SNPs at four loci near *TMEM212* (rs6794092), *ZNF518B* (rs7691990), *MFAP3-GALNT10* (rs2033195, rs815611) and *FER1L4* (rs6088887) were nominally associated with BMI in the meta-analysis of replication samples ($0.003 < P_{ADD} < 0.05$). They also showed consistent direction of association in all GWAS and replication samples. Meta-analysis of 4939 GWAS (S1 and S2) and replication samples (S3 to S6) showed increased association signals in these five SNPs ($2.1X10^{-6} < P_{ADD} < 2.3X10^{-5}$). The functions of most of these nearby genes are unknown except that *GALNT10* encodes a member of the GalNAc polypeptide N-acetylgalactosaminyltransferases involved in the synthesis of mucin-type oligosaccharides. Our results in AA suggest four novel putative BMI loci in African Americans that warrant further replication studies.

1032/T

Genetics of Meiotic Recombination: Genome Wide Association Studies for recombination phenotypes. *F. BEGUM[1], E. FEINGOLD[1,2], S.L. SHERMAN[3], V.G. CHEUNG[4], R. CHOWDHURY[5].* 1) Department of Biostatistics, University of Pittsburgh, Pittsburgh, PA, USA; 2) Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA, USA; 3) Department of Human Genetics, Emory University School of Medicine, Atlanta, Georgia, United States of America; 4) Department of Genetics, University of Pennsylvania, Philadelphia, PA, USA; 5) Department of Pediatrics, University of Pennsylvania, Philadelphia, PA, USA.

Meiotic recombination is critical to successful human reproduction. Absence of recombination and recombination in certain high-risk locations are both associated with aberrant meiotic outcomes, including trisomy and monosomy. Several genes have recently been associated with variability in meiotic recombination, including RNF212 for total recombination and PRDM9 for recombination hotspot usage. We have combined several large family GWAS datasets to confirm these associations and to find additional regions that are associated with various recombination phenotypes. Family datasets are necessary because recombination phenotypes are estimated for each parent in the dataset, based on their children. We have also expanded the sample size available for studying recombination by developing methods for using many different pedigree configurations for estimating recombination phenotypes; previous recent papers have relied on methods that only score recombination for parents in nuclear families with two or more children. Our goal is to identify genetic determinants of recombination in order to better understand the biology of recombination and its role in meiosis.

1033/T

Validation of a computationally feasible method for imputation in African Americans in the NHLBI CARe Project. *C.D. Palmer[1,2], G. Lettre[3,4], J.N. Hirschhorn[1,2,5] for the NHLBI CARe Consortium.* 1) Program in Genomics and Divisions of Genetics and Endocrinology, Children's Hospital Boston, Boston, MA, 02115, USA; 2) Program in Medical and Population Genetics, Broad Institute, Cambridge, MA, 02142, USA; 3) Faculté de médecine, Université de Montréal, Montréal, Québec, Canada H3C 3J7; 4) Institut de cardiologie de Montréal, Montréal, Québec, Canada H1T 1C8; 5) Department of Genetics, Harvard Medical School, Boston, MA, 02115, USA.

Genotype imputation has become a common tool in the analysis of genome-wide association studies and medical resequencing, but imputation in admixed populations presents additional challenges. Imputation involves generating probabilistic genotypes for densely genotyped individuals using a reference panel such as HapMap, but imputation quality is highly dependent on well-matched ancestry between sample and reference individuals. This is not always possible in data such as HapMap2 or current releases from 1000 Genomes, which are enriched for Northern European (CEU), West African (YRI) and East Asian (JPT+CHB) populations. For admixed populations with representation of ancestral populations in reference panels, such as African Americans (~80% African and ~20% European ancestry), an ideal solution involves dynamically selecting reference haplotypes based on local ancestry estimates. However, these methods are not yet computationally feasible.

Here, we present validation of a simpler method for imputation of an admixed sample. This method, similar to that of Huang et al. (2009), uses a combination of HapMap haplotypes broadly matched to the sample's ancestral populations. We imputed five African American cohorts from the NHLBI CARe (Candidate gene Association REsource) Project. Over 8000 individuals were represented from the ARIC, CARDIA, CFS, JHS, and MESA cohorts. Each cohort was genotyped on Affymetrix 6.0 (Affy6) and Illumina IBC (ITMAT, Broad, CARe) iSelect Infinium chips. Imputation was performed using MACH and combined HapMap2 CEU and YRI reference panels, to reflect the admixture in the samples. This method generated genotype estimates at ~2.7 million SNPs. For SNPs genotyped on one chip but absent from the other, the estimated allele dosages for the missing SNPs were compared to genotype calls for the same individuals on the other platform. With the Affy6 chip (~800K SNPs), imputed SNPs had ~95.6% concordance with genotypes at an expected-to-observed variance ratio (RSQ_HAT) ≥0.3, compared to published ~96% concordance in European samples. The combined reference panel yielded higher minor allele concordance than either CEU or YRI alone. With the IBC chip (~40K SNPs), allelic concordance was lower (~90%), and required RSQ_HAT ≥0.6 to match the concordance of the Affy6 imputation. These results demonstrate that imputation of admixed populations is computationally feasible using available software.

1034/T

Using whole-genome sequence data to expand the search for asthma risk variants: Australian Asthma Genetics Consortium. *M.A. Ferreira[1], M.C. Matheson[2], C.F. Robertson[3], G.B. Marks[4], L.J. Palmer[5], S.C. Dharmage[2], P. Le Souef[6], D.L. Duffy[1], M.J. Abramson[7], G.W. Montgomery[1], P.J. Thompson[8] on behalf of the Australian Asthma Genetics Consortium.* 1) Queensland Institute of Medical Research, Brisbane, Australia; 2) Centre for MEGA Epidemiology, The University of Melbourne, Australia; 3) Royal Children's Hospital, Melbourne, Australia; 4) Woolcock Institute of Medical Research, Sydney, Australia; 5) Genetic Epidemiology, University of Western Australia, Perth, Australia; 6) School of Paediatrics and Child Health, Princess Margaret Hospital for Children, Perth, Australia; 7) Department of Epidemiology & Preventive Medicine, Monash University, Melbourne, Australia; 8) Lung Institute of Western Australia, Perth, Australia.

Most genetic risk factors for asthma remain to be identified and it is now clear that these can only be detected through large international collaborative studies. In 2010 we established the Australian Asthma Genetics Consortium to generate and analyse whole-genome genotype data on up to 3,000 doctor-diagnosed cases and 4,000 controls of European descent. All samples have been genotyped using the Illumina platform. We expanded the genomic coverage by imputing ~8 million SNPs using the phased data for 56 unrelated CEPH individuals sequenced at a low coverage by the 1000 Genomes Project. Based on preliminary analyses of a subset of 986 cases and 1,846 controls, we have confirmed the association between variants in ORMDL3 and asthma (rs6503525, P=4.8x10-7). We also identified a new risk variant in IL1RL1 that is independent of that reported in a previous study (rs1420101), suggesting the presence of multiple independent risk variants for asthma in this region. We found no evidence for an increased number or size of copy number variants (CNVs) in cases when compared to controls, when considering large (>100kb), uncommon (MAF<0.05) CNVs. Genotype data from the remaining 2,000 cases and 2,000 controls, together with whole-genome sequence data that we are currently generating with deep coverage (30x) for a carefully selected reference panel of asthma cases, will be used to confirm these results and extend our analyses of rare variants.

1035/T

Phenotypic differences in genetically identical Monozygotic Twins. *L. Martelli[1,3], G. Maire[2], D.P. Pereira[3], R.S. Simao[3], J. Huber[3], A.L. Simoes[1], S. Choufani[4], R. Weksberg[4], J.A. Squire[5], M. Yoshimoto[5].* 1) Dept Genetics - School of Medicine, University of Sao Paulo, Ribeirao Preto, SP, Brazil; 2) Dept Paediatric Laboratory Medicine, The Hospital for Sick Children, Toronto, Ontario, Canada; 3) Division of Medical Genetics, Hospital das Clinicas - School of Medicine, Ribeirao Preto, SP, Brazil; 4) Dept Genetics and Genome Biology, The Hospital for Sick Children, Toronto, Ontario, Canada; 5) Dept Pathology and Molecular Medicine, Queen's University, Kingston, Ontario, Canada.

Monozygotic (MZ) twins represent an important resource in genetic studies related to normal development and disease. Consequently, studies of MZ twins have become a powerful tool for studying disorders and endophenotypes, highlighting the genotype-phenotype differences, and testing hypotheses regarding gene-environment interactions. Recently, CNV analysis of phenotypically discordant MZ twins has been applied for identifying disease predisposition loci. In this case report, we evaluated obesity-discordant MZ twins to determine whether distinct genetic events can influence the pathophysiological feature and characterize genetic predisposition factors. The proband (twin A), product of the second gestation of a 37 yo woman, was born at 38 weeks gestation after an uncomplicated pregnancy. The boy was delivered by C-section, weighting 3,340g and height of 49cm. Twin B birth weight was 2,360g and H= 47cm. Twin A weight with 17mo was 16,100 g (average for 47months), H = 75cm (p3-10) and OFC = 50,5 cm (p50-98). With 27 mo: W= 22,200 g (>> p97), H= 87,5 cm (p25-50). At the same age, twin B presented normal growth parameters, weighting 12,800g. Non dysmorphic features were detected. DNA zygosity test was compatible with monozygotic twins. Laboratorial tests showed normal basal ACTH levels; IGF1=12ng/mL; lipidogram with elevated triglycerides levels. Thyroid, renal and liver function tests were normal as well as basal cortisol, calcium, phosphorus, basal glucose and GTT. Leptin= 20 ng/ml and BMI> 23 (RV= 10-20). Serum copper was slightly elevated. Abdominal ultrasonography revealed discrete hepatomegaly. Both karyotypes were normal. The primary platform for analysis of genetic variants was the 6.0 SNP array (Affymetrix) using peripheral blood derived DNA obtained from the twin pair. We compared one twin versus its co- twin to assess the accurate genotype of the twins and putative imbalance within each twin pair. Importantly, the SNP analysis confirmed the monozygosity. However, the findings indicated that obesity-discordant MZ twins were genotypically concordant for all CNVs. According to the enrichment analysis, the CNV events were random and phenotypically neutral, as no obesity-related pathways were detected. Thus, a genetic mechanism origin for obesity-discordance in MZ twins was not detected in our study by SNP arrays methodology and further epigenetics or transcriptome investigations are necessary to yield molecular mechanisms underpinning disease.

1036/T

Identification of novel type 2 diabetes susceptibility loci by large-scale replication using the "Metabochip". *A. Kumar[1,2], A. Mahajan[1,3], I. Prokopenko[1,4], V. Lagou[1], N. Robertson[1], N.W. Rayner[1], R. Magi[1], C. Groves[4], K. Stirrups[5], S.E. Hunt[5], A. Bennett[4], S. Wiltshire[1], J. Trakalo[1], G. Mirza[1], D. Buck[1], K. Zhou[6], C.N.A. Palmer[6], A.D. Morris[6], T.M. Frayling[7], A. Hattersley[7], M. Weedon[7], A. Wood[7], C. Langford[5], S. Potter[5], P. Deloukas[5], P. Donnelly[1], A.P. Morris[1], C.M. Lindgren[1], M.I. McCarthy[1,4], The Wellcome Trust Case Control Consortium, The DIAGRAM Consortium.* 1) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom; 2) Swiss Tropical and Public Health Institute, University of Basel, Basel, Switzerland; 3) Institute of Genomics and Integrative Biology, CSIR, Delhi, India; 4) Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford, United Kingdom; 5) Wellcome Trust Sanger Institute, Hinxton, United Kingdom; 6) Biomedical Research Institute, University of Dundee, Ninewells Hospital and Medical School, Dundee, United Kingdom; 7) Institute of Biomedical and Clinical Science, Penninsular Medical School, Exeter, United Kingdom.

Over 30 common variant signals influencing type 2 diabetes (T2D) susceptibility have been identified, most through meta-analysis of genome-wide association studies. However, replication efforts have focussed only on the strongest associations, and have failed to fully exploit large-scale discovery meta-analyses such as that undertaken by the DIAGRAM consortium (12,057 cases, 56,071 controls of European descent). The Metabochip, a custom iSELECT array containing ~195,000 SNPs, was designed to support large-scale follow up of putative associations for T2D and other metabolic and cardiovascular traits.

We analysed Metabochip genotype data, called using GenCallv1.1, for an additional 4,434 T2D cases and 7,932 controls from UK cohorts (UK T2D Genetics Consortium, Warren 2 Collection and 1958 British Birth Cohort). We analysed 4,790 high-quality, statistically-independent SNPs which capture >95% of the strongest ~5,000 autosomal signals from the DIAGRAM meta-analysis. Association analyses in each cohort were performed under an additive model and combined via fixed effects meta-analysis.

We first compared patterns of replication between "Stage 1" DIAGRAM and "Stage 2" Metabochip data. Of 37 established autosomal T2D loci, 36 showed directional consistency (i.e. same high risk allele in both stages) including *TCF7L2* (p=7.3x10^{-30}), *FTO* (p=4.6x10^{-10}) and *KCNQ1* (p=2.4x10^{-9}). After excluding SNPs at these loci, there was a highly significant concordance in the direction of effects (2,569 [59.3%] of 4,332 SNPs, binomial test p=8.8x10^{-35}). The concordance was even stronger among the 314 SNPs demonstrating nominal evidence of association (p<0.05) in Stage 2 (233 [74.2%] concordant, binomial test p=4.8x10^{-18}). Subsequent meta-analysis of the two stages of our study revealed 5 novel loci exceeding genome-wide significance mapping near: *SPRY2* (p=5.5x10^{-11}, OR= 1.11[1.08-1.15]); *ANK1* (p=2.0x10^{-10}, OR=1.11[1.08-1.15]); *LINGO1* (p=5.6x10^{-10}, OR=1.10[1.07-1.14]); *ZMIZ1* (p=3.1x10^{-9}, OR=1.09[1.06-1.12]; and *BAT3* (p=3.1x10^{-8}, OR= 1.10[1.06-1.13]).

The concordance of results in the two stages of our study is consistent with a long-tail of modest effect common variant associations with T2D. Metabochip-genotyping underway in >30,000 T2D cases and >50,000 controls should add considerably to the tally of proven T2D-susceptibility loci.

1037/T

A Genome-wide association analysis in over 187,000 individuals identifies 14 loci contributing to variation in central obesity and fat-distribution. *C.M. Lindgren[1, 2, 3].* 1) on behalf of the GIANT (Genetic Investigation of ANthropmetric Traits) Consortium; 2) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom; 3) Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Churchill Hospital, Oxford, United Kingdom.

Obesity is an increasing public health issue, but not all forms of obesity carry the same risk. Individuals with high waist-to-hip ratio (WHR) have an increased risk of Type 2 Diabetes (T2D), hypertension, heart disease, stroke and certain cancers. WHR is one of the primary measures of fat distribution and has a substantial heritability (~50%), independent of overall adiposity. Hitherto, the genetic variants that contribute to variation in WHR have not been well characterized. To detect common variants we performed a meta-analysis of 32 genome-wide association studies comprising >77,000 individuals of European ancestry as part of the GIANT consortium. We tested ~2.8 million imputed and genotyped SNPs for association with WHR using an additive model, adjusted for age, BMI and sex. Our discovery analysis identified 16 independent loci associated with WHR with p<5x10-6. We took these loci forward for replication (*de-novo* and *in-silico*) in 30 additional cohorts (>110,000 individuals). In our analysis combining discovery and follow-up studies 14 loci reached genome-wide significance (p<5x10-8). We confirmed the known locus near *LYPLAL1* (1q41; p=6.9x10-21) and identified 13 novel associations, including: near *VEGFA* (6p12; p=5.9x10-25), *TBX15* (1p11; p=8.7x10-25), *GRB14* (2q24.3; p=2.1x10-24) and near *ADAMTS9* (3p14; p=9.8x10-14). *TBX15* is differentially expressed between subcutaneous and visceral fat and the expression is correlated with WHR. *GRB14* is reported to be a regulator of insulin-mediated signaling and is significantly associated with triglycerides in our data (p=7.4x10-9). *VEGFA* is a growth factor that has been suggested to play a role in diabetic nephropathy and retinopathy. *ADAMTS9* is significantly associated with T2D, possibly mediating an effect through decreased insulin sensitivity of peripheral tissues. Further, we find a directionally consistent enrichment of associations (P-nominal <0.05) with increased triglycerides, LDL-cholesterol, fasting insulin, HOMA-IR and T2D. We also observed a marked gender difference in our results; 7 of the 14 loci showed a stronger association in women than in men. Taken together, these results promise to enhance our knowledge of underlying biological pathways involved in fat-distribution and propose a genetic overlap with metabolic risk and T2D. Hopefully these advancements will support functional and translational advances in the management of obesity through development of novel diagnostic and therapeutic options.

1038/T

Rare variant SNP calling; performance of current genotype calling algorithms. *N.W. Rayner[1,2], N. Robertson[1,2], I. Prokopenko[1,2], C. Groves[2], C.M. Lindgren[1], G. Mirza[1], J. Trakalo[1], D. Buck[1], M.A. Eberle[3], T. Royce[3], K. Zhou[4], C.N.A. Palmer[4], A.D. Morris[4], T. Frayling[5], A. Hattersley[5], A. Woods[5], P. Deloukas[6], S. Potter[6], M.I. McCarthy[1,2].* 1) WTCHG, Univ Oxford, Oxford, United Kingdom; 2) OCDEM, Univ Oxford, United Kingdom; 3) Bioinformatics Department, Illumina Inc, San Diego, USA; 4) Biomedical Research Institute, University of Dundee, Ninewells Hospital & Medical School, Dundee, United Kingdom; 5) Institute of Biomedical and Clinical Science, Peninsula Medical School, Exeter, UK; 6) Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK.

With the growing focus on studies of low-frequency and rare alleles, the performance of alternative genotype-calling algorithms in this part of the allele frequency spectrum becomes critical. We evaluated three such algorithms (Illuminus, GenoSNP, Gencall) on data generated on the "metabochip" (a custom iSELECT array containing ~197k SNPs, of which 62,494 have MAF<1%, according to CEU HapMap data). The programs were all run with default settings, except GenoSNP (0.95 probability threshold cutoff). Genotypes were obtained for 30 CEPH HapMap Trios, and for 7,984 unrelated samples from the Dundee T2D case-control collection, and analysed without further QC for metrics including genotyping rate, mendelian inconsistencies (MI: in trios, contingent on both parents having genotypes) and between-methods concordance. Of the three methods, Illuminus performed least well judged by genotyping rate (94.9%) and MI count (59,229 MI counts across all SNPs, 51,396 of these in MAF<1% SNPs). The MI count was significantly reduced when we reclustered the trios with up to 17,000 other samples (2,954; 939 in MAF<1% SNPs), but still higher than the other methods. Gencall (run without Gentrain to reduce overfitting given overlap in the samples typed) achieved higher genotyping rate (99.2%) with relatively low MI counts (544 overall, 152 in MAF<1% SNPs), but discordancy analyses suggested that Gencall was generally conservative with respect to calling of small heterozygote clusters. GenoSNP might be expected to outperform other methods for rare SNPs: it clusters data on an "all SNPs per sample" basis (rather than "all samples per SNP"), and therefore is less reliant on observing all three genotype clusters for a given SNP. Indeed, we found that GenoSNP combined respectable genotyping rates (96.7%) with low MI rates (235 overall, 44 in MAF<1%), despite being less conservative than Gencall at defining small heterozygote clusters. Overall, therefore Gencall and GenoSNP performed well across all metrics, even for low-frequency SNPs. In the case-control data, Gencall and GenoSNP calls were highly concordant (99.8% where both made calls in 185,802 high-quality SNPs; 99.9% in MAF<1%) though there were more serious disparities at the remaining ~11k. One further attractive feature of GenoSNP is that the "per-sample" calling strategy makes calls more stable when new data are integrated.

1039/T

META-ANALYSIS OF GENOME-WIDE ASSOCIATION STUDIES OF DIABETIC NEPHROPATHY. *R.M. Salem[1,2,8], N. Sandholm[5,6], A.J. McKnight[3], T. Isakova[7], E. Brennan[4], C. Palmer[1,2], D. Sadlier[4], A. Taylor[1,7], V. Harjutsalo[5,6], C. Guiducci[1], W. Williams[1,7], S. Bain[3], M. Parkkonen[5,6], D. Savage[3], F. Martin[4], C. Forsblom[5,6], J. Florez[1,7], C. Godson[4], A.P. Maxwell[3], J. Hirschorn[1,2,8], P.H. Groop[5,6].* 1) Broad Institute, Cambridge, MA, US; 2) Children's Hospital, Boston, MA, US; 3) Queen's University of Belfast, NI, UK; 4) Conway Institute of Biomolecular and Biomedical Research, University College, Dublin, ROI; 5) Biomedicum Helsinki, Helsinki, FI; 6) Helsinki University Central Hospital, Helsinki, FI; 7) Massachusetts General Hospital, Boston, MA; 8) Harvard Medical School, Boston, MA.

Diabetic nephropathy (DN), often leading to end-stage renal disease (ESRD), is a significant complication of type 1 diabetes (T1D), with lifetime risk of approximately 30% among persons with T1D. We sought to improve understanding of the genetic contribution to DN by assembling the largest collection of individuals with T1D (n=6,462), with and without nephropathy. These samples are part of the GENIE (GEnetics of Nephropathy, an International Effort) Consortium and include the All Ireland-Warren3-UK GoKinD (UK/ROI, 823 cases and 903 controls) and FinnDiane (FD, 1,411 cases and 1,708 controls) collections, as well as publicly available data from the US GoKinD study obtained from dbGAP (US, 782 cases and 835 controls). Cases were patients with T1D and nephropathy (macroalbuminuria or ESRD) and controls were patients with T1D for at least 15 years and no evidence of renal disease (no microalbuminuria). The UK/ROI was genotyped on the Illumina Omni1-quad chip and FD on the Illumina 610Quad, while US was genotyped previously on the Affy 5.0 platform. We imputed genotypes using MACH with the HapMap2 CEU panel, and performed association tests using PLINK, with principal components of ancestry, age at recruitment, duration of diabetes, gender and study site as covariates. We meta-analyzed association statistics using METAL. In total, ~2.4 million single nucleotide polymorphisms (SNPs) with minor allele frequency > 1% and passing QC were analyzed. A total of 15 SNPs achieved a P-value of less than 10^{-6} in the combined analysis. Of these, multiple SNPs mapped to 2q34, 3q13.31, 4q34.3 and 20q11.21. Four of these SNPs were observed in a biologically plausible gene at 2q34. We are assembling additional samples to follow up the top SNPs and test whether any reach genome-wide significance. We are performing additional related analyses of extreme phenotypes and DN-related quantitative traits. FD also has longitudinal follow-up data that will facilitate investigation of the initiation and progression of diabetic nephropathy. Finally, we will compare our list of top signals with data from microarray analyses of in vitro models of DN. By combining genetic and expression data, this research has the potential to highlight novel biological pathways and improve our understanding of DN.

1040/T

Educational attainment: from a single marker association approach toward pathway analysis. *N.W. Martin[1,2], K.H. Verweij[1,2], S.E. Medland[1], D.R. Nyholt[1], P.A. Madden[3], A.C. Heath[3], G.W. Montgomery[1], M.J. Wright[1,2], N.G Martin[1,2].* 1) Genetic Epidemiology Laboratory, Queensland Institute of Medical Research, Brisbane, Australia; 2) School of Psychology, University of Queensland, Brisbane, Australia; 3) School of Medicine, Washington University, St Louis, Missouri, USA.

Background: Educational Attainment (EA) is measure of interest across multi-disciplinary research in economics, health and cognitive functioning. Correlations between EA and measures of cognitive performance are as high as 0.8. This makes EA an attractive alternative phenotype for studies willing to map cognitive genes due to the ease of collecting EA data compared to other cognitive phenotypes such as IQ. Methodology: In an Australian family sample of over 10000 individuals we performed a genome-wide association scan (GWAS) using the genotypes of ~2,5 million single nucleotide polymorphisms (SNP) for a 6-point scale measure of EA (from 1 = 7 years or less of schooling to 6 = university postgraduate training). A gene-based test of association was then applied to the GWAS results, followed by pathway analyses of the most associated genes. Results/Significance: No SNP reached a genome wide significant p-value (p £ 10-8) with the best SNP having a p-value of 1.1x10-6. The gene-based test of association showed only one gene with a p-value that passed correction got multiple testing, however, it had no relevance to brain phenotypes. Similarly, the pathway analyses of the top associated genes were mostly inconclusive with the exception of a hypothetical role for the glycosaminoglycan degradation pathway in the etiology of EA. However, this result should be taken with caution as further replications and meta analyses will be needed to draw firm conclusions. Despite having performed the largest GWAS for EA to our knowledge, our study demonstrated the high polygenic architecture of EA. Our results show that EA does not escape the debate of whether common variants of small effect or rare variants of large effect are responsible for explaining the phenotypic variance in complex traits.

1041/T

HLA Stratification Analysis of the Finnish Coeliac Disease GWAS Data. *K.I. Loytynoja[1], E. Einarsdottir[1], K. Kurppa[2], K. Kaukinen[3], M. Maki[2], A. Aromaa[4], V. Salomaa[4], L. Peltonen[5], P. Dubois[6], D.A. van Heel[7], P. Saavalainen[1].* 1) Medical Genetics, University of Helsinki, Helsinki, Helsinki, Finland; 2) Paediatric Research Centre, University of Tampere Medical School and Tampere University Hospital, University of Tampere, Finland; 3) Department of Gastroenterology and Alimentary Tract Surgery, Tampere University Hospital and Medical School, University of Tampere, Finland; 4) National Institute for Health and Welfare, Helsinki, Finland; 5) Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK, and Institute for Molecular Medicine Finland (FIMM), Helsinki, Finland; 6) Blizard Institute of Cell and Molecular Science, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK; 7) Centre for Gastroenterology, Institute of Cell and Molecular Science, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, United Kingdom.

Introduction: Coeliac disease (CD) is a multifactorial disorder caused by the ingestion of gluten in genetically susceptible individuals. The genes encoding the HLA-DQ2 and HLA-DQ8 heterodimers are the strongest genetic risk factors for the disease. Roughly percent of patients carry DQ2 in cis (DQ2.5) or trans (DQ2.2 and DQ7) and 5-10 percent have DQ8. These HLA variants are also common in the general population, implicating additional genetic factors in disease susceptibility. **Materials and methods:** A recent GWA study on CD in four European populations revealed multiple risk loci for CD, and included samples from 642 cases and 1 834 controls from the Finnish population (Dubois et al. 2010). Based on HLA-tagging SNPs (rs2187668 for DQ2.5 and rs3957148 for DQ8), 560 cases and 349 controls were DQ2.5 positive, and 56 cases and 405 controls were DQ8 positive/DQ2.5 negative. In addition, 26 cases and 1080 controls carried neither of these alleles. We performed independent analyses for DQ2.5 positive and DQ8 positive/DQ2.5 negative carriers, and also compared DQ2.5 positive and DQ8 positive/DQ2.5 negative cases against each other. For follow-up purposes we selected only those signals that reached p-values < 1.0*10-5. **Results:** We found novel associations on chromosomes 3 and 15 in DQ2.5 positive group and a significantly strengthened association for IRF4 gene on chromosome 6. In DQ8 positive/DQ2.5 negative group, novel associations located on chromosomes 1, 5, and 11. Novel associations on chromosomes 2, 3, and 17 were found in DQ2.5 positive versus DQ8 positive cases -analysis. **Conclusions:** In addition to the previously identified HLA-haplotype independent loci, there may be additional and unique susceptibility loci in carriers of specific HLA-haplotypes. HLA-stratification also strengthened signals in several previously found loci. Our data therefore suggests that HLA-stratification may reveal particularly interesting subgroups of patients, that helps understanding the interactions and shared biological pathways of the CD risk genes.

1042/T

Genetic variation that predicts white blood cell count and its differential, a marker of the health of the immune system. *D.R. Crosslin[1], N. Weston[2], K. Ehrlich[2], G. Hart[2], A. McDavid[3], K. Newton[2], C.S. Carlson[3], E.B. Larson[2], G.P. Jarvik[1], . eMERGE Network[1,2,3].* 1) Department of Medicine, University of Washington, Seattle, WA; 2) Group Health Research Institute, Center for Health Studies, Seattle WA; 3) Fred Hutchinson Cancer Research Center, Public Health Sciences Division, Seattle WA.

White blood cell count (WBC) is unique among the identified inflammatory predictors of chronic disease in that it has been routinely measured in healthy patients in an unbiased way for the duration of the electronic medical record (EMR) data at Group Health Cooperative (GH) and other eMERGE Network participating sites. We led genome wide association studies (GWAS) to identify variation that predicts WBC and its differential (neutrophils, lymphocytes, monocytes, eosinophils and basophils). As well as being a marker of the health of the immune system, WBC is emerging as a risk factor for chronic diseases, including vascular disease and renal failure. GWAS of total WBC and its differential counts can identify genes that predict WBC as the end result of a variety of inflammatory pathways. Within the eMERGE network, the GH, Marshfield Clinic and Mayo Clinic sites have >98% of subjects with WBC. Northwestern University has 92% and Vanderbilt University has 91%. In total, there were 19,000 subjects with WBC, and >18,000 with differential. The sample at GH was older (74.5±7.9), and subjects had multiple visits for the duration of the EMR, with a median number of visits of nine. The goal was to select WBCs that were not inflated by acute processes. We developed an algorithm to extract WBC and differential repeated measures from the EMR using subject-level and visit-level inclusion/exclusion criteria. All subjects in the eMERGE Network were genotyped on the Illumina Human660W-Quadv1_A platform. For the genetic association, we compared and contrasted different approaches utilizing repeated measures. These summary measures provided a cleaner set of phenotypes to analyze than cross-sectional data. The within-subject measures included the following: 1.median value for a subject; 2.within-subject predicted value at the overall mean age; 3.within-subject slope; and 4.the best unbiased linear predictor. WBC is a complex phenotype to analyze, and we have provided the framework and model for extracting and summarizing subject-level data from a repeated-measure EMR. Our algorithm has successfully been translated to other eMERGE sites. Initial association analyses are ongoing and replication is planned with other Network data, but preliminary results at GH are encouraging. There were ten genes mapped to SNPs with p-values <0.0001 across the phenotypes. Assessment of the contribution of top hits to inflammatory pathways and gene set enrichment will complete the analyses.

1043/T

Whole genome-scan of genetic determinants for dental caries in the primary dentition. *E. Feingold[1], J.R. Shaffer[1], X.J. Wang[1], M.K. Lee[1], F. Begum[1], D.E. Weeks[1], M. Barmada[1], K. T.Cuenco[1], S. Wendell[1], D. Crosslin[2], C. Laurie[2], K. Doheny[3], E. Pugh[3], F. Geller[4], B. Feenstra[4], H. Zhang[4], H. Boyd[4], M. Melbye[4], R.J. Weyant[1], R. Crout[5], D. McNeil[5], S.M. Levy[6], R.L. Slayton[6], M. Willing[7], B. Broffitt[6], M.L. Marazita[1].* 1) Univ Pittsburgh, Pittsburgh, PA; 2) Univ of Washington, Seattle, WA; 3) Johns Hopkins University, Baltimore, MD; 4) Statens Serum Institut, Copenhagen, Denmark; 5) University of West Virginia, Morgantown WV; 6) University of Iowa, Iowa City, IA; 7) Washington University, St. Lous, MO.

Objective: Despite advances in dental care, dental caries (also known as tooth decay) remains one of the most common chronic diseases of human beings, and the incidence of caries in young children has actually increased in recent years. The etiology of dental caries is thought to involve a complex interplay of environmental and genetic factors, with heritabilities of caries phenotypes ranging from 30-60%. As part of the NIH Genes and Environment Initiative (GENEVA), we performed the first genome-wide association study (GWAS) of dental caries in the primary dentition to identify genomic regions harboring genes responsible for caries susceptibility or resistance in children. Subjects and Methods: 1,643 subjects between the ages of 3 and 12 years with primary teeth were drawn from larger family cohort studies in Pennsylvania, West Virginia, and Iowa. The majority of subjects were Caucasian. All tooth surfaces were evaluated by trained dental examiners, and scored as sound, white spot (non-cavitated), decayed (cavitated), or filled. Individuals with > 1 lesion were considered “affected” (n=705), and those with no lesions were considered “unaffected” (n=938). Each subject was genotyped for the Human610-Quad SNP panel by the Center for Inherited Disease Research, and the data were cleaned in collaboration with the GENEVA Data Coordinating Center. Associations between dental caries and each SNP were first assessed using the trend test (PLINK) ignoring familial relationships, then repeated with adjustments for relatedness (variance components-SOLAR). Finally, we stratified the sample based on household water fluoride levels, and repeated the analyses in each subset as a preliminary investigation of gene by environment interaction. Results: A region on chromosome 11 was significantly associated with caries, with several SNPs showing unadjusted p-values < 10-6. Regions on chromosome 1 and 17 also showed multiple SNPs with p-values < 10-5 in the vicinity of plausible candidate genes. After stratification on household water fluoride levels, a region on chromosome 9 was also significant ((p-values < 10-6). Replication is now in progress in an additional cohort. NIH grants U01DE018903, R01DE014899, R01DE09551, R01DE012101, U01HG004423, U01HG004446, NIH contract HHSN268200782096C.

1044/T

Rare variant analysis of the mitochondrial genome in age-related hearing impairment. *E. Fransen, S. Bonneux, G. Van Camp.* Center for Medical Genetics, University of Antwerp, Antwerp, Belgium.

Age-related hearing impairment (ARHI) is a condition whereby hearing acuity declines with ageing. Heritability studies indicated that about half of the phenotypic variance is attributable to genetic factors. Previous studies on nuclear genes showed association with GRHL2 and GRM7, but much of the variance remains unexplained. Since alterations in the mitochondrial genome have been implicated in various ageing diseases, we also performed an analysis of mitochondrial variants by completely resequencing the mitochondrial genome in 200 affected persons and 200 controls. In a first round of analyses, we tested association between the phenotype and the common mt variants or mt haplogroups, but no significant associations were found. Moreover, we did not find any common variant to be associated with ARHI. The average mutation load per individual, calculated as the number of rare alleles carried by an individual, did not differ significantly between cases and controls, even when common variants (MAF>0.05) were excluded from this calculation. The previous tests have limited power to detect the influence of rare variants on disease risk. Here we have used the collapsing technique proposed by Li and Leal (2010), to perform a gene-based analysis of rare mitochondrial variants. For each of the mitochondrial genes separately, we subdivided the individuals into two groups according to their mutational status: individuals carrying no minor allele were scored as wildtype, whereas individuals with at least one mutation were scored as mutant. This analysis was performed on i) all variants, ii) only rare variants (MAF<0.05) and iii) variants only found once in the entire dataset. Within each mt gene, association between mutational status and case control status was tested using the Fischer’s exact test. Reference Li, B. and Leal, S.M. (2008) “Novel Methods for Detecting Associations with Rare Variants for Common Diseases: Application to Analysis of Sequence Data.” Am. J. Hum. Genet. 83(3): 311-21.

1045/T

Genetic loci for BMI and BMI Change in the Transition from Adolescence to Young Adulthood. *M. Graff[1], P. Gordon-Larsen[1], C.C. White[2], J. Ngwa[2], T. Esko[3, 4], N. Amin[5], P. Scheet[6], C. Schurmann[7], A. Teumer[7], C.S. Fox[8], L. Qi[9, 10], R.M. van Dam[9, 11], D. Strachan[12], A. Metspalu[3, 4], C.M. van Duijn[5], D. Schlessinger[13], H. Völzke[14], K.E. North[15], S. Berndt[16], L.A. Cupples[2, 8].* 1) Carolina Population Center and Department of Nutrition, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA; 2) Department of Biostatistics, Boston University School of Public Health, University Medical Campus, Boston, Massachusetts, USA; 3) Estonian Genome Center and Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia; 4) Estonian Biocenter, Tartu, Estonia; 5) Department of Epidemiology, Erasmus Medical Center, Rotterdam, The Netherlands; 6) Center for Statistical Genetics, Department of Biostatistics, University of Michigan, Ann Arbor, Michigan, USA; 7) Interfaculty Institute for Genetics and Functional Genomics, Ernst-Moritz-Arndt-Universität Greifswald, Greifswald, Germany; 8) Framingham Heart Study, Framingham, Massachusetts, USA; 9) Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts, USA; 10) Channing Laboratory, Department of Medicine, Brigham and Women’s Hospital, Boston, Massachusetts, USA; 11) Departments of Epidemiology and Public Health and Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; 12) Division of Community Health Sciences, St. George’s, University of London, London, SW17 0RE, UK; 13) Gerontology Research Center, National Institute on Aging, Baltimore, Maryland, USA; 14) Institute for Community Medicine, Ernst-Moritz-Arndt-Universität Greifswald, Greifswald, Germany; 15) Department of Epidemiology and Carolina Center for Genome Sciences, School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA; 16) Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland, USA.

The period between late adolescence and young adulthood is a period with a high risk for weight gain and development of obesity. Information on genetic determinants of body mass index (BMI) or change in BMI during this particular period of life may yield important insight into the etiology of obesity. The purpose of the current study was to identify genetic loci influencing cross-sectional observations of BMI (BMI level) and change in BMI (BMI change), during late adolescence and early adulthood in Caucasians considering potential gender differences. We performed a meta-analysis of 9 genome-wide association studies that included observations of BMI level between ages 16 and 21 years (N = 13530) and BMI change between ages 16 and 35 years (N = 6626). Each study imputed~2.5 million SNPs in HapMap and tested for association assuming an additive genetic model, stratified by gender and/or case-control status and adjusted for age, center, and principal components reflecting racial variation. Family studies also adjusted for relatedness. Multiple BMI observations at different ages were used to perform a growth curve analysis for BMI change. Inverse normal transformed residuals were used for level and change as the outcomes. The meta-analysis was conducted using the inverse variance weighted method. For BMI level, we identified 5 independent loci (P< 5.0 x 10-8) in men and women combined near TNNI3K (p=1.9x10-12), TMEM18 (p= 3.0x10-11), MC4R (p=3.4x10-09), FTO (p=2.2x10-09), and PMAIP1 (p= 4.2x10 -09); 2 in men only (near PMAIP1, p=5.5x10-10 and MC4R, p= 3.2x10-08); and 1 in women only (near TNNI3K, p=9.6x10-09)In the smaller longitudinal sample, we found no significant loci for BMI change. In summary, TNNI3K and PMAIP1 are novel hits, whereas FTO, MC4R, and TMEM18 are already well replicated. During a narrow period of the life course, late adolescence and early adulthood, we find several significant loci related to body mass index that partly vary by gender. These results suggest that the expression of genetic effects may relate to particular periods of the life course and can vary by gender.

1046/T

Does the Bunion come from the Genes or High Heels? The Healthy Twin Study, Korea. *D.H. Lee[1], J. Sung[1], M.K. Lee[1], Y.M. Song[2], K. Lee[3], J.H. Hwang[4].* 1) Complex Disease and Genetic Epidemiology Branch, Department of Epidemiology and Institute of Environment and Health, School of Public Health Seoul National University; 2) Department of Family Medicine, Samsung Medical Center, and Center for Clinical Research, Samsung Biomedical Research Institute, SungKyunKwan University School of Medicine, Seoul, Korea; 3) Department of Family Medicine, Busan Paik Hospital, Inje University College of Medicine, Korea; 4) Department of Rehabilitation Medicine, Samsung Medical Center, SungKyunKwan University School of Medicine, Seoul, Korea.

Buion, or hallux valgus is a common foot condition characterized by lateral displacement deformity of the first metatarsophalangeal joint in great toe. Hallux valgus is associated with wearing high-heeled shoes especially in women, but its genetic susceptibility is not well known. We are determined to estimate genetic contributions to the hallux valgus angle (HVA) and the hallux valgus (defined by HVA >15 degree in either foot) after considering the individual preference to the heel size.1,288 Korean adult twins and their families (183 monozygotic and 32 dizygotic twin pairs, and 857 individuals of their family took foot examination, (502 men and 786 women, average age 44.06 years). The HVA was radiologically evaluated from the weight-bearing anteroposterior (AP) foot X-ray views. The mean HVA was 14.15 degree for men, and 16.63 degree for women, and 47.0 % of men (236 persons), and 55.9 % of women (439 persons) were classified as hallux valgus. A variance component method was used to estimate heritability. After consideration of shared environmental effects and adjustment for age and sex effects, the heritability estimates ± s.e. were 0.52 ± 0.047 for HVA, and 0.74 ± 0.063 for hallux valgus. When we considered the preferred heel height, asked as “what is your typical heel size?”, the women preferring higher (>5 cm) heels showed significant associations with the bunion status, and also showed evidence of the interaction between additive genetic effects Our findings suggest that HVA and hallux valgus have a strong genetic influence and for someone who are vulnerable to hallux valgus, wearing high heels will more likely to produce bunions.

1047/T

A two-stage genome-wide association study of mean telomere length. *K.A. Pooley[1], J. Morrison[1], A.A. Al Olama[1], M. Shah[2], B.A.J. Ponder[3], P.D.P. Pharoah[2], D.E. Neal[3,4], F.C. Hamdy[5], J.L. Donovan[6], Z. Kote-Jarai[7], R.A. Eeles[7,8], D.F. Easton[1], A.M. Dunning[2].* 1) CR_UK Genetic Epidemiology Unit, Dept of Public Health, University of Cambridge, Strangeways Res Lab, Cambridge, United Kingdom; 2) Dept of Oncology, University of Cambridge, Strangeways Res Lab, Cambridge, United Kingdom; 3) Cancer Research UK Cambridge Research Institute, Li Ka Shing Centre, Robinson Way, Cambridge, United Kingdom; 4) Depts of Oncology and Surgery, Addenbrooke’s Hospital, Hills Road, Cambridge, United Kingdom; 5) Nuffield Department of Surgery, University of Oxford, United Kingdom; 6) Department of Social Medicine, University of Bristol, United Kingdom; 7) Translational Cancer Genetics Team, The Institute of Cancer Research, Sutton, Surrey, United Kingdom; 8) Royal Marsden NHS Trust Foundation, Sutton, Surrey, United Kingdom.

Telomeres protect against DNA damage that may result in genome instability. Mean telomere length is heritable and progressively shortens with age, and there is evidence for marked telomere length attrition in cancer patients, post-diagnosis. Recently, loci have emerged that may start to explain telomere length homeostasis and biology. To investigate the genetic basis of mean telomere length, we performed a genome-wide association study (GWAS), using an Illumina 550K array. Participants were 1,540 disease-free men from the ProtecT prostate cancer case-control study on which we had also measured mean telomere length using a quantitative Real Time PCR assay. In the second stage, we genotyped by Taqman® assay the 12 loci most strongly associated with mean telomere length in stage 1, in a further 15,606 samples on which we also had telomere length measurements. This stage comprised cancer-free individuals (n=9,708) from the SEARCH study, the Sisters in Breast Screening (SIBS) study, and the MAPLES melanoma family study; SEARCH breast cancer cases (n=6,338) and MAPLES and SEARCH melanoma cases (n=560). Of the initial GWAS top 12 SNPs, 11 demonstrated a change in mean telomere length (ΔCt), whereby the rare allele was associated with shorter mean telomere length (p-trend=$1.7x10^{-6}$ to p-trend=$3.2x10^{-5}$, adjusted for age, study plate, gender and family). In the Stage 2 replication, one of the top 12 variants showed evidence of replication (stage 2, p-trend=0.007; combined stage 1 & 2, p-trend=$3.3x10^{-4}$). None of the 12 SNPs in the second stage of the study were significantly associated with risk of breast cancer (6,367 cases, 6,483 controls), or melanoma (562 cases, 778 controls). In addition to our GWAS hits, we also found weak evidence of association with mean telomere length for two recently published loci (rs12696304, p-trend=0.017; rs16847897, p-trend=0.014). These loci are worthy of further investigation, and if confirmed, may provide novel insights into the determinants of telomere length control and attrition, and its role in ageing and common disease progression.

1048/T

Genome-wide association study and pathway analysis in alopecia areata implicates innate and adaptive immune responses. *H. Kim[1], L. Petukhova[1], M. Duvic[2], M. Hordinsky[3], D. Norris[4], V. Price[5], Y. Shimomura[1], P. Sin[1], A. Lee[6], W.V. Chen[7], K.C. Meyer[8], R. Paus[8,9], C.A.B. Jahoda[10], C.I. Amos[7], P.K. Gregersen[6], A.M. Christiano[1, 11].* 1) Department of Dermatology, Columbia University, New York, NY; 2) Department of Dermatology, M. D. Anderson Cancer Center, Houston, Texas; 3) Department of Dermatology, University of Minnesota, Minneapolis, Minnesota; 4) Department of Dermatology, University of Colorado, Denver, Colorado; 5) Department of Dermatology, UCSF, San Francisco, California; 6) The Feinstein Institute for Medical Research, North Shore LIJHS, Manhasset, New York; 7) Department of Epidemiology, M. D. Anderson Cancer Center, Houston, Texas; 8) Department of Dermatology, University of Lübeck, Lübeck, Germany; 9) School of Translational Medicine, University of Manchester, Manchester, UK; 10) Department of Biological Sciences, University of Durham, Durham, UK; 11) Department of Genetics and Development, Columbia University, New York, New York.

Alopecia areata (AA) is among the most highly prevalent human autoimmune diseases, leading to disfiguring hair loss due to the collapse of immune privilege of the hair follicle and subsequent autoimmune attack. The genetic basis of AA is largely unknown. We undertook a genome-wide association study in an initial discovery sample of 250 unrelated cases and 1049 controls, and replicated our findings in an independent sample of 804 cases and 2229 controls. Joint analysis identified 139 SNPs that are significantly associated with AA (p£5x10-7) and which implicate at least eight genomic regions, including the HLA. The regions outside of the HLA harbor several key components of Treg activation and proliferation (CTLA4, IL-2/IL-21, IL-2RA, and Eos), as well as three ligands of NKG2D, an activating receptor of cytotoxic lymphocytes (MICA, ULBP3, and UPBP6). We also found evidence for genes expressed in the hair follicle itself (PRDX5 and STX17). Next, we reduced the stringency threshold (p<0.01) to identify a set of SNPs that mapped onto 2929 genes, on which we performed pathway analysis. This analysis identified several immune related pathways with significant representation in our data set, including NK cell mediated cytotoxicity, T cell receptor signalling, cytokine-receptor interaction, Jak-STAT signalling and antigen presentation. Additionally several pathways that appear to be aligned with hair follicle specification and maintenance were also identified, including WNT signalling, ErbB signalling, and cell adhesion. Finally, a subset of SNPs mapped onto disease processes for type I diabetes, autoimmune thyroid disease, systemic lupus erythematosus and asthma, supporting the common cause hypothesis of autoimmune diseases. We have defined the genetic underpinnings of AA, placing it within the context of shared pathways among autoimmune diseases, and implicating a novel disease mechanism, the upregulation of ULBP ligands, in triggering autoimmunity.

1049/T

Genomewide association study in Systemic Sclerosis. *Y. Allanore[1,2], M. Saad[3], P. Dieude[4], C. Boileau[5], M. Martinez[3], GENESYS consortium.* 1) INSERM U781, Hôpital Necker, Université Paris Descartes, Paris, France; 2) Rhumatologie A, Hôpital Cochin, Université Paris Descartes, APHP, Paris, France; 3) INSERM U563 Université Paul Sabatier, Toulouse, France; 4) Rhumatologie, INSERMU699, Hôpital Bichat, Paris Diderot université, Paris, France; 5) Université VSQ, Biochimie A, Hôpital A. Pare, Boulogne, France.

Systemic sclerosis (SSc) is an orphan multi-organ disease affecting the immune system, the microvascular network and the connective tissue. SSc leads to profound disability and premature death. Epidemiological data suggest a complex genetic etiology. To dissect genetic susceptibility to SSc we have performed a genome-wide association study in 654 SSc patients of French Caucasian origin from the GENESYS study, and 2,543 French controls (subjects without SSc from GENESYS and neurologically healthy subjects from the Three-City cohort- Lambert et al, Nat Genet. 2009;41:1094-9). Subjects were genotyped using the Illumina Human 610 QUAD bead chip. The final post-QC discovery (stage-1) sample comprised 564 SSc cases and 2,466 controls, and a total of 489,814 SNPs passed quality control criteria. Association was tested using logistic regression assuming additive genetic effects and adjusted for the two principal components to account for population substructure. The genome-wide association results revealed a number of SNPs with strong evidence (P<10-5) of association. Interestingly, in our discovery sample, stronger signals were detected for 6 non-HLA SNPs that spanned 5 distinct genomic loci. Two SNPs belong to a candidate gene previously reported associated to other autoimmune disease of high relevance with regards to SSc pathogenesis. Furthermore, the remaining 4 SNPs are located on 3 previously unreported putative SSc loci. 2 SNPs are close to genes coding for neuro-mediators and 1 SNP is close to a metabolic gene recently showed to deeply contribute to extra-cellular matrix remodeling. Follow-up and replication analysis of these promising and new association signals are under way in a second French/Italian/German case-control sample (>1,750 SSc cases and 3,200 controls). Our preliminary GWAS results identified a tractable number of novel candidate genes for SSc that warrant further investigation. All top best associated SNPs will be reported in the meeting.

1050/T

Genome-wide association study for sarcoidosis identifies novel risk locus at 12q14.1. *S. Hofmann[1], A. Fischer[1], M. Nothnagel[2], G. Jacobs[1], P. Rosenstiel[1], K. Gaede[3], M. Schuermann[4], J. Mueller-Quernheim[5], S. Schreiber[1].* 1) Institute of Clinical Molecular Biology, CAU Kiel, Germany; 2) Institute of Medical Informatics and Statistics, CAU Kiel, Germany; 3) Reserch Center Borstel,Germany; 4) Institute of Human Genetics, University Lübeck, Germany; 5) Department of Pneumology, Medical University Hospital Freiburg, Germany.

Genetic factors are important to the development of sarcoidosis, and recent advantages in genome-wide screening and candidate gene experiments have lead to the identification of several sarcoidosis susceptibility loci. Although, these loci have considerably improved and changed the understanding of the sarcoidosis pathophysiology they explain only a minor part of the heritability underlying the disease. We aimed on the identification of further susceptibility loci for sarcoidosis and performed a genome-wide association analysis of 934,968 SNPs in 641 German sarcoidosis cases and 1,263 control individuals. We discovered a new association at chromosome 12q14.1. Subphenotype-specific analysis revealed a stronger association of the lead SNP with acute sarcoidosis (OR=1.30) compared to the chronic phenotype (OR=1.16). Fine mapping of the novel locus and quantitative mRNA expression studies point to a candidate gene that plays an important role in the ER-associated degradation of misfolded or unassembled proteins and functions in the maturation of dendritic cells.

1051/T

A Genome-wide association study identifies susceptibility variants for Graves' disease. *J. Wu[1, 2], C. Yang[1], F. Tsai[2], R. Chen[2], C. Chen[1], Y. Chen[1].* 1) Institue Biomedical Sci, Academia Sinica, Taipei, Taiwan; 2) China Medical University Hospital, Taichung, Taiwan.

Graves' disease (GD) is a common autoimmune thyroid disease affecting 0.5-1% of the general population. To investigate susceptibility genes that increase the risk of GD in Han Chinese, a genome-wide association study was conducted in which 596 GD cases and 804 controls were genotyped with Affymetrix SNP6.0 genechips. Graves' disease was diagnosed according to the American Thyroid Disease Association Criteria. All cases were recruited from the China Medical University Hospital, Taichung, Taiwan. The controls were randomly selected from the Taiwan Han Chinese Cell and Genome Bank. All of the participating cases and controls were of Han Chinese origin, which is the origin of 98% of the Taiwan population. We excluded SNPs from further analyses by three major criteria: (1) total call rate <98%, (2) minor allele frequency <5%, and (3) p-value of Hardy-Weinberg Disequilibrium test <10-4. A total of 694,436 SNPs (79.99%) passed the quality control filter with an average call rate of 99.8% and subjected to association analysis using trend (Cochran-Armitage) test. Eleven SNPs within three loci outside the MHC showed associations with p value between 10-5and 10-6. Two of the three loci were novel and one was located within the thyroid stimulating hormone receptor (TSHR) gene that encodes the primary autoantigen in GD. We also demonstrated a positive association of GD with HLA-DQB1 and DRB1 alleles in Han Chinese. In summary, we have identified novel SNPs/loci associated with Graves' disease in Han Chinese. Our study may lead to a better understanding of the underlying mechanism of Graves' disease pathogenesis.

1052/T

Genome-wide association study with gender adjustment to search for genetic variants responsible for intracranial aneurysms in Japanese population. *K. Akiyama, A. Narita, H. Nakaoka, T. Cui, A. Tajima, I. Inoue.* Department of Molecular Life Sciences, Basic Medical Science and Molecular Medicine, Tokai University School of Medicine, Isehara, Kanagawa, Japan.

Intracranial aneurysm (IA) (MIM105800) is a cerebrovascular disease showing a reported prevalence of 3-6 %. The rupture of an IA leads to subarachnoid hemorrhage (SAH) which often results in death or severe physical disability, therefore the disease is a major public health concern. A large-scale GWAS in European cohorts has revealed SNPs responsible for predisposition to IA, locating on 18q11.2, 13q13.1, 10q24.32, 8q11.23-q12.1 and 9p21.3. To identify novel genetic susceptibility loci for Japanese IA, we carried out a multistage association study using genome-wide SNPs in Japanese case-control subjects.

IA is most likely a multifactorial etiology, involving complex interactions of genetic and environmental risk factors. There is a possibility that non-genetic risk factors for aneurysm formation, such as smoking habits, hypertension, frequent alcohol intake and female gender, might hinder the identification of genetic effects. Therefore, additional tests with adjusting sex effects which act between genetic effect and IA were performed at a genome-wide level.

In the first stage of our GWAS, we genotyped over 300,000 tag SNPs spanning the whole genome for 250 IA cases and 249 control subjects. In the second stage, we genotyped 2,304 SNPs in additional subjects. In the following intensified study, we genotyped 22 SNPs in further additional subjects. We assessed evidence for association using standard approaches, and additional tests by adjusting sex effects which act between the genetic effect and disease in order to evaluate the genuine genetic effect of SNPs on IA. Consequently, five SNPs ($p=1.31\times10^{-5}$ for rs1930095 of intergenic region; $p=1.32\times10^{-5}$ for rs4628172 of TMEM195; $p=2.78\times10^{-5}$ for rs7781293 of TMEM195; $p=4.93\times10^{-5}$ for rs7550260 of ARHGEF11; $p=3.63\times10^{-5}$ for rs9864101 of IQSEC1) with probabilities of being false positives < 0.5 were associated with IA in Japanese population. Furthermore, when performing a multivariate logistic regression with gender, hypertension and smoking habit as covariates, we still observed significant associations of the five SNPs. This study indicates the presence of several susceptibility loci which deserve further investigation in the Japanese population.

1053/T

Genome-wide association study identifies two novel regions at 11p15.5-p13 and 1p31 with major impact on acute-phase Serum Amyloid A. *E. Albrecht[1,20], C. Marzi[1,20], P.G. Hysi[2], V. Lagou[3,4], M. Waldenberger[1], A. Tönjes[5,6], I. Prokopenko[3,4], K. Heim[7], H. Blackburn[8], J.S. Ried[1], M.E. Kleber[9], M. Mangino[2], B. Thorand[1], A. Peters[1], C.J. Hammond[2], B.O. Boehm[10], P. Kovacs[11], L. Geistlinger[1], H. Prokisch[7,12], B.R. Winkelmann[13], T.D. Spector[2], H.-E. Wichmann[1,14,15], M. Stumvoll[5], N. Soranzo[2,8], W. März[16,17,18], W. Koenig[19], T. Illig[1,20], C. Gieger[1,20].* 1) Institute of Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany; 2) Dept of Twin Research and Genetic Epidemiology, King's College London, London, UK; 3) Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford, UK; 4) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK; 5) Department of Medicine, University of Leipzig, Leipzig, Germany; 6) Coordination Centre for Clinical Trials, University of Leipzig, Leipzig, Germany; 7) Institute of Human Genetics, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany; 8) Wellcome Trust Sanger Institute Genome Campus, Hinxton, UK; 9) LURIC non-profit LLC, Freiburg, Germany; 10) Division of Endocrinology, Department of Medicine, University of Ulm, Germany; 11) Interdisciplinary Centre for Clinical Research, Department of Internal Medicine III, University of Leipzig, Leipzig, Germany; 12) Institute of Human Genetics, Klinikum Rechts der Isar, Technische Universität München, Germany; 13) Cardiology Group Sachsenhausen, Germnany; 14) Institute of Medical Informatics, Biometry and Epidemiology, Chair of Epidemiology, Ludwig-Maximilians-Universität, Munich, Germany; 15) Klinikum Grosshadern, Munich, Germany; 16) synlab Medizinisches Versorgungszentrum Heidelberg, Eppelheim, Germany; 17) Institut für Public Health, Sozialmedizin und Epidemiologie, Medizinische Fakultät; 18) Clinical Institute of Medical and Chemical Laboratory Diagnostics, Graz, Austria; 19) Department of Internal Medicine II - Cardiology, University of Ulm Medical Center, Ulm, Germany; 20) These authors contributed equally.

Serum amyloid A (SAA), an acute-phase protein, is causally involved in the pathogenesis of amyloidosis and has been found to be implicated in obesity, atherosclerosis and its clinical complications, and various malignancies. We conducted the first genome-wide association study on baseline acute-phase SAA (A-SAA) concentrations in three population-based studies (KORA, TwinsUK, Sorbs) and one prospective case cohort study (LURIC) including a total of 4,212 participants of European descent and identified two genetic susceptibility regions at chromosome 11 and 1. The region at chromosome 11 contains serum amyloid A1 (SAA1) and the adjacent general transcription factor 2 H1 (GTF2H1), Hermansky-Prudlak Syndrome 5 (HPS5), lactate dehydrogenase A (LDHA), and lactate dehydrogenase C (LDHC). The high degree of explained variance (10.84%) of the total variation of A-SAA suggests that the region is of key importance in the regulation of inflammation. The second region encloses the leptin receptor (LEPR) gene at chromosome 1. As this region has been found to be associated with other acute-phase proteins in previous studies, our finding indicates a close interplay between A-SAA, leptin, and other inflammatory proteins and a larger role of the LEPR gene locus in inflammatory processes as it has been assumed in the past.

1054/T

Genome-wide association study identifies a second independent genetic variant in BCL11A influencing F cells in sickle cell disease. *P. Bhatnagar[1], S. Purvis[2], E. Barron-Casella[2], M.R. DeBaun[3], J.F. Casella[2], D.E. Arking[1], J.R. Keefer[2].* 1) McKusick-Nathans Institute of Genetic Medicine, School of Medicine, Johns Hopkins University, MD; 2) Department of Pediatrics, Division of Pediatric Hematology, School of Medicine, Johns Hopkins University, MD; 3) School of Medicine, Washington University, St. Louis, MO.

Sickle cell disease (SCD) is the most common autosomal recessive blood disorder in the United States, affecting about 1 in 400 African Americans. SCD is a widely studied with marked phenotypic heterogeneity that results from environmental as well as genetic factors. Variation in the level of fetal hemoglobin (HbF) accounts for much of the clinical heterogeneity associated with incidence of pain episodes. Therefore, HbF level has emerged as an important prognostic factor in SCD and can be measured by the proportion of Fcells. The genetic regulation of Fcells levels is complex and quantitative trait loci (QTLs) at chromosome 6q23, 11p15 and Xp22.2 have been reported. To map additional QTLs, we performed a genome-wide association study (GWAS) in 440 African-ancestry SCD patients from the Silent Infarct Transfusion Trial cohort. Genotyping was performed using Illumina HumanHap650Y arrays, and principal component analysis (PCA) was used to correct for potential population stratification. Fcells quantitation was done in a single laboratory. All the analyses were performed by adjusting age, sex and top ten principal components. The average age of the cohort was 9.15 yrs, with 53% males. Our results replicate the previously implicated chromosome 2p15 region that includes BCL11A, confirming the association of BCL11A in the modulation of HbF levels. In earlier GWAS, an intronic BCL11A SNP, rs766432 was the most significant SNP associated with Fcell levels. In our study, we mapped another novel BCL11A intronic SNP, rs6706648 (~2 kb downstream to rs766432, r^2=0.25), associated with lower Fcell counts (P=4.71x10^{-14}). Interestingly, the effect of this SNP is in the opposite direction observed for rs766432; the minor T allele of rs6706648 is associated with lower levels of Fcells, whereas minor C allele of rs766432 is associated with higher levels. Using conditional linear regression and haplotype analyses, we show that these two variants represent independent genetic effects. The variance explained independently by rs6706648 and rs766432 are ~12% and ~11%, respectively, and combined, these SNPs explain ~16%. These results highlight the importance of denser genetic screens, and suggest that analytical methods that specifically look for multiple genetic effects within a gene may improve the ability to detect genes associated with Fcell levels in SCD. Further exploration of the region containing rs6706648 may help explain negative regulation of Fcell production.

1055/T

Meta analysis of African American Genome Wide Association Studies of type 2 diabetes: The CARe T2D Plus Study. *D.W. Bowden[1], S.J. Bielinski[2], L. Kao[3], D. Siscovick[4], S.R. Patel[5], J.R. Zmuda[6], J.B. Meigs[7], M. Sims[8], D. Sarpong[9], S.S. Rich[10], B.I. Freedman[11], M.O. Goodarzi[12], S.F.A. Grant[13], C.D. Langefeld[14], N.D. Allred[1], J.S. Pankow[16], Y. Li[17], L.A. Lange[17], J.G. Wilson[15], M.C. Ng[1], the Candidate Gene Association Resource.* 1) Center for Human Genomics, Wake Forest University School of Medicine, Winston-Salem, NC; 2) Division of Epidemiology, Mayo Clinic College of Medicine, Rochester, MN; 3) Department of Epidemiology, Johns Hopkins School of Medicine, Baltimore, MD; 4) Departments of Medicine and Epidemiology, University of Washington, Seattle, WA; 5) Center for Clinical Investigation, Case Western Reserve University, Cleveland, OH; 6) Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; 7) Department of Medicine, Harvard Medical School, General Medicine Division, Massachusetts General Hospital, Boston MA; 8) Jackson Heart Study, Department of Medicine, University of Mississippi Medical Center, Jackson, MS; 9) Jackson State University, Jackson Heart Study Coordinating Center, Jackson, MS; 10) Center for Public Health Genomics, University of Virginia, Charlottesville, VA; 11) Department of Internal Medicine, Wake Forest University School of Medicine, Winston-Salem, NC; 12) Division of Endocrinology, Diabetes & Metabolism, Cedars-Sinai Medical Center, Los Angeles, CA; 13) Center for Applied Genomics, Division of Human Genetics, Children's Hospital of Philadelphia & Dept. of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia PA; 14) Department of Biostatistical Sciences, Wake Forest University School of Medicine, Winston-Salem, NC; 15) V.A. Medical Center and University of Mississippi Medical Center, Jackson, MS; 16) Division of Epidemiology and Community Health, University of Minnesota, Minneapolis, MN; 17) Department of Genetics, University of North Carolina, Chapel Hill, NC.

A meta-analysis of 6 African American genome wide association studies: Atherosclerosis Risk in Communities (ARIC), Coronary Artery Risk Development in Young Adults (CARDIA) Cleveland Family Study (CFS), Jackson Heart Study (JHS), Multi-Ethnic Study of Atherosclerosis (MESA), and Wake Forest University, has been performed for type 2 diabetes (T2D). Samples were genotyped on the Affymetrix 6.0 platform with imputation of genotypes performed with MACH using the HapMap Phase II CEU and YRI (1:1) haplotypes as reference resulting in ~3M SNP genotypes for analysis (MAF > 0.01). The initial analysis was performed on a total of 8936 DNA samples (2229 prevalent T2D cases at baseline and 6707 non-diabetic controls; Baseline analysis: BSL). A second analysis (Diabetes Working Group analysis: DWG) encompassed 3121 cases with prevalent and incident T2D and 3792 non-diabetic controls without impaired glucose tolerance (fasting glucose <100mg/dl and/or 2hr glucose<140mg/dl) or T2D. Association analysis was performed on 3 million directly genotyped and imputed SNPs in each cohort separately using PLINK or SNPGWA programs with adjustment for admixture using principal components. Meta-analysis was then carried out using METAL. There are both qualitative and quantitative differences between the BSL and DWG analyses. The TCF7L2 SNP rs7903146 was imputed and results in the strongest evidence of association in African Americans (P=4.3X10-12 and OR [95%CI]=1.35[1.24-1.47] BSL; P=1.7X10-9 and OR[95%CI]=1.32[1.20-1.44] DWG) consistent with numerous prior reports. Other, more nominal evidence of association was observed near TAS2R16-SLC13A1 (P=3.2X10-7; OR [95%CI]=0.74[0.66-0.83];BSL). With the exception of TCF7L2 there is limited evidence of association of T2D SNPs identified from prior studies in European-derived populations: CDKAL1, JAZF1, KCNQ1, KCNJ11-ABCC8 with index SNPs showing modest association with T2D (0.001<P<0.05). However, in gene or locus-wide analyses of these "European" loci, suggestive evidence for association was observed in KCNQ1 independent of the index SNP (most significant SNP OR=1.19(CI 1.09-1.29), P=0.0001). The common variant genetic architecture of T2D in African Americans remains elusive. Larger sample sizes achievable through meta analysis or independent replication analyses will be needed to follow-up borderline-significant genetic variants that appear to contribute to T2D susceptibility in the African American population.

1056/T

Meta-analysis of type 1 diabetes GWAS datasets reveals novel loci. *J.P. Bradfield[1], H.Q. Qu[2], K. Wang[1], H. Zhang[1], P.M. Sleiman[1], C.E. Kim[1], J.T. Glessner[1], H. Qiu[1], F. Mentch[1], K. Thomas[1], E.C. Frackelton[1], R. Chiavacci[1], M. Imielinski[1], D.S. Monos[3,4], S.F.A. Grant[1,3,5], H. Hakonarson[1,3,5].* 1) Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, PA; 2) Departments of Pediatrics and Human Genetics, McGill University, Montreal, QC, CA; 3) Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, PA; 4) Department of Pathology and Laboratory Medicine, Abramson Research Center, Children's Hospital of Philadelphia, Philadelphia, PA; 5) Division of Human Genetics, Abramson Research Center, Children's Hospital of Philadelphia, Philadelphia, PA.

Type 1 diabetes is an autoimmune disease leading to the destruction of the pancreatic islets. The prevalence of T1D in the United States in individuals below the age of 20 is 1.7 per 1000. Genome wide association studies (GWAs) have been particularly valuable in identifying the genetic susceptibility loci underlying type 1 diabetes with over 40 associations described in the last two years. One inherent limitation to GWAs is the large sample sizes required to detect loci of modest effect. Meta-analyses are the most efficient and economical way to assemble such large cohorts. In an effort to find more loci, we analyzed the largest combined T1D cohort to date, totaling 10,000 cases and 17,500 controls, comprising ~14000 samples (~3500 cases, ~10500 controls) genotyped on Affymetrix 500K chips (GoKinD, WTCCC) and ~13500 samples (~6500 cases, ~7000 controls) genotyped on Illumina 550K chips (McGill/CHOP, DCCT-EDIC, T1DGC). A cohort of ~1000 independent trios (T1DGC/McGill) was used for replication of all previously unknown T1D loci with p<1.0 x 10e-05. Power calculations based on these sample sizes indicate we have greater than 80% power to detect a variant with relative risk of 1.13 and above assuming a minor allele frequency of 20%. Methodologically, we initially imputed all datasets up to the 2 million CEU Hapmap SNPs using Mach 1.0 so that platform independent comparisons could be made. To control for population stratification, we used principal components anaysis (PCA) in the Illumina and Affymetrix cohorts separately. The results of the PCA analysis were then used as covariates in a logistic regression. Summary stats were then computed on the Illumina and Affymetrix cohorts and combined at all 2 million SNPs using fixed-effects meta-analysis. Three SNPs in previously unknown T1D loci replicated with a fishers combined p<5.0 x 10e-08 and four SNPs in previously unknown loci showed suggestive replication in which the replication p-value was less than 0.05 but the combined p-value was more than the genome wide cutoff. One replicated SNP resides in region 13q22.2 in the gene LMO7 and another in region 2p23.3 in the gene EFR3B. The last replicated SNP doesn't reside in a gene but is in region 6q27.

1057/T

Genomewide association study demonstrates the power of extreme truncate selection, identifying novel genes controlling bone mineral density and fracture. *M.A. Brown[1], E.L. Duncan[1], P. Danoy[1], J. Kemp[2], P. Leo[1], B. Richards[3,4], F. Rivadeneira[5], T.D. Spector[4], A.G. Uitterlinden[5], J. Wark[6], E. Dennison[7], R. Eastell[8], K. Estrada[5], G. Jones[9], E. McCloskey[8], G. Nicholson[10], R.L. Prince[11], P.N. Sambrook[12], J.A. Eisman[13,14], D.M. Evans[2].* 1) University of Queensland Diamantina Institute, Brisbane, Queensland, Australia; 2) MRC Centre for Causal Analyses in Translational Epidemiology, University of Bristol, Bristol, United Kingdom; 3) Departments of Medicine, Human Genetics, Epidemiology and Biostatistics, Lady Davis Institute, Jewish General Hospital, McGill University, Montreal, Quebec, H3T 1E2, Canada; 4) Department of Twin Research and Genetic Epidemiology, King's College London, London, WC2R 2LS, United Kingdom; 5) Department of Internal Medicine and Epidemiology, Erasmus Medical Center, Rotterdam, 3015, The Netherlands; 6) University of Melbourne Department of Medicine and Bone & Mineral Service, Royal Melbourne Hospital, Victoria 3050, Australia; 7) Medical Research Council Epidemiology Resource Centre, Southampton SO16 6YD, United Kingdom; 8) Academic Unit of Bone Metabolism, Metabolic Bone Centre, University of Sheffield, United Kingdom; 9) Menzies Research Institute, University of Tasmania, Hobart, 7000, Australia; 10) The University of Melbourne, Dept of Clinical and Biomedical Sciences: Barwon Health, Geelong, Victoria 3220, Australia; 11) School of Medicine and Pharmacology, University of Western Australia, Perth, 6009, Australia; and Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Perth, Australia; 12) Kolling Institute, Royal North Shore Hospital, University of Sydney, Sydney, Australia; 13) St Vincent's Clinical School, St. Vincent's Campus and University of New South Wales, Sydney, NSW, Australia; 14) Garvan Institute of Medical Research, Sydney, 2010, Australia.

BMD is strongly correlated with fracture risk, and is highly heritable. This study aimed to identify genes associated with BMD, using a unique design of extreme truncate selection in a cohort ascertained for BMD at one skeletal site (total hip (TH)), and one gender and age/menopausal status group (postmenopausal women age 55-85 years). A GWAS was performed in 1952 women of white western European descent, with either extreme high (z=+1.5 to +4, n=1053) or low bone mineral density (BMD) (z=-1.5 to -4, n=899), with follow-up in a cohort of 14,396 participants, assessing both BMD and fracture risk. Following imputation, 2,543,109 SNPs were tested for association with TH BMD. 124 SNPs were studied in the replication cohorts. Our dataset replicated at P<0.05 previously associated SNPs in 21 of 26 BMD genes reported from previous GWAS (*ARHGAP1, CTNNB1, ESR1, FAM3C, FLJ42280, FOXL1, GPR177, HDAC5, JAG1, LRP5, MARK3, MEF2C, MEPE, OPG, RANK, RANKL, STARD3NL, SOST, SOX6, SP7 (Osterix)* and *ZBTB40*). Fracture association was observed (P<0.05) with SNPs at *GALNT3, CLCN7, FLJ42280* and *GPR177* loci. Two genes (*GALNT3, RSPO3*) were identified with $P<5x10^{-8}$ and independently confirmed, and three other genes (*LTBP3, CLCN7* and *SOX4*) were identified with suggestive association ($5x10^{-8}<P<5x10^{-5}$) and confirmed in the replication cohorts. *GALNT3* belongs to the FGF23 pathway, a key pathway involved in control of phosphate and vitamin D levels that has never previously been associated with any common human disease. Mutations of *GALNT3* cause familial tumoral calcinosis and hyperostosis-hyperphosphataemia syndrome through effects on FGF23 glycosylation. RSPO3 is a Wnt-pathway agonist which has not previously been associated with any human disease. Mutations of *LTBP3* cause dental agenesis and high BMD through effects on TGFB1 bioavailability. Mutations of *CLCN7* cause osteopetrosis due to effects on osteoclastic bone resorption. Our study also strongly confirms the previously reported suggestive association of *TGFBR3* with BMD (P=6.7x10-5), indicating that this is a true BMD-associated gene. The extreme-truncate ascertainment scheme used in this study resulted in markedly increased study power, with the GWAS having similar statistical power to studies of ~16,000 unselected cases, and gains further power relative to previous screens through its site, gender and age focus. This study design is efficient and effective for gene discovery in quantitative traits such as BMD.

1058/T

Biological Pathway-based Genome-wide Association Analysis Indentified the Importance of Inflam Pathway for Femoral Neck Bone Geometry in Chinese males. *J. Chen[1, 2], YJ. Liu[1], YF. Pei[1], J. Li[1], J. Hamilton[1], HW. Deng[1, 2].* 1) University of Missouri - Kansas City, School of Medicine, Kansas City, MO 64108, USA; 2) Laboratory of Molecular and Statistical Genetics, College of Life Sciences, Hunan Normal University, Changsha, Hunan 410081, P R China.

Femoral neck (FN) bone geometry parameters are an important predictor of bone strength and fracture risk. These bone geometry parameters, including CSA (Cross Sectional Area), CT (Cortical Thickness), ED (Endosteal Diameter), Z (Section Modulus) and BR (Buckling Ratio), are under strong genetic control. However, the majority of the underlying genetic factors remain to be identified. In this study, we performed pathway-based genome-wide association analysis in a cohort of 1627 (Male: 802; Female: 825) unrelated Chinese adults to search for genes underlying variation in FN bone geometry parameters. The pathway-based analysis method used in this study is a modification of the Gene Set Enrichment Algorithm. A total of 1007 pathways extracted from the MSigDB database (*http://www.broadinstitute.org/gsea/msigdb/index.jsp*) were analyzed. Among all of the pathways analyzed, the Inflam pathway was most strongly associated with FN CSA in males only even after multiple testing adjustments ($p = 0.0009$, $q_{fdr} = 0.001$, $p_{fwer} = 0.0009$). CSA refers to the area with mineralized bone tissue excluded bone marrow space. Earlier studies have demonstrated that the Inflam pathway is involved in Interleukins and TNF serve as signals to coordinate the inflammatory response, in which macrophages recruit and activate neutrophils, fibroblasts, and T cells. Some of the genes in the Inflam pathway (e.g., TGFB1, IL-6, TNF) play important roles in osteoblastogenesis or osteoclastogenesis. In summary, our study suggests the importance of the Inflam pathway on variation in FN bone geometry parameters of males.

1059/T

Genome-wide Copy-Number-Variation Analysis Identified the *DOK6* Gene to Be Associated with Obesity in Chinese. *Y. Cheng[1,2], T. Xu[1,2], TL. Yang[1,3], YF. Pei[1,3], L. Zhang[1,3], N. Yu[1], HB. Liu[1,4], J. Li[1], K. Redger[1], Q. Tian[1], P. Yu[1], J. Hamilton[1], C. Papasian[1], YJ. Liu[1], HH. Zhou[2], HW. Deng[1].* 1) School of Medicine, University of Missouri-Kansas City, Kansas City, MO; 2) Pharmacogenetics Research Institute, Institute of Clinical Pharmacology, Central South University, Changsha, Hunan 410078, P.R.China; 3) Institute of Molecular Genetics, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an 710049, P R China; 4) Key Lab of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education, HuaZhong Agricultural University, 430070, PR China.

Obesity is a major public health problem with strong genetic determination. Copy number variations (CNVs) have been suggested to contribute to complex human diseases. However, whether CNV is associated with obesity remains largely unknown. In this study, we performed genome-wide CNV analyses for body mass index (BMI) and body fat mass in a sample of 1,627 unrelated Chinese subjects. All the subjects were genotyped for the Affymetrix genome-wide human SNP array 6.0 which includes more than 946,000 probes for the detection of CNVs. Body fat mass was measured by a dual-energy X-ray absorptiometry scanner (Hologic Discovery A system). Among the 217 CNVs that passed the quality control, we found that a CNV (CNP12610) located at chromosome 18q22.2 was associated with body mass index (BMI) and body fat mass. This CNV achieved an experiment-wise P-value of 4.61×10^{-5} for BMI and of 1.4×10^{-4} for body fat mass. Compared with normal diploid (copy number=2), CNV loss (copy number=0 or 1) confers an average increase in BMI and body fat mass of 2.27 kg/m^2 and 3.86 kg, respectively. CNP12610 covers the docking protein 6 (*DOK6*) gene which was reported to played a role in the *RET* (ret proto-oncogene) signaling cascade. The *RET* gene encodes one of the receptor tyrosine kinases, which are cell-surface molecules that transduce signals for cell growth and differentiation. Mutations in the *RET* gene were reported to be associated with endocrine disorders such as multiple endocrine neoplasia. Our study is the first suggesting the potential contribution of the *DOK6* gene to obesity in Chinese.

1060/T

Design of a replication study in advanced age-related macular degeneration Mexican patients based on genome-wide association studies. *A. Contreras[1], J.C. Fernandez-Lopez[1], F. Morales-Mandujano[1], L. Sebastian-Medina[1], U. Rodriguez-Corona[1], J.C. Canseco-Mendez[1], A. Ramos-Perez[1], J.C. Zenteno-Ruiz[2], D. Ochoa-Contreras[3], K. Estrada[1], S. March[1], E. Graue[2,4], G. JImenez-Sanchez[1], I. Silva-Zolezzi[1].* 1) INMEGEN, Mexico DF, Mexico; 2) Conde de Valenciana Hospital, IAP. Mexico DF, Mexico; 3) School of Medicine, UNAM, Mexico DF, Mexico; 4) Asociacion para Evitar la Ceguera en Mexico, AC., Mexico DF, Mexcio.

Age-related macular degeneration (AMD) is the most common cause of central blindness in the elderly population. Four genome-wide association studies, two with ~100,000 SNPs, one with ~600,000 SNP and one with ~2.5 x 106 SNPs (imputed) have demonstrated the clear association (p£7E-7) of 8 genes with AMD: Complement Factor H (*CFH*), complement component 2 and 3 (*C2* and *C3*), complement factor B and I (*CFB* and *CFI*), age-related maculopathy susceptibility (*ARMS2*), TIMP metallopeptidase inhibitor 3 (*TIMP3*) and Lipase C (*LIPC*). In order to search for new genes and variants associated to AMD in an admixed we genotyped ~1 x 10^6 SNPs in 100 unrelated Mexican patients with advanced AMD, 100 unrelated healthy controls and 300 population controls from the Mexican Genome Diversity Project (MGDP). Our GWAS results replicated the association found in ARMS2 rs1040924 (p-value<4.0E-7). Additional signals with suggestive p-values<5.0E-5 were identified in regions not previously associated to AMD in chromosomes 3, 5 and 7. The association all regions identified in Caucasians except rs10490924, did not achieve GWAS statistical significance because our GWAS study is underpowered. We are currently performing a replication study in a larger case-control group in candidate regions based on the results from GWAS in other populations and in Mexicans in 200 AMD patients, 200 healthy controls and 300 population controls from the MGDP. This study includes 134 SNPs in the candidate regions and 250 AIMs to correct for population stratification. Our study suggests that contribution of genetic variants to disease-risk in AMD in Mexicans may differ to that of other populations and that the genomic structure of admixed populations may allow the identification of new disease-associated genes.

1061/T

A Genome-wide Association Study of Human Intelligence. *G. Davies[1], A. Tenesa[2], A. Payton[3], X. Ke[3], K.A. McGhee[1], M. Luciano[1], S.E. Harris[1,4], A.J. Gow[1], D. Liewald[1], A. Christoforou[11,12], V.M. Steen[11,12], T. Espeseth[13], A.J. Lundervold[14], I. Reinvang[13], S. Le Hellard[11,12], H. Fox[10], P. Haggarty[9], G. McNeill[9], W.E.R. Ollier[3], A. Pickles[8], D. Porteous[4], J.M. Starr[7], M. Horan[6], N. Pendleton[6], P.M. Visscher[5], I.J. Deary[1].* 1) University of Edinburgh Centre for Cognitive Ageing and Cognitive Epidemiology, Department of Psychology, University of Edinburgh, 7 George Square, Edinburgh, UK; 2) Colon Cancer Genetics Group, Institute of Genetics and Molecular Medicine, University of Edinburgh and MRC Human Genetics Unit, Edinburgh, UK; 3) Centre for Integrated Genomic Medical Research, Stopford building, The University of Manchester, Oxford road, Manchester, M13 9PT; 4) Medical Genetics Section, University of Edinburgh Molecular Medicine Centre and Institute of Genetics and Molecular Medicine, Edinburgh, UK; 5) Genetic Epidemiology, Queensland Institute of Medical Research, Brisbane, Australia; 6) Clinical Neurosciences, School of Translational Medicine, The University of Manchester, Hope Hospital, Salford, Greater Manchester, UK; 7) University of Edinburgh Centre for Cognitive Ageing and Cognitive Epidemiology, Geriatric Medicine, University of Edinburgh, Royal Victoria Hospital, Edinburgh, UK; 8) Biostatistics Group, Community Based Medicine, SCAN Building Complex, The University of Manchester, Manchester, UK; 9) Environmental & Occupational Medicine, Liberty Safe Work Research Centre, Foresterhill Road, Aberdeen, UK; 10) Scottish Dementia Research Network, Royal Victoria Hospital, Edinburgh, UK; 11) Bergen Mental Health Research Center, Department of Clinical Medicine, University of Bergen, Bergen, Norway; 12) Dr Einar Martens' Research Group for Biological Psychiatry, Center for Medical Genetics and Molecular Medicine, Haukeland University Hospital, Helse Bergen HF, Bergen, Norway; 13) Center for the Study of Human Cognition, Department of Psychology, University of Oslo, Oslo, Norway; 14) Department of Biological and Medical Psychology, University of Bergen, Bergen, Norway.

General cognitive ability has a high heritability through the human lifecourse from adolescence to old age. The field of cognitive genetic research aims to identify the genetic variants responsible for high cognitive functioning and successful cognitive ageing. To date, there are almost no replicated variants from candidate gene studies that contribute to variation in cognitive functioning. An exception is the contribution of APOE at older ages. Genome-wide studies have been inconclusive, but suggest large numbers of genes with very small effect. Limitations with studies to date include inadequate sample size, population stratification effects, and differences in cognitive measurements. A collaborative project between the University of Edinburgh and the University of Manchester examines data from five Caucasian cohorts. Genotyping has been performed on approximately 3800 DNA samples obtained from elderly volunteers from the Greater Manchester, Tyne and Wear, Aberdeen and Lothian (Edinburgh) areas of the UK using the Illumina 610 microarray. Mental and physical health, personality traits and socioeconomic status are available in addition to detailed cognitive measurements. Initial analyses focused on fluid and crystallized intelligence phenotypes. Fluid intelligence was based on either latent traits from a number of non-verbal tests, or from reasoning tests. Crystallized intelligence was based on vocabulary tests. Fluid-type intelligence declines with age and crystallized intelligence holds up better. Association analysis, of each cohort, was performed in PLINK using linear regression analysis under an additive genetic model. Meta-analyses of these association results were then carried out in METAL, using an inverse standard error method. Preliminary GWAS meta-analysis results for fluid and crystallised intelligence suggest evidence of polygenic control. Replication in an independent Norwegian population has produced positive results.

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Genome-Wide Association Scan Identified UQCC Locus for Spine Bone Size in Humans. *F.Y. Deng[1, 2], S.F. Lei[1, 2, 3], X.G. Liu[4], T.L. Yang[4], Y. Guo[4], S.S. Dong[4], X.H. Xu[4], Q. Tian[1, 2], Y.J. Liu[1, 2], Y.Z. Liu[1, 2], J. Li[1, 2], H. Shen[1, 2], H.W. Deng[1, 2, 3, 5].* 1) Human Genetics/Genomics Program, Department of Orthopedic Surgery, UMKC, Kansas City, MO; 2) Department of Basic Medical Science, School of Medicine, UMKC, Kansas City, MO; 3) College of Life Sciences, Hunan Normal University, Changsha, Hunan 410081, P. R. China; 4) School of Life Science and Technology, Xi'an Jiaotong University, Xi'an, Shaanxi 710049, P. R. China; 5) Center of Systematic Biomedical Research, Shanghai University of Science and Technology, Shanghai 200093, P. R. China.

Bone size (BS) is one of the major indexes for bone strength, and BS contributes significantly to the pathology of osteoporotic fracture. Osteoporotic fracture at spine is one of the most disabling outcomes of osteoporosis in humans. To identify genomic loci underlying spine BS variation, we performed a genome-wide association scan (GWAS) in a sample of 2,286 unrelated Caucasian subjects using Affymetrix 6.0 SNP arrays which cover 909,622 SNPs across the human genome. Areal BS (cm^2) at lumbar spine was measured using daily calibrated dual energy X-ray absorptiometry machines. PLINK tool set was used to conduct the genotypic association analyses. Principle component analyses method was utilized to correct for population stratification for the GWAS analyses. Out of a total of 92 SNPs with p <10e-4 in the initial GWAS test, a group of 8 contiguous SNPs (rs6060369, rs6088791, rs6060373, rs2425062, rs2248393, rs4911178, rs1570004, rs6142358), which cluster at UQCC gene locus (ubiquinol-cytochrome c reductase complex chaperone), was identified. The association of the above identified SNPs with spine BS were further replicated in a second independent sample of Caucasians (one-sided $p < 0.1$), as well as in a third sample of Chinese (one-sided $p < 0.05$). With meta-analyses, more significant p values were attained for the 8 SNPs, providing further evidence of their associations with spine BS in humans. The group of SNPs, with strong linkage disequilibrium with each other, tightly form one haplotype block. This study identified UQCC as a novel locus underlying spine BS variation in humans. The mechanism, by which the UQCC locus regulates bone growth and development, still awaits demonstration by future functional studies.

1063/T

Bivariate Genome-Wide Association Analyses for Obesity and Osteoporosis in Chinese. *H.W. Deng[1,2,3], X. Li[1], L.J. Tan[1], X.G. Liu[1], S.F. Lei[1,2], X.D. Chen[1].* 1) Laboratory of Molecular and Statistical Genetics, College of Life Sciences, Hunan Normal University, Changsha, Hunan, 410081, P. R. China; 2) School of Medicine, University of Missouri-Kansas City, 2411 Holmes Street, Kansas City, MO 64108, USA; 3) Systematic Biomedicine Research Center, University of Shanghai for Science and Technology, Shanghai, 200093, P.R. China.

Obesity and osteoporosis are two common and closely genetically correlated diseases of major public health importance. Although a number of genome-wide association studies (GWAS) have been conducted separately for one of these two diseases, GWAS considering the potential genetic correlation between obesity and osteoporosis are rare. In this study we have performed a bivariate GWAS of obesity and osteoporosis, in order to identify the pleiotropic genes and the genetic mechanisms linking these two diseases. With Affymetrix Genome-Wide Human SNP Array 6.0 genotyped in ~1,600 unrelated Chinese subjects, we focused on two phenotypes, body mass index for obesity and bone mineral density for osteoporosis. Our preliminary results found that a number of SNPs may play pleiotropic roles for obesity and osteoporosis such as rs9503404 on chromosome 6. Our findings, together with the prior biological evidence, will advance our understanding on the co-regulation of obesity and osteoporosis, and aid in the management and treatment for the two diseases.

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Empirical power of regression methods for cluster-correlated data of quantitative traits in GWAS Based on the Electronic Medical Record; A Mayo eMERGE Study. *K. Ding[1], K.R. Bailey[2], I.J. Kullo[1].* 1) Division of Cardiovascular Diseases, Mayo Clinic, Rochester, MN; 2) Division of Biomedical Statistics and Informatics, Mayo Clinic, Rochester, MN.

The Electronic Medical Record (EMR) is potential source of high throughput phenotyping to conduct genome-wide association studies (GWAS) including those of medically relevant quantitative traits. An important methodological challenge is dealing with multiple observations often noted in an EMR for a quantitative trait in an individual. We describe empirical power of regression methods for cluster-correlated data of quantitative traits, using six red blood cell (RBC) traits (hemoglobin, hematocrit, RBC count, MCV, MCH, and MCHC) as an example. For 3,012 patients in the Mayo electronic medical records and genomics (eMERGE) cohort, we extracted and compiled 20,650 measurements of six RBC traits from the Mayo EMR between 01/01/1994 to 09/30/2009. Several regression methods, including an aggregate regression analysis [ie, constructing a summary statistic (mean or median) in the same individuals], a weighted least square (WLS), and a generalized estimating equation (GEE), were used in cluster-correlated data analysis. In the context of GWAS, we used a variance-component model to simulate cluster-correlated data, and, for each method, construct the test statistic under the null hypothesis of no association. To quantify the statistical power of different methods, we simulated cluster-correlated data 1,000 times by incorporating the effect of a given genetic marker in the variance-component model. For most RBC traits, there was greater variance between than within clusters such that ~70% variation is attributed to differences between clusters. The exception was MCHC for which only ~45% variation could be attributed to differences between clusters. Under the null hypothesis of no association, we generated the distribution of test statistics under four different regression methods in 10 times simulation in the context of GWAS, i.e., 4,769,900 test statistics were generated for each trait under each regression method. We tabulated the empirical power of different methods by calculating the proportions of times that the critical value was exceeded at the significance level of 0.05 (two sided). We did not find that any one of the regression methods to be more powerful than the remaining methods by pairwise comparison using the McNemar's test. We suggest the use of empirical power to compare regression methods for cluster-correlated data of quantitative traits in GWAS.

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Analysis of known asthma-associated variants in population-based cohorts. *Z.K.Z. Gajdos[1, 2, 3], A. Ramasamy[4], M. Kuokkanen[5], S. Vedantam[1,2], H. Lyon[2], C. Palmer[1,2], E. Widen[6], J. Eriksson[7], P. Jousilahti[5], T. Laitinen[7], V. Salomaa[5], J.N. Hirschhorn[1,2,3], M.-R. Jarvelin[4], APCAT Consortium.* 1) Broad Institute, Cambridge, MA; 2) Children's Hospital Boston, Boston, MA; 3) Harvard Medical School Department of Genetics, Boston, MA; 4) Imperial College London, London, UK; 5) The National Institute for Health and Welfare, Helsinki, Finland; 6) The Institute for Molecular Medicine, Helsinki, Finland; 7) University of Helsinki, Helsinki, Finland.

Asthma is a major public health concern. Although environmental factors play a prominent role, about half of asthma susceptibility is due to inherited factors. Discovery of the genetic contributors to asthma could provide important insights into why patients get asthma, with potential implications for prognosis, diagnosis, treatment and prevention. Genome-wide association (GWA) studies have identified a number of asthma-associated loci, primarily through studies of cohorts ascertained for asthma or of population-based studies of asthma-related traits such as eosinophil counts. As with most polygenic traits, the power of GWA studies of asthma is limiting. To see if we could improve power using existing data, we utilized the fact that many population-based studies with genome-wide genotype data have also asked participants about physician-diagnosed asthma. Analyzing these data could supplement current studies by helping to validate known loci and increasing power to identify novel loci. To explore this possibility, we formed the Analysis in Population-based Cohorts of Asthma Traits (APCAT) consortium, which currently includes participants from Finland (Northern Finnish Birth Cohort of 1996, Finrisk cohorts, Health 2000, Helsinki Birth Cohort, and Young Finns Study) and the United States (Framingham Heart Study). Information from 2,091 physician-diagnosed asthmatics and 15,463 healthy controls were available for analyses. We are currently analyzing the association of physician-diagnosed asthma with each of ~2.2 million SNPs, assuming an additive genetic model, and stratifying by smoking status (never, former, and current smokers). To confirm the utility of these population-based cohorts, we first examined eight variants with strong evidence from the literature for association with asthma, at or near ORMDL3, IL1RL1, WDR36, IL33, MYB, IL13, PDE4D, and DENND1B. The direction of association for all eight SNPs was consistent with the prior literature. Four of the eight SNPs were nominally associated with asthma (1-tailed P value < 0.05), with the most significant association at IL33 (rs3939286, 1-tailed $P = 8.63 \times 10^{-6}$). These results provide good evidence that population-based samples can be helpful to validate SNPs identified in case-control studies, and suggest that GWA studies in these samples can serve as a useful complement to GWA studies of samples specifically ascertained for asthma.

1066/T

Genes regulating skin color in Koreans: The Health Twin Study. *M. Han[1], J. Sung[1], Y.M. Song[2], K. Lee[3], M. Lee[1], D.H. Lee[1].* 1) Seoul National University, Seoul, Korea; 2) Department of Family Medicine, Samsung Medical Center, SungKyunKwan University School of Medicine; 3) Department of Family Medicine, Busan Paik Hospital, School of Medicine, Inje University.

Variations in human skin color are but only inborn, not also adaptive traits that correlate closely with the sun's ultraviolet (UV) radiation. The skin's pigment is Melanin that protects peoples from the many harmful effects of ultraviolet (UV) rays. But it is very diverse with individual life style besides race. Recently genetic studies have identified a subset of genes influencing the extent of skin pigmentation; MATP, IRF4, TYR, OCA2, and MC1R were associated with skin colors in a Genome-wide association studies (GWAS) in Europian population. But little is known about the genetic regulators in skin pigmentation difference in Asian family-based population. Specific aims of the study was 1) to test whether there are genetic influences on the skin colors of both ultraviolet light(UV)-exposed (cheek) and UV-unexposed (arm, flank) in Asians, and 2) to identify the genes which are responsible for the degree of skin pigmentation in both body areas. A total of 1440 individuals of Korean twins and their families (543 men and 897 women aged from 17 to 80 years, mean age: 44.1 ± 0.4) join this study. The skin surface pigmentation, were measured using MPA 5, Courage-Khazaka electronic. The average skin pigmentation in cheek, arm and flank were 166.01±55.5, 155.48±49.3 and 134.07±51.9. Pearson correlation coefficients between skin brightness on the cheek and arm, the flank and arm, the flank and cheek are respectively 0.62 , 0.47 and 0.35. The heritabilities of skin pigmentation were 0.55 (p = 6.63e-39) for cheek and 0.66 (p = 3.02e-67)for arm, 0.58 (p = 1.00e-44) for flank area. Genome-wide association test was conducted with Affymetrix GeneChip version 6 (with 541,000 effective SNP markers), using FBAT. We identified rs7956106(12, p12.3,) rs12117410(1,-p31.1), rs11162211(1,-p31.1) SNPs associated with the skin pigmentation on the cheek , rs17016276(1,-q32.2), rs2501892(1,+q32.2), rs1542140(1,-q32.3) SNPs on the arm, and rs8060157(16, q12.1), rs9940645(16, +q12.1), SNPs on the flank. While these genes await replication or further validation, we concluded that different sets of genes may regulate the skin pigmentation according to the UV exposure status.

1067/T

Neurotransmitter receptor gene polymorphisms are associated with widespread pain: findings from the first genome-wide association study of musculoskeletal pain. *K.L. Holliday[1], T. Neogi[2], D.T. Felson[1,2], K. Wang[3], W. Thomson[1], J. McBeth[1].* 1) Arthritis Research UK Epidemiology Unit, University of Manchester, Manchester, United Kingdom; 2) Boston University School of Medicine, Boston, Massachusetts, USA; 3) Department of Biostatistics, Boston University School of Public Health, Boston, Massachusetts, USA.

Purpose: Musculoskeletal pain disorders are prevalent with 10% of the general population reporting widespread pain. Heritability estimates for widespread pain are around 50% although the susceptibility loci have yet to be identified. The purpose of this study was to identify susceptibility loci by conducting the first genome-wide association study (GWAS) of widespread pain. Methods: As part of the Framingham SHARe project, subjects from 3 generations were genotyped using Affymetrix 500k and 50k gene-focused SNP chips. Samples were excluded for low call rate (<95%), excess heterozygosity and mendelian errors. SNPs were excluded for deviation from HWE ($p<10^{-6}$), low call rate (<97%), MAF<1% and mendelian errors (>100). Whole genome imputation was carried out using HapMap CEU data and MACH v1.0.15. Data on musculoskeletal pain was available for a subset of these subjects (n=3850) from 2 generations, which was used to classify cases with widespread pain (reporting pain in contra-lateral body quadrants above and below the waist and in the axial skeleton) and controls (reporting no pain). Generalised estimating equation regression analysis was used to perform a case-control GWAS analysis under an additive genetic model on autosomal SNPs with a MAF>3%, while accounting for relatedness between subjects and using genomic control to adjust for population stratification in R. Results: Imputation was conducted on 378,163 SNPs meeting quality control criteria providing 2,321,937 SNPs for analysis in 572 cases and 1587 controls. The genomic inflation factor was 1.02. Using a p-value cut off of 1×10^{-5}, we identified 52 SNPs in association with widespread pain which comprise 8 individual loci, many of which are strong candidate loci for susceptibility to widespread pain. In particular, an intronic SNP in the neurotransmitter receptor gene *GABA B receptor 2* (*GABBR2*) was associated with an increased risk of having widespread pain (OR=1.37 (1.24, 1.51) $p=6.7 \times 10^{-6}$). A SNP 100 Kb downstream of *glutamate receptor 2* (*GRIA2*), was also associated with an increased risk of having widespread pain (OR= 1.54 (1.35, 1.73) $p=8.96 \times 10^{-6}$). Conclusion: This study, the first GWAS of widespread pain, implicates a number of biologically relevant loci including neurotransmitter genes that are directly linked to pain pathways. Validation of these findings is required in independent cohorts to ascertain the true impact of these loci on widespread pain susceptibility.

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PRIMe: A Method for Characterization and Evaluation of Pleiotropic Regions from Multiple Genome-wide Association Studies. *J. Huang[1,2], A.D. Johnson[1,2], C.J. O'Donnell[1,2].* 1) Framingham Heart Study, National Heart Lung & Blood Institute, framingham, MA; 2) Center for Cardiovascular Genomics, Division of Intramural Research, National Heart, Lung and Blood Institute, Bethesda, MD.

Background: The concept of pleiotropy was first defined a century ago as one mutation resulting in multiple independent phenotypes. The current abundance of genome-wide association studies (GWAS) results provides an unprecedented opportunity to fully examine this phenomenon in a systematic manner. **Methods & Results**: We developed the Pleiotropic Region Identification Method (PRIMe) to systematically identify and characterize genomic regions where significant association p-values are observed with multiple traits. PRIMe iteratively finds SNPs with the lowest association p-value as the *driver*, and SNPs whose r^2 with the *driver* is above a user specified threshold (≥ 0.8 by default) as *passengers*. Regions are defined by one *driver* and zero or more *passengers*. We denote Pleiotropy Index as the number of traits with significant association p-values at an identified genomic region. For GWAS conducted on traits with known correlation coefficients and for SNPs with known linkage disequilibrium (LD) structure, simulations were performed to derive the statistical distribution of the Pleiotropy Index. The simulation is based on a mathematical derivation that the correlation matrix between all *K* x *M* test statistics is simply the (kronecker) product of the *K* dimensional correlation matrix of the traits (ρ) and the *M* dimensional correlation matrix of the SNPs (*r*). Separately, we evaluated an approximation approach for independent traits and SNPs. For *K* independent traits each with *M* independent SNPs in the region, the number of traits with at least one SNP below a p-value threshold P_S follows a binomial distribution $B(n,p)$, with *n* equal to *K* and *p* equal to $1 - (1- P_S)^M$. Using our simulation approach, we identified pleiotropic regions including *SH2B3* and *BRAP* (both at 12q24.12) for GWAS of hematological and blood pressure traits. By the approximation approach, we confirmed the genome-wide significant pleiotropy of these two regions based on the NHGRI GWAS catalog, and identified further plausible regions of potential pleiotropy including the *FTO* and *ABO* regions. **Conclusion**: Our method can be used to generate further gene hypotheses and may facilitate targeting of pleiotropic regions for resequencing and functional follow-up. This new approach, together with continuing efforts to centralize GWAS results into accessible repositories, is well suited to advance the study of the genetic architecture of complex diseases beyond individual investigators or individual datasets.

1069/T

Discovering combined causal signals from genome-wide association studies by network module based approach. *P. Jia[1,2,3], S. Zheng[1,3], J. Sun[1,2,3], J. Rong[4], W. Zheng[4,5], Z. Zhao[1,2,3,5].* 1) Biomedical Informatics, Vanderbilt University, Nashville, TN; 2) Psychiatry, Vanderbilt University, Nashville, TN; 3) Bioinformatics Resource Center, Vanderbilt University, Nashville, TN; 4) Vanderbilt Epidemiology Center, Vanderbilt University, Nashville, TN; 5) Vanderbilt-Ingram Cancer Center, Vanderbilt University, Nashville, TN.

Genome-wide association studies (GWAS) have revealed hundreds of common variants susceptible to common diseases. While GWA studies have suggested hundreds of single markers/genes that are associated with various diseases, an important emerging question now is how to detect genetic signal beyond single markers/genes in order to explore their combined effects on mediating complex diseases/traits. Here, we introduced a module based method, dense module searching (DMS), to identify candidate subnetworks or genes for complex diseases by integrating the association signal from GWAS datasets into the human protein-protein interaction (PPI) network. The DMS method extensively searches for subnetworks enriched with low P-value genes in GWAS datasets. Specifically, we can apply this method to either single or multiple GWAS datasets. In the latter case, additional GWAS dataset(s) can be used for evaluation/validation of the modules found by the discovery GWAS dataset. The top ranked modules are evaluated by using permutation data so that significant modules can be selected for further analysis and validation. Compared with pathway-based approaches, this method introduces flexibility in defining a gene set and can effectively utilize local PPI information. We demonstrated DMS in two complex diseases, schizophrenia and breast cancer, which have different characteristics of pathology and heritability. We showed that disease candidate genes could converge on PPI subnetworks mediating critical biological functions. For each disease, the DMS method successfully identified a set of candidate genes, including some well studied genes that could not be detected in the original GWA studies. Our functional evaluation revealed that 1) for schizophrenia, several neurodevelopment-related functional categories were consistently enriched; and 2) for breast cancer, Wnt/β-catenin signaling and HER-2 signaling in breast cancer stood out as two of the most enriched pathways. These results indicate that the DMS method and strategy is effective in detecting real effect of susceptibility genes through their interaction in the context of PPI network.

1070/T

A QTL in the MHC class II gene region influences generalized vitiligo age-of-onset. *Y. Jin, P.R. Fain, R.A. Spritz.* Human Med Gen, Univ Colorado School of Medicine, Aurora, CO 80045.

Generalized vitiligo is a common autoimmune disease in which acquired patchy depigmentation of skin, hair, and mucous membranes results from loss of melanocytes from involved areas. Age-of-onset of generalized vitiligo involves a substantial genetic component, but has not previously been studied systematically. To identify loci that influence generalized vitiligo age-of-onset, we carried out a genome-wide association study in 1339 Caucasian generalized vitiligo patients, with replication in an independent cohort of 677 cases, treating age-of-onset as a quantitative trait. We identified a major association signal in the MHC class II gene region, in the vicinity of c6orf10-BTNL2 (rs7758128; genome-wide association $P = 9.94 \times 10^{-9}$ and replication $P = 2.23 \times 10^{-3}$). We previously identified this same region as a locus contributing to generalized vitiligo susceptibility per se in the same patient cohort. These findings highlight the critical role of the MHC class II gene region in both the pathogenesis and natural history of generalized vitiligo, and perhaps other autoimmune diseases, possibly via mediating recognition and presentation of environmental triggers of disease by the immune system.

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Trait-stratified genome-wide association study identifies novel and diverse genetic associations with serologic and cytokine phenotypes in systemic lupus erythematosus. *S.N. Kariuki[1], B.S. Franek[1], A.A. Kumar[1], J. Arrington[1], R.A. Mikolaitis[2], T.O. Utset[1], M. Jolly[2], M.K. Crow[3], A.D. Skol[4], T.B. Niewold[1].* 1) University of Chicago Pritzker School of Medicine, Section of Rheumatology and Gwen Knapp Center for Lupus and Immunology Research, 924 E 57th Street, Chicago, IL 60637; 2) Rush University Medical Center, Section of Rheumatology, 600 South Paulina Street, Chicago, IL 60612; 3) Hospital for Special Surgery, Mary Kirkland Center for Lupus Research, 535 East 70th Street, New York, NY 10021; 4) University of Chicago Pritzker School of Medicine, Section of Genetic Medicine, 5841 South Maryland Ave, Chicago, IL 60637.

Introduction: Systemic lupus erythematosus (SLE) is a highly heterogeneous disorder, characterized by differences in autoantibody profile, serum cytokines, and clinical manifestations. SLE-associated autoantibodies and high serum interferon alpha (IFN-α) are important heritable phenotypes in SLE which are correlated with each other, and play a role in disease pathogenesis. These two heritable risk factors are shared between ancestral backgrounds. The aim of the study was to detect genetic factors associated with autoantibody profiles and serum IFN-α in SLE. Methods: We undertook a case-case genome-wide association study of SLE patients stratified by ancestry and extremes of phenotype in serology and serum IFN-α. Single nucleotide polymorphisms (SNPs) in seven loci were selected for follow up in a large independent cohort of 538 SLE patients and 522 controls using a multi-step screening approach based on novel metrics and expert database review. The seven loci were: leucine-rich repeat containing 20 (LRRC20); protein phosphatase 1H (PPM1H); lysophosphatidic acid receptor 1 (LPAR1); ankyrin repeat and sterile alpha motif domain 1A (ANKS1A); protein tyrosine phosphatase, receptor type M (PTPRM); ephrin A5 (EFNA5); and V-set and immunoglobulin domain containing 2 (VSIG2). Results: SNPs in the LRRC20, PPM1H, LPAR1, ANKS1A, and VSIG2 loci each demonstrated strong association with a particular serologic profile (all OR>2.2 and p<3.5x10-4). Each of these serologic profiles was associated with increased serum IFN-α. SNPs in both PTPRM and LRRC20 was associated with increased serum IFN-α independent of serologic profile (p=2.2 x 10-6 and p= 2.6 x 10-3 respectively). None of the SNPs were strongly associated with SLE in case-control analysis, suggesting that the major impact of these variants will be upon subphenotypes in SLE. Conclusions: This study demonstrates the power of using serologic and cytokine subphenotypes to elucidate genetic factors involved in complex autoimmune disease. The distinct associations observed emphasize the heterogeneity of molecular pathogenesis in SLE, and the need for stratification by subphenotypes in genetic studies. We hypothesize that these genetic variants play a role in disease manifestations and severity in SLE.

1072/T

Family-based genome-wide association study for length or height from infancy to early childhood. *H. Kim[1], E. Lee[2], H. Kim[1], S. Jung[1], B. Han[2], J. Lee[2], H. Chung[3].* 1) 1Department of Biochemistry, School of Medicine, Ewha Womans University, Seoul, Korea; 2) Center for Genome Science, Korea National Institute of Health, Korea Centers for Disease Control and Prevention, Seoul, Korea; 3) Department of Obstetrics and Gynecology, School of Medicine, Ewha Womans University, Seoul, Korea.

With increasing intermarriage and the number of admixed infants, understanding the anthropometric variation of admixed infants and children is important. To identify genetic factors that influence infant and childhood height, family-based genome-wide association analyses were conducted using 269,888 single nucleotide polymorphisms (SNPs) in 165 trios composed of a Korean father, a Vietnamese mother, and Vietnamese-Korean offspring of a marriage-based immigrant cohort in Korea. In a single-SNP-based analysis, the six SNPs in or near the genes BMP4, MAF, MAGI2, and PTPN7 showed consistent suggestive associations at all height standard deviation scores using Korean, World Health Organization, and Vietnamese growth references. We did not find genome-wide significant associations with height after multiple-testing correction in a single SNP-based analysis. However, the haplotypes in linkage disequilibrium block, which contained the SNPs near the suspected loci, were significantly associated with height. Similar to the results of contiguous haplotype analysis using tagged SNPs, noncontiguous haplotypes of variable length also showed a significant association near the suspected loci. Our results demonstrate that infant and childhood height may be regulated by genetic variations that differ from those of adults and remind us of the need to look at human height from a different perspective, namely age. This study is the first genetic association analysis on cross-sectional infant and childhood height in admixture families, and it provides a basis for future investigations into the genes acting at each stage of height growth.

1073/T

Using shared controls in genome-wide association studies. *R.J. Klein[1], S. Mukherjee[1,2], R. Kurtz[3], S.H. Olson[4].* 1) Program in Cancer Biology and Genetics, Memorial Sloan-Kettering Cancer Center, New York, NY; 2) Gerstner Sloan-kettering Graduate School of Biomedical Sciences, New York, NY; 3) Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY; 4) Department of Epidemiology and Biostatistics, Memorial Sloan-Kettering Cancer Center, New York, NY.

Though genome-wide association studies have successfully identified numerous susceptibility loci for common diseases, their use is limited due to the expense of collecting and genotyping large cohorts of individuals. In a hospital-based setting recruitment of controls to studies can be especially difficult. One potential solution to these problems is to use “shared controls,” or data from control individuals genotyped as part of other studies that have been deposited in public repositories. While such an approach has been successfully used by several groups, the genetically heterogeneous nature of the US population makes this approach potentially problematic in the US. Even if analysis is restricted to individuals with a single continental ancestry, if cases and controls are recruited from different places in the U.S. underlying population substructure may appear and lead to false positive findings. To investigate the utility of this approach in US-based GWAS, we applied this approach to an ongoing GWAS of pancreatic cancer. Using analytical calculations, we estimate that once the case:control ratio exceeds 1:10, additional controls do not significantly add to the power of the study. We demonstrate that in our cohort of 263 pancreatic cancer cases and 203 controls from the New York area, the proportions of individuals whose genetic data suggests southeastern Europe or Ashkenazi Jewish ancestry is larger than in individuals in the dbGaP database. When we add up to 5629 additional controls from dbGaP, we find that there is a large inflation of the test statistic that is properly corrected for using eigenvectors from principal components analysis as covariates. Most of this correction is achieved just by using the first six principal components. While the four SNPs identified as pancreatic cancer risk factors by the PanScan study show hardly any association with pancreatic cancer in our cohort alone, results that are trending significant are observed when we add in shared controls from dbGaP. While several SNPs reach a level of genome-wide significance in our analysis, none of them are significant at the P=0.05 level in the PanScan data, suggesting that false positives are a concern with this kind of approach. These data suggest a potential new approach to GWAS in which a smaller number of cases are genotyped genome-wide, analyzed in conjunction with controls from dbGaP, and then the top SNPs are genotyped in a larger case-control set.

1074/T

Genetic factors contributing to Metabolic syndrome: the Healthy Twin Study in Korea. *J. Lee[1], J. Sung[1], Y.M. Song[2], K. Lee[3], M.K. Lee[1], D.H. Lee[1].* 1) Department of Epidemiology, Seoul National University of Public Health; 2) Department of Family Medicine, Samsung Medical Center, SungKyunKwan University School of Medicine; 3) Department of Family Medicine, Busan Paik Hospital, School of Medicine, Inje University.

Metabolic syndrome (MetS) is one of the major health problems, due to its increasing prevalence and association with chronic disease. Numerous studies have reported genetic and environmental contributions to MetS, and several susceptibility loci for MetS have been identified through genome-wide association (GWA) studies. While MetS definition is useful for educational and preventive purposes, the scientific validity of these criteria has been argued, and some suggested that MetS components can be classified into two heterogenous components; 1)obesity—glucose intolerance—dyslipidemia; 2)hypertension mainly by covariance analysis. This study aims to estimate genetic effects on each MetS trait—waist circumference, HDL cholesterol, triglycerides, blood pressure (BP), fasting blood glucose—and to test whether each component shares overall genetic effects or specific genetic loci. In addition, we attempted to replicate reported loci and identify new region for this end from the Healthy Twin Study in Korea. 1,857 individuals from 635 pedigrees were genotyped with Affymetrix GeneChip v6.0 (1M). Overall genetic influences on MetS were measured by heritabilities (h2), and correlation of MetS traits between cotwins was also considered. 4 traits except for TG showed significant h2 (>.5) after adjustment, and all traits showed higher correlation between MZ cotwins than between DZ cotwins, further suggesting influence of genetic factors on MetS. Since MetS traits tend to cluster in individuals, we generated new factors by Principal Component (PC) analysis, and PCs also showed high h2 (>.5). GWA analyses for each trait showed significant associations with SNP markers, some of which were replication. The 1p21.2 (rs11166440) and 13q13.3 (rs6563539) regions were associated with diastolic and systolic BP level in this study, which were previously reported associations with aortic root and heart-rate respectively. We also attempted to identify susceptibility loci using latent composite phenotypes of MetS and PC1 exhibited significant p-values in 2p22.1, implying there are latent factors in developing MetS. According to previous reports, genetic associations with MetS were found in chromosome 2p23 and 2p24, supportive to this finding in terms of physical distance on chromosome. Our results suggest some components of current MetS, but not all, may have specific genetic ground, especially between obesity and lipid levels, but another genetic component will regulate BP levels.

1075/T

Genome-Wide Association Study of Saliva Flow Rates from the COHRA-GENEVA Study. *M. Lee[1], K.T. Cuenco[1], X. Wang[1], J.R. Shaffer[1], F. Begum[1], E. Feingold[1], D.E. Weeks[1], M.M. Barmada[1], S. Wendell[1], D. Crosslin[2], C. Laurie[2], B. Weir[2], K.F. Doheny[3], E. Pugh[3], R.J. Weyant[1], R.J. Crout[4], D.W. McNeil[4], M.L. Marazita[1].* 1) University of Pittsburgh, Pittsburgh, PA; 2) University of Washington, Seattle, WA; 3) Johns Hopkins, Baltimore, MD; 4) West Virginia University, Morgantown, WV.

Background and Objective: The Appalachian region experiences limited healthcare access and negative health outcomes including oral disease. As a result of disproportionate levels of dental caries found in the Appalachians, the Center for Oral Health Research in Appalachia (COHRA) began evaluating underlying risk factors for dental caries in families. COHRA participants, as part of the NIH Gene, Environment Association Studies Consortium (GENEVA), were included in a genome-wide association study (GWAS) to identify genetic variants and assess environmental factors related to dental caries. Dental caries are heritable (29%~40%) even after adjusting for shared environmental factors in this population. Caries risk may be influenced by saliva flow altering the oral environment. Saliva flow itself may also be under genetic influence. **Samples and Methods:** Measures included caries status, demographics, and unstimulated saliva flow rates (ml/min). The estimated heritability of saliva flow is 49% in COHRA subjects. A GWAS was conducted of unstimulated saliva flow rates (mean=0.581(ml/min), sd= 0.418) in COHRA-GENEVA subjects (n = 1506; 647 males and 859 females) who are self-reported Caucasian, and 5 to 75 years of age. The Center for Inherited Disease Research generated subject genotypes with the Illumina 610-Quad platform. Genotype quality control was performed by both GENEVA Coordinating Center and our analytic group (548,874 SNPs with Minor Allele Frequency (MAF)>0.02 and Hardy-Weinberg Equilibrium (HWE) p-value>0.0001). The association between each SNP and transformed saliva flow rate was assessed using regression analysis ignoring familial relationships but adjusted for gender and age under an additive genetic model and will be repeated with adjustments for relatedness (variance components-SOLAR). **Results:** Preliminary GWAS results suggest regions of chromosomes 2, 8, and 13 may be associated with a cubic-root transformed saliva flow rate. We are currently assessing the utility of applying a two-stage sampling methodology which involves repeated split samples. This method may be a useful alternative for other seldom measured traits for which independent replication populations are unavailable. These data also provide the initial findings for specific gene regions associated with unstimulated saliva flow rates in this underserved population. NIH grants DE014899, DE018903.

1076/T

Identificaiton of candidate variant for Intraocular Pressure using Korean twin families and Mongolian large families: The Healthy Twin Project and GENDISCAN Project. *M. Lee[1], J. Sung[1], Y. Song[2], K. Lee[3], S. Cho[1], D. Ham[4], D.H. Lee[1], J. Seo[5].* 1) Complex Disease and Genetic Epidemiology Branch, Department of Epidemiology and Institute of Environment and Health, School of Public Health Seoul National University; 2) Department of Family Medicine, Samsung Medical Center, and Center for Clinical Research, Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, Seoul, Korea; 3) Department of Family Medicine, Busan Paik Hospital, Inje University College of Medicine, Korea; 4) Department of Ophthalmology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; 5) Department of Molecular Medicine, School of Medicine, Seoul National University, Korea.

Intraocular pressure (IOP) is one of the major risk factors for Primary open-angle glaucoma (POAG): the second most frequent cause for visual impairment worldwide. Genetic studies of IOP have been mainly accomplished in glaucomatous families among Caucasians therefore searching for causative variants for IOP variation using healthy population will provide further insight.Particularly, studies from Asians, who show different epidemiologic characteristics for IOP and glaucoma will bear academic and public health significance. The Healthy Twin study and The GENDISCAN study recruited large families from Korea and two isolated regions (Selenge and Dashibalbar) in Mongolia. Clinical information and epidemiologic data were collected and IOP values were measured using non-contact method. For Selenge population with 1451 individuals from 142 families we had genotyped 390 short tandem repeat (STR) markers and 370K genome-wide SNPs (Illumina). In Dashibalbar we collected 1490 individuals from 95 families and the genotyping was done with 1097 STR markers, and 610K genome-wide SNPs (Illumina) for 652 individuals. In Korean twin-families, 1502 individuals from 396 families were genotyped using Affymetrix GeneChip version 6.0 for 1376 individuals. Family-based genome-wide linkage and association approach was adopted for investigating causative variants regulating IOP variation. For regions with replicated by linkage analysis, we further tested for associations in both populations. The heritabilities of IOP level were 0.48, 0.50, and 0.62 adjusted for age, sex and age by sex interaction for two populations in Mongolia in Koreans. The GWAS analysis revealed several candidate genes. SNPs with genome wide significance in two populations were closely located to POAG candidate gene on Chromosome 5: Zinc Finger protein 608 (ZNF608) and WD repeat domain 36 (WDR36). In those two regions, there are several significant SNPs and haplotypes associated with IOP elevation: C alleles on rs7723819 increased IOP level by 7% and 2% for having one more C allele (WDR36, C allele frequency 39%). Our study showed that QTL regulating IOP in general populations from Korea and Mongolia are linked to regions harboring POAG genes. Furthermore we suggest that the QTL from our analysis can influence both normal variation and disease occurrence and some genes may regulate both normal variation and the development of disease.

1077/T

Genome-wide association Study for Renal Traits in African Americans: the CARe Renal Consortium. *C.-T. Liu[1], A. Tin[2], A. Kottgen[3], X. Lu[1], I. de Boer[4], N. Franceschini[5], C. Peralta[6], E. Akylbekova[7], S.-J. Hwang[8], T.S. Leak[9], J. Mychaleckyi[10], M. Reilly[11], H. Kramer[12], R. Townsend[13], A. Dreisbach[14], M. Shlipak[15], M. Flessner[16], D. Siscovick[17], A. Cupples[1], W.L Kao[18], C.S. Fox[8] on behalf of the CARe and CKDGen Consortiums.* 1) Department of Biostatistics School of Public Health Boston University, Boston, MA; 2) Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, 615 N. Wolfe Street, Baltimore, MD; 3) Department of Epidemiology, Johns Hopkins University, 615 N. Wolfe St., Room 6021, Baltimore, MD 21205; 4) Division of Nephrology, University of Washington, Box 357183, 1959 NE Pacific St, Seattle, WA 98195; 5) Department of Epidemiology, University of North Carolina at Chapel Hill, 137 E. Franklin St., Suite 306 CB#8050 Chapel Hill, NC 27514; 6) Division of Nephrology University of California, San Francisco Medical School and San Francisco VA Medical Center, 4150 Clement Street, San Francisco, CA 94121; 7) Jackson State University, 350 West Woodrow Wilson Drive, Suite 701, Jackson, MS 39213; 8) NHLBI's Framingham Heart Study and the Center for Population Studies, 73 Mt Wayte Ave Suite #2, Framingham MA 01702; 9) Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Graduate School of Public Health, 130 N. Bellefield Avenue, Room 512, Pittsburgh PA 15213; 10) Center for Public Health Genomics, University of Virginia, PO Box 800717, Charlottesville, VA 22908-0717; 11) University of Pennsylvania Division of Cardiology, Perelman Center for Advanced Medicine, East Pavilion, 2nd Floor; 3400 Civic Center Boulevard, Philadelphia PA 19104; 12) Loyola University, 2160 South First Avenue, Bldf 105 Rm 3385, Maywood, IL 60153; 13) University of Pennsylvania Renal Electrolyte and Hypertension Division, 3400 Spruce St, Philadelpha PA 19104; 14) University of Mississippi Division of Nephrology, 2500 North State Street, University of Mississippi, Jackson, MS 39216; 15) General Internal Medicine, University of California, San Francisco, 4150 Clement St. , San Francisco, CA 94121; 16) Department of Medicine, University of Mississippi Medical Center, 2500 North State Street, Jackson, MS 39216-4505; 17) Departments of Epidemiology and Medicine, University of Washington, Seattle, WA, USA, 1730 Minor Ave, Suite 1360, Seattle, WA, 98101; 18) Department of Epidemiology and the Welch Center for Prevention, Epidemiology, and Clinical Research, Johns Hopkins University, 615 N. Wolfe St., Room 6513, Baltimore, MD 21205.

Background: Chronic kidney disease (CKD) is an increasing global public health problem. Although hypertension and diabetes are major risks factors for CKD, there is considerable evidence that genetic factors also contribute. To date, most studies have reported results for individuals of European ancestry; few studies have reported results in those of non-European ancestry. Here we present the results of a genome-wide association (GWA) study from the CARe Renal Consortium in African Americans. Methods: Using estimated glomerular filtration rate (eGFRcrea) defined by the MDRD equation, we performed a meta-analysis of GWA data in 7382 individuals of African ancestry from four CARe studies (ARIC, CARDIA, JHS and MESA) to identify potentially susceptibility loci for eGFRcrea and CKD (eGFRcrea < 60 ml/min/1.73m2). We also developed an algorithm to interrogate and validate signals reported in European ancestry individuals by interrogating their associated regions in African ancestry participants. Results: No individuals SNPs reached genome-wide significance. However, we identified four potential novel SNPs, rs6581768 (chr 12, p=3.0E-07, eGFRcrea), rs7784820 (chr 7, p=5.3E-07, eGFRcrea), rs1257581 (chr 11, p=9.97E-07, CKD) and rs6428106 (chr 1, p=2.95E-07, CKD), which are respectively near DYRK2 (related to glycogen synthesis regulation), GNAT3 (related to gut capacity to absorb sugars), B3GAT1 and RGS1 (highly expressed in renal cell carcinoma tissues) region. We also interrogated 24 previously published loci associated with eGFRcrea identified by the CKDGen Consortium. Of the 24 lead SNPs identified in participants of European ancestry, 23 had direction-consistent beta coefficients among our AA samples. We subsequently interrogated the 250 kb flanking regions for the best SNP in each region, and identified 8 SNPs in participants of African ancestry that reached significance after Bonferroni correction by the number of independent corrected SNPs within the region. Conclusion: In 7382 African Americans, we identified 4 potential novel loci and replicated eight loci previously associated with eGFRcrea in whites. While our findings require replication, our study supports the importance of multi-ethnic GWAS in the identification of novel loci and underscores the similarity of genetic architecture among European and African populations.

1078/T

Using data mining to detect main effects and interactions in GWAS. *G. Lubke, R. Walters, C. Laurin.* University of Notre Dame, Notre Dame, IN.

The common approach in genome-wide association studies (GWAS) is to test single nucleotide polymorphisms (SNPs) univariately, thus necessitating substantial corrections for multiple testing. We investigated the precision rate of a data mining procedure to detect small main effects ($<10^{-7}$) and 2-way interaction effects using simulated genome-wide data. The procedure is carried out in a parallelized way, and is computationally feasible on clusters with 32Gb RAM. The procedure can be used as a screen to select a small proportion of promising SNPs for further parametric testing.

1079/T

A genome-wide association analysis of gender-specific effects of fasting glycaemic traits. *R. Magi[1], J.J. Hottenga[2], I. Prokopenko[1,3], MAGIC investigators.* 1) WTCHG, University of Oxford, Oxford, United Kingdom; 2) Department of Biological Psychology, VU University Amsterdam, Amsterdam, Netherlands; 3) OCDEM, University of Oxford, Oxford, United Kingdom.

Meta-analyses of genome-wide association (GWA) studies for glycaemic traits have described 16 loci influencing fasting glucose (FG) and two loci influencing fasting insulin (FI) levels. Within the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) we aimed to investigate gender-specific differences in genetic regulation of FG/FI levels. To do this, we performed gender-stratified meta-analyses of 36 genome-wide association (GWA) studies informative for FG and FI within MAGIC in up to 32,993 and 27,870 non-diabetic men and 42,149 and 34,940 non-diabetic women, respectively.

Among previously described loci *DGKB-TMEM195I* demonstrated the largest gender-specific effect (heterogeneity Q P=0.026, I^2=80%) showing stronger association in men (β=0.031 [SE=0.004], P=9.1x10^{-14}) than in women (β=0.019 [SE=0.004], P=1.1x10^{-7}). Gender-specific heterogeneity for *MTNR1B* was also high (Q P<0.05, I^2=75%) with larger effects for men (β=0.080 [SE=0.005], P=3.7x10^{-56}) than for women (β=0.067 [SE=0.004], P=3.3x10^{-51}). Effects estimates in women were higher for *C2CD4B* and *GCKR*, where the gender heterogeneity was present, but not significant (I^2~60%, Q P>0.05). *G6PC2* and *ADCY5* showed moderate and *GCK*, *PROX1*, *SLC30A8* low between-gender heterogeneity for FG (I^2£50% and I^2<25%, respectively). In the FG meta-analysis we uncovered a novel locus near *PCSK1* showing strong gender heterogeneity (Q P=0.055, I^2=73%) with women showing β=0.023 [SE=0.003], (P=3.0x10^{-9}) and men β=0.012 [SE=0.004], (P=0.008); overall β=0.018 [SE=0.003], (P=9.0x10^{-10}). The FI meta-analysis revealed higher effect estimates in men (β=0.034 [SE=0.006], P=3.3x10^{-8}) compared to women (β=0.018 [SE=0.006], P=0.001) at rs860598 near *IGF1* locus (overall β=0.024 [SE=0.004], P=3.6x10^{-9}, heterogeneity Q P=0.042, I^2=76%).

Our analysis showed the novel FG-associated locus near *PCSK1* and four known FG- or FI-associated loci have differential gender-specific effects. These findings may provide new insights into the physiological mechanisms linking these loci to the physiological regulation of fasting glycaemic traits and to gender-specific differences in T2D pathogenesis.

1080/T

Overcoming heterogeneity to indentify genes influencing handedness: results from the International Handedness Consortium. *S.E Medland[1,9], R. Magi[2,3,4], D.M Evans[5], A. Kumar[2,3,4], H. Stefánsson[8], B.M Neale[6,7], C.M Lindgren[2,3,4] for the International Handedness Consortium.* 1) Gen Epidemology, Queensland Inst Med Res, Brisbane, Australia; 2) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK; 3) Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford, UK; 4) Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia; 5) MRC Centre for Causal Analyses in Translational Epidemiology, Department of Social Medicine, University of Bristol, Bristol, UK; 6) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA USA; 7) Program in Medical and Population Genetics, The Broad Institute, Boston, MA USA; 8) deCODE Genetics, Reykjavik, Iceland; 9) School of Psychology, University of Queensland, Brisbane, Australia.

Background: The prevalence of left-handedness (~10%) is increased in a number of psychiatric, neurologic, and learning disorders suggesting that cerebral asymmetry may play a role in these disorders. Handedness is moderately heritable and is related to the lateralization of language functions within the brain. The International Handedness Consortium was formed to conduct large scale meta-analyses to ameliorate the low power associated with GWAS in unselected samples with low prevalence. Here we present the results of the third round of analysis which included data from 32 samples comprising results from 55,399 individuals (5,429 cases, 49,970 controls). Methods: Writing hand was used to classify participants as left- or right-handers, and sex and year of birth were used as covariates. All samples were derived from European descent populations. We tested for trait associations under an additive model for a total of ~2.5 million common HapMap SNPs (directly genotyped and imputed). Weighted Z-score based meta-analysis was conducted using METAL with weights defined by effective sample size. Results: The initial meta-analysis results for the 32 samples were extremely disappointing (the strongest p-value was 1.197e-06). To ascertain whether we had a complex heterogeneity problem we undertook a series of novel multi-dimensional scaling analyses. The results of these analyses identified four samples which were outliers compared to the remaining 28. Re-running the analysis with these four samples removed vastly improved the results, yielding a p-value of 6.092e-08. Conclusions: We have identified a region on chromosome 5 that is potentially associated with handedness. The region encompasses SLIT3 which plays a role in the axon-guidance-pathways that guide motor neuronal development during fetal development. In addition, our study demonstrates that meta-analyses may benefit from examination of heterogeneity at the whole genome as well the SNP level.

1081/T

Efficient Testing of Gene-Environment Interactions Affecting Asthma Susceptibility in the Children's Health Study. *C.E. Murcray, J. Baurley, F. Gilliland, W.J. Gauderman.* University of Southern California, Los Angeles, CA.

Asthma is a growing health concern for children, with prevalence of asthma and asthma-related symptoms increasing over the last few decades. Although the etiology of asthma is largely unknown, it is widely accepted that asthma is a result of the interplay of environmental exposures and genetic factors. Genome-wide association studies of asthma have already uncovered novel genetic regions related to asthma susceptibility. However, little work has been done to allow for the possibility of heterogeneity of genetic risk by subgroups defined by environmental exposures in these large studies. Emerging evidence suggests that smoking, air pollution, traffic, and other personal exposures affect asthma risk and symptoms. It may therefore be important to include these environmental risk factors in the search for novel genetic variants. The Children's Health Study (CHS) is a prospective cohort study of southern California children that began in 1992. Children enrolled in the study have been followed for up to 8 years, with data collected on a large number of respiratory related exposures, including yearly regional air pollution measures, traffic-related exposures, and personal environmental factors, such as in utero and passive tobacco smoke exposure. In addition to this collection of comprehensive environmental data, genome-wide genotyping was conducted on the Illumina 550K genotyping chip for 2,841 children (1,248 asthmatic cases/1,593 non-asthmatic controls). This resource offers the unique opportunity to scan the genome for genes associated with asthma susceptibility whose effects are modified by environmental factors. To analyze this data, we apply a two-step procedure developed by Murcray et al (2009). Their method uses a screening step to prioritize SNPs that are likely to be involved in a GxE interaction by modeling the induced G-E association in the combined case-control sample. We optimize this procedure to efficiently scan for GxE interactions that affect asthma susceptibility for binary (e.g. in utero tobacco smoke, close proximity to a major road) and continuous (e.g. PM2.5, ozone, traffic-related pollution) exposures. For a scan of Gx in utero tobacco smoke exposure, we identify a genome-wide statistically significant SNP on chromosome 6, rs807532, using the Murcray et al two-step approach. This SNP fails to reach genome-wide significance using the standard GxE approach for case-control studies.

1082/T

Within the mTOR pathway, translation regulator EIF4EBP3 sequencey variants associate with healthy human aging. *S.S. Murray[1], B.A. Patay[1,2], E. Smith[1], S. Topol[1], M. Shaw[1], C. Bloss[1], A. Torkamani[1], N. Villarasa[1], K. Wang[3], G. Tranah[4], H. Hakonarson[3], E. Orwoll[5], S. Cummings[4], N. Schork[1], S. Levy[1], E.J. Topol[1,2].* 1) Scripps Genomic Medicine, Scripps Translational Science Institute, La Jolla, CA; 2) Scripps Clinic, La Jolla, CA; 3) Children's Hospital of Philadelphia, Philadelphia, PA; 4) California Pacific Medical Center Research Institute, San Francisco, CA; 5) Oregon Health Sciences University, Portland, OR.

While there has been considerable application of genome-wide association studies to elucidate genes and pathways related to diseases, this methodology has yet to be used to understand the phenotype of healthspan. We have used the Illumina Human1M to genotype over 1 million single nucleotide polymorphisms (SNPs) in 397 DNA samples from individuals who were at least age 80 and had no chronic disease ("wellderly" cases) and 386 individuals who were deceased from various chronic diseases, matched for birth year, gender and ancestry ("illderly" controls). We conducted a genome-wide association from the whole-genome genotyping data, and multiple SNPs in the translator regulator EIF4EBP3 were associated (peak SNP rs250431 P = 1.8 X 10^{-5}; 21 SNPs < 10^{-4}) where the allele frequency at rs250431 was higher in the "illderly" control versus the "wellderly" case groups (0.39 and 0.29, respectively). The top genome-wide associated SNPs were assessed in a second healthy aging cohort (N=284) and compared with the same set of illderly controls, and strengthened the evidence for association (peak SNP rs250431 P = 7.9 X 10^{-6}, combined sample P = 3.1 X 10^{-6}). The finding of significant association with EIF4EBP3 is particularly intriguing as there is an established strong relationship between dietary restriction and aging and longevity. EIF4EBP3 with EIF4EBP1 and EIF4EBP2 produce proteins that bind to eIF4E which prevent eIF4G binding thus inhibiting translation and affects cell proliferation. The ortholog to this gene is 4E-BP, which was recently demonstrated to regulate lifespan in Drosophila subjected to caloric restriction. Since the allele frequency of the associated SNPs is higher in the illderly versus the wellderly groups, this finding raises the possibility of an accelerated aging disorder with the illderly having a higher rate of increase in mortality with age. We are currently sequencing the EIF4EBP3 gene region as well as other key genes in the mTOR pathway in both wellderly and illderly samples. We will compare the spectrum of variants between both wellderly and illderly groups by using single locus tests for common variants, and methods to test association by collapsing rare variants based on function or being present in a contiguous window.

1083/T

Genome-wide association study identified the association of IL28B with response to PEG-IFN-α/RBV therapy for HCV patients. *N. Nishida[1], Y. Tanaka[2], M. Sugiyama[3], A. Koike[4], M. Sageshima[1], Y. Ogasawara[1], Y. Ishibashi[1], Y. Uehara[1], M. Mizokami[3], K. Tokunaga[1].* 1) Department of Human Genetics, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; 2) Department of Clinical Molecular Informative Medicine, Nagoya City University, Nagoya, Japan; 3) Research Center for Hepatitis and Immunology, International Medical Center of Japan Konodai Hospital, Ichikawa, Japan; 4) Central Research Laboratory, Hitachi Ltd., Kokubunji, Japan.

Together with technology developments on large-scale single nucleotide polymorphism (SNP) genotyping, genome-wide association studies (GWAS) with hundreds of thousands of SNPs allow the identification of candidate genetic loci for multifactorial diseases in different populations. We conducted GWAS to identify the host genes associated with response to PEG-IFN-α/RBV treatment in 142 Japanese patients with HCV genotype 1 (78 with null virological response (NVR) and 64 with virologic response (VR)). We found that the minor alleles of two SNPs near the gene IL28B (a member of interferon lambda family) on chromosome 19 were strongly associated with NVR (rs8099917; P = 3.11 × 10^{-15}, OR=30.0 and rs12980275; P = 1.93 × 10^{-13}, OR=20.3). We confirmed these associations with independent patient panels of 50 NVR and 122 VR (combined P = 2.68 × 10^{-32} (OR = 27.1) and 2.84 × 10^{-27} (OR = 17.7), respectively). In the fine mapping of the region, seven SNPs located in the IL28B region showed the strongest associations (P = 5.52 × 10^{-28}-2.68 × 10^{-32}; OR = 22.3-27.1). Quantitative PCR assays in peripheral blood mononuclear cells showed lower IL28 expression level in patients possessing the minor alleles, suggesting that variant(s) regulating interferon-lambda expression is associated with the response to PEG-IFN-α/RBV treatment. The strong association of IL28B with response to PEG-IFN-α/RBV treatment was also found among HCV-infected individuals of European and African ancestry. In these populations, the strongest association was observed from rs12979860 (located about 3 kb upstream of IL28B), which shows to be in linkage disequilibrium with rs8099917 (r^2=0.98). The frequency of minor allele-positive patients were much higher in the NVR group than in the VR group for both SNPs (74.3% in NVR, 12.5% in VR for rs12980275; 75.6% in NVR, 9.4% in VR for rs8099917). Further studies following our report of this robust genetic association to NVR may make it possible to develop a pre-treatment predictor of which individuals are likely to respond to PEG-IFN-α/RBV treatment. This would remove the need for the initial 12-24 weeks of treatment that is currently used as a basis for a clinical decision about whether treatment should be continued.

1084/T

Genome-wide association studies of hematological, biochemical and physical traits in the Japanese population identified 49 novel loci. *Y. Okada[1,2], Y. Kamatani[3], A. Takahashi[1], K. Matsuda[4], N. Hosono[5], H. Ohmiya[1], Y. Daigo[4], K. Yamamoto[2], M. Kubo[5], Y. Nakamura[4], N. Kamatani[1].* 1) Lab for Statistical Analysis, CGM, RIKEN, Japan; 2) Department of Allergy and Rheumatology, Graduate School of Medicine, the University of Tokyo, Japan; 3) Centre d'Etude du Polymorphisme Humain (CEPH), Paris, France; 4) Lab of Molecular Medicine, HGC, IMS-UT, Japan; 5) Lab for Genotyping Development, CGM, RIKEN, Japan.

Recent development of genome-wide association studies (GWASs) led to the identification of numerous quantitative trait loci (QTL). Here, we report GWASs for hematological, biochemical and physical traits in the Japanese populations. We enrolled ~20,000 Japanese individuals under the support of BioBank Japan Project (http://biobankjp.org). We evaluated 9 hematological traits (white blood cell count: **WBC**, neutrophil count, red blood cell count: **RBC**, hemoglobin concentration: **Hb**, hematocrit: **Ht**, mean corpuscular volume: **MCV**, mean corpuscular hemoglobin: **MCH**, mean corpuscular hemoglobin concentration: **MCHC**, and platelet count: **PLT**), 15 biochemical traits (urate: **UA**, high-density lipoprotein: **HDL-C**, low-density lipoprotein: **LDL-C**, triglyceride: **TG**, gamma glutamyl transferase: **GGT**, alkaline phosphatase: **ALP**, aspartate aminotransferase: **AST**, alanine aminotransferase: **ALT**, creatine kinase: **CK**, total protein: **TP**, albumin: **ALB**, blood urea nitrogen: **BUN**, serum creatinine: **sCr**, serum Calcium: **Ca**, and serum Phospate: **P**), and 1 physical trait (adult height). We identified 99 significant associations ($P < 5\times10^{-8}$), including 49 novel associations.

Our study also provides the following topics. (i): Ethnically different genetic backgrounds of QTL. Although *IGF1* is a well-known gene that plays an important role in skeletal development, the association of *IGF1* with height had not been established in Europeans. Our study identified that the multiple SNPs were independently associated with height in *IGF1* locus, and their minor allele frequencies were high in Asian populations but quite low in Europeans and Africans. (ii): Most of the identified QTL included the genes that were functionally related with the traits. We identified that the SNP in *ABO* gene that determine ABO blood type of human was associated with RBC, Hb and MCHC. It suggested that the proportion of the individuals suffering from anemia would be different according to ABO blood type. (iii): Even though a number of QTL were identified, most of the QTL would be still unidentified. We evaluated the combined effects of the height-associated loci incorporating a total of 51 loci. However, the explained proportion of the variance of height was as low as 4.8%, suggesting the missing heritability of the trait. Our study would enhance our knowledge for QTL and motivate the accumulations of the further studies.

1085/T

A Genome-wide Association Study of von Willebrand Factor Plasma Levels and other Hematological Traits in a Healthy Young Cohort. *A. Ozel[1], K. Desch[2], D. Siemieniak[3], D. Ginsburg[1,3], J. Li[1].* 1) Department of Human Genetics, University of Michigan, Ann Arbor, MI; 2) Department of Pediatrics, University of Michigan, Ann Arbor, MI; 3) HHMI, University of Michigan, Ann Arbor, MI.

von Willebrand factor (VWF) is an abundant plasma glycoprotein that regulates hemostasis. Plasma levels of von Willebrand Factor (VWF) are highly variable among healthy people, with ~70% of this variability attributed to heritable factors. von Willebrand disease (VWD), the most common inherited bleeding disorder in humans, results from deficiencies in VWF function. Conversely, elevated plasma VWF levels are associated with an increased risk for deep vein thrombosis. We performed a genomewide association study involving genotyping >800,000 common SNPs (Illumina Omni1-Quad) in a healthy sibling cohort of 1146 subjects (ages 14-35) in 499 sibships. Approximately 80% of subjects are of European ancestry. We collected a total of 62 traits, including plasma VWF antigen level, peripheral blood cell counts (RBC, WBC, platelets), blood cell indices (hematocrit, hemoglobin, MCV, MCH, MCHC, RDW, MPV), as well as common traits such as hair color, eye color, weight, height, and handedness. Many traits show strong familial clustering, suggesting substantial genetic contribution to their variation. Data clean-up for genotypes and phenotypes is near completion. Initial analyses reveal a significant association of plasma levels of VWF with the ABO locus ($p\sim10^{-30}$), consistent with previous reports. Suggestive evidence of association and linkage is found for the VWF gene and a number of other loci. Analyses of other hematological traits (peripheral blood cell counts and platelet factors) are currently underway, as are meta-analyses involving another healthy young cohort and data from the CHARGE consortium. These data provide new insight into the regulation of hemostasis and may identify novel genetic modifiers of bleeding and thrombosis risk. The study is supported by NIH grant HL039693 and is part of the GENEVA (Gene Environment Association Studies) Consortium.

1086/T

Genome-wide association study identifies a locus at 7p15.2 associated with the development of moderate-severe endometriosis. *JN. Painter[1], CA. Anderson[2,3], DR. Nyholt[4], S. MacGregor[5], SH. Lee[5], PM. Visscher[5], P. Kraft[6,7], NG. Martin[8], AP. Morris[2], SA. Treloar[1,9], SH. Kennedy[10], SA. Missmer[6,11,12], GW. Montgomery[1], KT. Zondervan[2,10].* 1) Molecular Epidemiology, Queensland Institute of Medical Research, Brisbane, QLD, Australia; 2) Genetic and Genomic Epidemiology Unit, Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, OX3 7BN, UK; 3) Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, CB10 2HH, UK; 4) Neurogenetics Laboratory, Queensland Institute of Medical Research, 300 Herston Rd, Herston, QLD 4006, Australia; 5) Queensland Statistical Genetics, Queensland Institute of Medical Research, 300 Herston Rd, Herston, QLD 4006, Australia; 6) Department of Epidemiology, Harvard School of Public Health, Boston, MA; 7) Department of Biostatistics, Harvard School of Public Health, Boston, MA; 8) Genetic Epidemiology, Queensland Institute of Medical Research, 300 Herston Rd, Herston, QLD 4006, Australia; 9) Centre for Military and Veterans' Health, The University of Queensland, Mayne Medical School, 288 Herston Road, QLD 4006, Australia; 10) . Nuffield Department of Obstetrics and Gynaecology, University of Oxford, John Radcliffe Hospital, Oxford, OX3 9DU, UK; 11) Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA; 12) Department of Obstetrics, Gynecology and Reproductive Biology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

Endometriosis is a common gynaecological disease associated with severe pelvic pain and sub-fertility. We conducted a genome-wide association (GWA) study using 540,082 SNPs in 3,194 surgically confirmed endometriosis cases and 7,060 controls from Australia and the UK. There is currently considerable debate as to whether different endometriosis stages represent disease progression or whether the milder form of the disease is an epiphenomonen. We used novel statistical methods to estimate the proportion of variation explained by all markers and perform polygenic predictive modelling for disease stage, both of which showed significantly increased genetic loading among the 42% of cases with moderate-severe endometriosis. The strongest signals of association were also observed for moderate-severe disease. We subsequently genotyped 72 SNPs in an independent US dataset consisting of 2,392 endometriosis cases and 1,646 controls. An association with rs7798431 on 7p15.2 for moderate-severe endometriosis (P = 6.0 x 10-8, OR = 1.34 (1.21-1.49)) was replicated, reaching combined genome-wide significance (P = 1.7 x 10-9; OR = 1.26 (1.17-1.35)). Following analysis of imputed SNPs the strongest evidence of association was seen for rs12700667. Both SNPs are located in a 48 kb segment of high LD in an intergenic region upstream of plausible candidate genes, NFE2L3 and HOXA10.

1087/T

Replication of SLE loci in European-American Males. *A. Rasmussen[1], J.A. Kelly[1], R.P. Kimberly[2], J.C. Edberg[2], E.E. Brown[2], G. McGwin Jr[2], M. Petri[3], R. Ramsey-Goldman[4], J.D. Reveille[5], L.M. Vila[6], P.K. Gregersen[7], C.O. Jacob[8], G.S. Gilkeson[9], J.A. James[1], P.A. Gaffney[1], K.L. Moser[1], M.E. Alarcón-Riquelme[1], J.T. Merrill[1], T.J. Vyse[10], R.H. Scofield[1], S.K. Nath[1], E.K. Wakeland[11], B.L. Cobb[1], L.A. Criswell[12], B. Tsao[13], K.M. Kaufman[1], C. Langefeld[14], J.B. Harley[15].* 1) Arthritis and Immunology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK; 2) University of Alabama at Birmingham, Birmingham, AL; 3) Division of Rheumatology, Department of Medicine, Johns Hopkins University, Baltimore, MD; 4) Northwestern University, Chicago, IL; 5) University of Texas Health Sciences Center, Houston, TX; 6) University of Puerto Rico School of Medicine, San Juan, PR; 7) Feinstein Institute of Medical Research, Manhasset, NY; 8) University of Southern California, Los Angeles, CA; 9) Medical University of South Carolina, Charleston, SC; 10) Imperial College London, UK; 11) University of Texas Southwestern Medical Center, Dallas, TX; 12) University of California San Francisco, San Francisco, CA; 13) University of California Los Angeles, Los Angeles, CA; 14) Wake Forest University Health Sciences, Winston-Salem, NC; 15) Cincinnati Children's Hospital, Cincinnati, OH.

Objectives: Systemic Lupus Erythematosus (SLE) is an autoimmune inflammatory disease with a strong genetic component, which predominantly affects women with a 9:1 female:male ratio. A genome wide association study (GWAS) and its replication cohort, the Large Lupus Association Study #1 (LLAS1), were performed in women of European-American (EA) ancestry and identified SLE susceptibility variants in ITGAM, PXK, KIAA1542 and confirmed several previously associated loci (Nat Genet 40:204-210, 2008). Here, we report confirmation of genetic associations with SLE in the EA males from LLAS1. Methods: We studied 8633 SNPs selected from the female LLAS1-GWA case-control study in a replication cohort of 213 EA males affected with SLE and 709 EA male controls. We performed a standard chi-square analysis in the males and conducted a Fisher's combined p-value for the combined result of males and females. We required primary associations to be significant for the risk allele in the males cohort at $P<0.05$ and $P<10^{-6}$ in the combined analysis of the four female cohorts plus male cohort. Results: The most significant associations were within the MHC region in chromosome 6p21, where all prior associations in females were confirmed in males; this was also the case for the association with IRF5. We also identified highly significant associations with markers in a 700 Kb segment on chromosome 8p23.1 containing XKR6, BLK and C8orf12. The associations with PXK and KIAA1542 did not reach statistical significance in the male-only cohort. Table 1 shows the most relevant associations identified. Conclusions: The EA male replication cohort supports many of the associations previously identified in the LLAS1-GWA study in females, and suggests that some sex differences may exist. These include a tendency for larger odds-ratios in males than those observed in females at several loci, and a candidate association to USH2A in males-only. Furthermore, we found significant association to multiple SNPs in a segment of 8p23.1 that encodes for at least three transcripts that are expressed in immune cells. This region has been proposed as a candidate for SLE-association mainly in Asian populations.

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Genome wide association study identifies three loci associated with psoriatic arthritis. *A. Reis[1], U. Hüffmeier[1], S. Uebe[1], A.B. Ekici[1], J. Bowes[2], E. Giardina[3], E. Korendowych[4], K. Juneblad[5], M. Apel[1], R. McManus[6], P. Ho[2], I.N. Bruce[2], A.W. Ryan[6], F. Behrens[7], J. Lascorz[1], B. Böhm[7], H. Traupe[8], J. Lohmann[9], C. Gieger[10], H.E. Wichmann[10,11,12], C. Herold[13], M. Steffens[13], L. Klareskog[14], T.F. Wienker[13], O. FitzGerald[15], G.M. Alenius[5], N.J. McHugh[4,16], G. Novelli[3], H. Burkhardt[7], A. Barton[2].* 1) Inst. of Human Genetics, Univ. Erlangen-Nuremberg, Erlangen, Germany; 2) ARC Epidemiology Unit, Univ. of Manchester, UK; 3) Dept. of Biopathology, University of Rome "Tor Vergata", Italy; 4) Royal National Hospital for Rheumatic Diseases, Bath, UK; 5) Dept. of Public Health and Clinical Medicine/ Rheumatology, Univ. Hospital, Umeå, Sweden; 6) Dept. of Clinical Medicine, Trinity College Dublin, Ireland; 7) Div. of Rheumatology, Univ. Frankfurt (Main), Germany; 8) Dept. of Dermatology, University of Münster, Germany; 9) Psoriasis Rehabilitation Hospital, Bad Bentheim, Germany; 10) Inst. of Epidemiology, Helmholtz Center Munich, Germany; 11) Inst. of Medical Informatics, Biometry and Epidemiology, Univ. of Munich, Germany; 12) Klinikum Grosshadern, Univ. of Munich, Germany; 13) Inst. of Medical Biometry, Informatics and Epidemiology, Univ.of Bonn, Germany; 14) Karolinska Institute, Stockholm, Sweden; 15) Dept. of Rheumatology, University College Dublin, Ireland; 16) Dept. of Pharmacy and Pharmacology, Univ. of Bath, UK.

Psoriatic arthritis (PsA) is an inflammatory joint disease distinct from other chronic arthritides and frequently accompanied by psoriasis vulgaris and seronegativity for rheumatoid factor. In order to identify susceptibility genes, we performed a SNP array based genome wide association study in 609 German patients and 990 control individuals. Analysis of 1,585,307 confidently imputed SNPs revealed three loci achieving genome-wide significance ($p<5x10^{-8}$) that were replicated in independent study groups of 1,761 patients and 3,727 control individuals of European origin. We confirmed HLA-C ($p=2.63x10^{-23}$) and IL12B ($p=5.60x10^{-13}$) as PsA susceptibility genes and observed several independent signals within the HLA region. The most associated IL12B variant, located 72kb upstream of the gene, was independent of previously reported psoriasis-associated IL12B-SNPs. Association to several intragenic variants of the TRAF3IP2 gene ($p=5.48x10^{-9}$) including the missense-variant R74W (rs13190932) was detected implicating this component of the IL-17 receptor signalling pathway as a PsA susceptibility locus. Functional studies of two missense variants within this gene showed altered binding to molecules TRAF3 and TRAF6. Our study implicates new susceptibility variants at known susceptibility loci and in addition identifies a novel pathway for PsA.

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Variants within the *CBLB* gene are associated with multiple sclerosis. *S. Sanna[1], M. Pitzalis[2], M. Zoledziewska[2], I. Zara[3], C. Sidore[1,2], R. Murru[5], M.B. Whalen[4], L. Scirru[5], M.A. Secci[5], F. Deidda[2], L. Corrado[6], N. Barizzone[6], F. Poddie[2], L. Morelli[2], G. Farina[7], M. Dei[1], S. Lai[1], A. Mulas[1], Y. Li[8,9,10], M. Pugliatti[7], S. Traccis[11], A. Angius[4], S. D'alfonso[6], M. Melis[12], G. Rosati[7], G.R. Abecasis[8], M. Uda[1], M.G. Marrosu[5], D. Schlessinger[13], F. Cucca[1,2].* 1) Istituto di Neurogenetica e Neurofarmacologia, CNR, Monserrato, Cagliari, Italy; 2) Dipartimento di Scienze Biomediche, Università di Sassari, Sassari, Italy; 3) CRS4, Laboratorio di Bioinformatica, Parco tecnologico della Sardegna, Pula (CA), Italy; 4) CRS4, Laboratorio di Genomica, Parco tecnologico della Sardegna, Pula (CA), Italy; 5) Centro Sclerosi Multipla, Dipartimento di Scienze Neurologiche e Cardiovascolari, Università di Cagliari, Cagliari, Italy; 6) Department of Medical Sciences and Interdisciplinary Research Center of Autoimmune Diseases (IRCAD), University of Eastern Piedmont, Novara, Italy; 7) Istituto di Neurologia Clinica, Università di Sassari, Sassari, Italy; 8) Center for Statistical Genetics, Ann Arbor, University of Michigan, MI, USA; 9) Department of Genetics, University of North Carolina, Chapel Hill, North Carolina, USA; 10) Department of Biostatistics, University of North Carolina, Chapel Hill, North Carolina, USA; 11) Presidio Ospedaliero, Divisione Neurologia, Ozieri, Italy; 12) Azienda Ospedaliera Brotzu, S.C. Neurologia, Cagliari, Italy; 13) Laboratory of Genetics, National Institute on Aging, Baltimore, MD, USA.

Multiple sclerosis (MS) is a multi-factorial neuroinflammatory and autoimmune disorder. A primary cause of disability in young adults, it results from interactions between unknown environmental factors and alleles of many susceptibility loci across the genome. Recent investigations of the genetics of MS have resulted in important advances, driven largely by completion of the first genome-wide association scans (GWAS). To detect additional loci, we performed a GWAS in 882 Sardinian Multiple Sclerosis (MS) cases and 872 controls genotyped with the Affymetrix 6.0 chip, using 575,678 SNPs that passed quality checks. We then successfully imputed 6,031,588 SNPs using haplotypes available from HapMap II, HapMap III and 1000 Genomes projects, and tested for association ~6.6 million variants. The strongest signal (OR=2.05, $p=1.45x10^{-20}$) was observed at a SNP tag for the HLA-DRB1*0301-DQB1*0201 allele. We then selected 9 SNPs outside of the HLA locus for validation and follow-up, based on their level of significance, proximity to functional candidate genes, and quality of imputation. Of those, SNP rs9657904 on chr3q13 was successfully confirmed in the GWAS samples and replicated in an independent set of 1,775 MS cases and 2,005 controls ($p=9.4x10^{-6}$). Notably, this variant is absent from the HapMap II reference panel, and we were able to fully assess it only after imputation with HapMap III and 1000 Genomes haplotypes. Combining all available genotypes, the observed pvalue was $1.6x10^{-10}$ (OR=1.40). The most associated variants at this locus fall in the promoter of a gene, *CBLB*, which encodes a negative regulator of adaptive immune responses. In support of its involvement in MS, mice lacking the ortholog are prone to experimental autoimmune encephalomyelitis, the animal model of multiple sclerosis. The strongest associated variant was also replicated in 1,441 cases and 1,465 controls from central-northern Italy with a similar effect size, hence confirming the role of this marker in increased risk for multiple sclerosis in another southern European population. Finally, we sequenced by the Sanger method the coding regions and promoter of the gene in 96 patients, observing novel variants that are potentially causative, and will now be assessed with focused biological studies.

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A new method for association studies allowing for multiple causal alleles. *B. Servin[1], P. Scheet[2].* 1) Dept Animal Genetics, INRA, Castanet-Tolosan, France; 2) The University of Texas Health Science Center at Houston M. D. Anderson Cancer Center Department of Epidemiology, Houston, TX.

Although hundreds of variants for a variety of traits have been identified using genetic association studies, only a proportion of the genetic variance expected from familial clustering can be explained. Finding this "missing heritability" is a current challenge in the genetics of complex traits. One possible extension of current approaches for detecting association is to model more complex genetic architecture, in particular in allowing for multiple causal alleles. This will become particularly relevant for the analysis of large scale resequencing data that unravel the full genotypic diversity of the study sample. We present a contribution to the resolution of this problem with a new multipoint method for the analysis of genetic association studies that allows for multiple causal alleles. Current approaches, including imputation-based approaches, still usually rely on testing a SNP genotype, potentially imputed, for association with a phenotype. Our new approach uses a test that allows for multipe alleles in a genomic region. It exploits a model for haplotype diversity in a genomic region to extract relatedness between individuals, and test for its association with the phenotype using a variance components approach. We developped a new statistic for association (a Bayes Factor) for this model that can be computed rapidly. We will show how our method leads to (i) increased power and (ii) more clear association signals than current approaches. We show that out new method has equivalent power than current tests (BF or p-value based) when the causal variant is bi-allelic and a much greater power when a large number of alleles contributes to the trait, therefore providing a robust inference for the analysis of genetic association studies.

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Genome-wide studies of idiopathic scoliosis susceptibility implicate genes encoding neural cell adhesion proteins. *S. Sharma[1], X. Gao[1], D. Londono[2], S.E. Devroy[1], K.N. Mauldin[1], J.T. Frankel[1], J.M. Brandon[1], D. Zhang[1], Q. Li[3], M.B. Dobbs[4,5], C.A. Gurnett[4,6,7], S.F.A. Grant[8], H. Hakonarson[8], J.P. Dormans[9], J.A. Herring[10,11], D. Gordon[2], C.A. Wise[1,11,12].* 1) Seay Center for Musculoskeletal Research, Texas Scottish Rite Hospital for Children, Dallas, Texas, USA; 2) Department of Genetics, Rutgers, The State University of New Jersey, Piscataway, New Jersey, USA; 3) UTSW Microarray Core, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas, USA; 4) Department of Orthopaedics, Washington University School of Medicine, St. Louis, Missouri, USA; 5) St. Louis Shriners Hospital for Children, St. Louis, Missouri, USA; 6) Department of Pediatrics, Washington University School of Medicine, St. Louis, Missouri, USA; 7) Department of Neurology, Washington University School of Medicine, St. Louis, Missouri, USA; 8) Division of Human Genetics, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA; 9) Department of Orthopaedic Surgery, The Children's Hospital of Philadelphia, Pennsylvania, USA; 10) Department of Orthopaedics, Texas Scottish Rite Hospital for Children, Dallas, Texas, USA; 11) Department of Orthopaedics, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas, USA; 12) McDermott Center for Human Growth and Development, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas, USA.

Idiopathic scoliosis (IS) affects ~3% of school age children and usually occurs coincident with adolescent growth. Heritability estimates are significant, yet pre-disposing alleles are lacking. We performed a genome-wide association study (GWAS) with the Illumina HumanCNV370-quad platform in 419 Texas trios, with follow-up in IS cases from Texas and other U.S. regions. For the discovery phase we applied the TDT statistic that is robust to population stratification and observed top IS-associated loci on chrs 3, 4, 5, 6, and 21. Restricting the analysis to genotyped and imputed SNPs in the non-Hispanic white subset, our largest ethnic group, produced top clustered signals on chrs 3, 7, 14, and 21, with strongest results for chr 3 loci in the proximity of the *CHL1* gene [rs1400180 $P=7.91 \times 10^{-8}$; OR(95%CI)= 2.13(1.60-2.82); rs10501081 $P=2.56 \times 10^{-6}$; OR(95%CI)=2.03(1.50-2.75)]. Genotyping chr 3 SNPs in independent, non-Hispanic white cohorts from Texas and other U.S. regions (total unrelated cases: controls = 562:666) produced the same direction of effect for each SNP, where combined evidence of replication was greatest for rs10510181 [$P_{Fisher}=2.58 \times 10^{-8}$; OR(95%CI)=1.49(1.29-1.73)]. However these SNPs were not significantly associated with IS in comparison with a separate GWAS (N=137 cases, 2,126 controls), likely reflecting disease complexity. *CHL1* encodes Close Homolog of L1, a neural cell adhesion protein involved in axon guidance and a plausible risk factor for IS, given the known causal relationship between Mendelian axon guidance deficiency and scoliosis. We obtained suggestive evidence of linkage (maximum HLOD = 1.93, P=0.001 at rs1400180) with IS in families (N>90) and expect that this cohort will be useful in sequence-based searches for disease alleles. We recently expanded our genome-wide studies (N=702 Texas trios) and noted top clustered associations with variants in additional neural cell adhesion and axon guidance genes including the gene encoding Down syndrome cell adhesion molecule (*DSCAM*) on chromosome 21. This is of particular interest, as increased incidence of scoliosis is reported in Down syndrome patients. Follow-up of promising loci is ongoing, including application of recent TDT and case-control methods that allow for locus heterogeneity to potentially increase power for association. Our data implicate variation affecting neural cell adhesion genes in IS susceptibility and suggest new testable hypotheses relating to disease pathogenesis.

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Genome-wide association study of the timing of the pubertal growth spurt in healthy children from the Fels Longitudinal Study. *B. Towne[1], J.W. Kent, Jr.[2], C. Bellis[2], J.E. Curran[2], E.W. Demerath[3], K.D. Williams[4], T.D. Dyer[2], A.C. Choh[1], W.C. Chumlea[1], R.M. Siervogel[1], J. Blangero[2], S.A. Czerwinski[1].* 1) Wright State University School of Medicine, Dayton, OH; 2) Southwest Foundation for Biomedical Research, San Antonio, TX; 3) University of Minnesota, Minneapolis, MN; 4) Temple University, Philadelphia, PA.

Stature is a highly heritable trait, and several recent genome-wide analyses have identified QTL and SNPs linked or associated with adult or childhood stature. But, very little is known of genetic influences on specific features of individual growth patterns during childhood. Presented here are results of an initial genome-wide association analysis of the timing of the pubertal growth spurt in healthy children from the Fels Longitudinal Study. We applied a triple logistic curve-fitting method to extensive serial stature data from 492 subjects aged 2 years to early adulthood (245 males and 247 females) to identify in each of them the timing of the initiation of the pubertal growth spurt (age at pre-pubertal minimum growth velocity or "age at take-off" - ATO) and the timing of the pubertal growth spurt while at its most intense (age at peak height velocity - APHV). Mean ATO and APHV in these males and females were 10.66 and 8.74 years, and 13.72 and 11.55 years, respectively. The heritability of ATO was high in magnitude and significance ($h^2 = 0.66 \pm 0.10$; $p < 0.0001$), as was the heritability of APHV ($h^2 = 0.70 \pm 0.10$; $p < 0.0001$). All 492 individuals were genotyped with the Illumina Human 610-Quad BeadChip containing more than 550,000 SNPs. Association analyses were conducted using measured genotype analysis implemented in SOLAR allowing for residual non-independence among relatives. In this sample, genome-wide significance was indicated by a chi-square $\geq$ 27.85 and suggestive evidence by a chi-square $\geq$ 22.70. Highly suggestive association of SNP rs6016887 (chromosome 20) with both ATO (chi-square = 25.54) and APHV (chi-square = 27.00) was found. Likewise, highly suggestive association of SNP rs1155563 (chromosome 4) with both ATO (chi-square = 26.14) and APHV (chi-square = 24.48) also was found. And, highly suggestive association of SNP rs7629566 (chromosome 3) with ATO was observed (chi-square = 27.07), as was a suggestive association of SNP rs4622992 (chromosome 4) with APHV (chi-square = 23.49). In previous analyses we had found suggestive linkages of both ATO and APHV to markers on chromosome 20 in the same region as SNP rs6016887, indicating that future combined linkage/association analyses may better clarify these findings. In sum, these results demonstrate the feasibility of locating and identifying genes that influence different aspects of childhood growth. Supported by NIH grants R01HD12252, R01HD40377, F32HD053206, and R37MH59490.

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Whole genome-scan of genetic determinants for dental caries in the permanent dentition. *X. Wang[1], J.R. Shaffer[1], E. Feingold[1], M. Lee[1], K.T. Cuenco[1], F. Begum[1], D.E. Weeks[1], M.M. Barmada[1], S. Wendell[1], D. Crosslin[2], C. Laurie[2], K. Doheny[3], E. Pugh[3], R.J. Weyant[1], A.R. Vieira[1], R.J. Crout[4], D.W. McNeil[4], M.L. Marazita[1].* 1) Univ Pittsburgh, Pittsburgh, PA; 2) University of Washington, Seattle, WA; 3) Johns Hopkins, Baltimore, MD; 4) West Virginia University, Morgantown, WV.

Background and Objective: Despite advances in dental care, dental caries remains one of the most common diseases throughout the world. The etiology of dental caries is thought to involve a complex interplay of environmental and genetic factors, with heritability of caries phenotypes ranging from 30-60%. However, specific susceptibility genes affecting dental caries are not well studied. Moreover, recent findings have revealed that genes affecting susceptibility to caries in the permanent dentition (in adults) may differ from those in primary teeth (in children). Moreover, recent findings have revealed that genes affecting susceptibility to caries in the permanent dentition (in adults) may differ from those in primary teeth (in children). Therefore, we performed the first highly dense genome-wide association study (GWAS) of dental caries in the permanent dentition to identify genomic regions likely to harbor genes responsible for caries susceptibility or resistance, particularly in adults. **Methods**: Dental exams on 2,219 Caucasian subjects (ages 14+, a sub-cohort from NIH funded GENEVA caries project) were performed to assess evidence of caries (e.g., decay, missing due to decay, or fillings) on all surfaces of each permanent tooth. Trend tests for association between caries phenotypes and 548,948 SNPs (genotyped by CIDR on the Illumina Human610-Quad SNP platform and passing quality control filters) were performed first using PLINK for three different age groups, 14+, 17+, and 20-60 years (sample sizes range from 1,965 to 2,219). Then the repeated analysis in SOLAR with adjustments for relatedness was conducted to verify the significance of association signals. **Results**: Genome-wide scans for different caries phenotypes (e.g. total number of teeth or surfaces with caries, percentage of teeth or surfaces affected etc.) and for the three age groups were compared, and "top hits" (P<1.0 x 10^-05) were inspected for nearby genes with plausible effects on tooth decay. Several of these signals were consistent across the three age groups and across the different caries phenotypes. In addition, several of the top signals were found within or near genes previously shown to be involved in tooth development or the disease processes. Replication in three additional cohorts is ongoing. *NIH grants DE018903, DE 014899, HG004438, HG004446, RR024153,; NIH contract HHSN268200782096C.*.

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Combining association evidence across populations with diverse LD patterns: an eigen-weighted regional method. *X. WANG[1], H.Y. XU[1], X.L. SIM[1], C. SUO[1], W.T. TAY[2], T.H. ONG[3], E.S. TAI[4,5], T.Y. WONG[2,6], K.S. CHIA[1,4], Y.Y. TEO[4,7].* 1) Centre for Molecular Epidemiology, National University of Singapore, Singapore; 2) Singapore Eye Research Institute, Singapore National Eye Centre, Singapore; 3) NUS Graduate School for Integrative Science and Engineering, National University of Singapore, Singapore; 4) Department of Epidemiology and Public Health, National University of Singapore, Singapore; 5) Department of Medicine, National University of Singapore, Singapore; 6) Centre for Eye Research Australia, University of Melbourne, Australia; 7) Department of Statistics and Applied Probability, National University of Singapore, Singapore.

Meta-analysis of genome-wide association studies (GWAS) across tens to hundreds of thousands of samples from multiple populations is now the preferred strategy to locate and verify new loci for phenotype-genotype associations. This approach generally assumes that: (i) the same causal variants exist across multiple populations; (ii) the same SNPs are assayed in all the populations; (iii) the LD structure between the causal variants and the assayed SNPs is similar across the different populations. In practice, different studies may rely on different genotyping platforms with moderately low degree of overlapping SNPs across the different technologies. Imputation strategies are subsequently utilized to generate a standardized set of SNPs for meta-analysis, although this can be sub-optimal without the availability of population-specific reference haplotypes. Also, heterogeneous patterns of LD with the causal variants may result in different SNPs emerging from the individual GWAS, further confounding meta-analyses. Here we introduce a region-based analysis that does not require the latter two assumptions to be met, by effectively searching for an over-representation of genotyped SNPs with suggestive evidence of disease association in a genomic region. While such analyses can be affected by SNPs in LD, we implement a novel eigen-decomposition method to estimate variable SNP weightings in order to minimize "double counting" due to LD. Crucially, this method avoids the assumption that the same markers have to display similar disease effects across multiple populations - which can be penalizing for increasingly large meta-studies. In regions of large heterogeneous patterns of LD across multiple populations, this method has the statistical power to detect disease associations that standard meta-analysis will have missed.We first implemented a series of simulations under different scenarios to assess the power and false positive rates, observing significantly higher power to detect disease associations that will otherwise have been missed by standard meta-analytic approaches. We subsequently applied this method to genome-wide data to at least half a million SNPs across three populations in Singapore (with Southern Chinese, South-East Asian Malay and Asian Indian genetic ancestries) , identifying novel genomic regions that are putatively implicated with type 2 diabetes, hypertension and hyperlipidemia.

1095/T

Common variants explain the same amount of variation in fasting glucose levels in Pakistani individuals as they do in Europeans. *A.R. Wood[1], M. Islam[2], B. Chaudhary[3], S. Hashmi[1], N. Chaturvedi[4], M.N. Weedon[1], T.M. Frayling[1], T.H. Jafar[2].* 1) Genetics of Complex Traits, Peninsula Medical School, Exeter, United Kingdom; 2) Department of Community Health Sciences, Aga Khan University, Karachi, Pakistan; 3) Department of Biological and Biomedical Sciences, Aga Khan University, Karachi, Pakistan; 4) International Centre for Circulatory Health, National Heart and Lung Institute, Imperial College London, United Kingdom.

It has recently been suggested that associations between common variants and phenotypes will differ between populations, for example due to "synthetic" common variant associations caused by multiple rare variants at any one locus, as well as differences in environmental factors between populations.

To test this hypothesis we genotyped 16 common variants associated with fasting glucose levels in Europeans in a population of 1500 individuals from Pakistan. We compared the total variation in fasting glucose explained by all 16 SNPs with that in Europeans.

We used a multiple linear regression with fasting glucose as the dependent variable with all 16 SNPs acting as the independent variables. Twelve of the 16 SNPs (P=0.038) had the same glucose raising allele as in Europeans and 4 of these were nominally significantly associated with fasting glucose levels in the expected direction (P<0.05). We found that the proportion of the variance explained was similar to that identified in the European population (3.2% - 4.4%), with an adjusted R^2 of 0.03 (95% CI: 0.01, 0.05).

This study provides evidence that the effects of SNPs associated with fasting glucose in the European population have similar effects in the Pakistani population. This finding suggests that population differences for common variants on complex traits may not be as dramatic as some have suggested.

1096/T

Genome-wide association study for adiponectin levels in Filipino women identifies *CDH13* and a novel uncommon haplotype at *KNG1* - *ADIPOQ*. *Y. Wu[1], Y. Li[1,2], E.M. Ethan[1,2], D.C. Croteau-Chonka[1], C.W. Kuzawa[4,5], T.W. McDade[4,5], L. Qin[1], G. Curochicin[1,6], J.B. Borja[7], L.A. Lange[1], L.S. Adair[3], K.L. Mohlke[1].* 1) Department of Genetics, Univ of North Carolina, Chapel Hill, Chapel Hill, NC; 2) Department of Biostatistics, Univ of North Carolina, Chapel Hill, Chapel Hill, NC; 3) Department of Nutrition, University of North Carolina, Chapel Hill, NC; 4) Department of Anthropology, Institute for Policy Research, Northwestern University, Evanston, IL; 5) Cells 2 Society: The Center for Social Disparities and Health at the Institute for Policy Research, Northwestern University, Evanston, IL; 6) Department of Family Medicine, State Medical and Pharmaceutical University, Chisinau, Moldova; 7) Office of Population Studies Foundation, University of San Carlos, Cebu City, Philippines.

Adiponectin is an adipocyte-secreted protein involved in a variety of metabolic processes, including glucose regulation and fatty acid catabolism. Plasma adiponectin is under substantial genetic influence, however, the common variants identified to date cannot explain the majority of the estimated 30-70% heritability. In addition, the loci associated with adiponectin remain unclear in populations of non-European ancestry. Therefore, we conducted a genome-wide association study (GWAS) to investigate the genetic loci associated with plasma adiponectin in 1,776 unrelated Filipino women from the Cebu Longitudinal Health and Nutrition Survey (CLHNS). A multiple linear regression model with covariates of age, age^2, socialeconomic status and menopausal status was used to evaluate the association between adiponectin and 2,073,674 SNPs. The strongest signal mapped to the gene *CDH13* (rs3865188, $P = 7.2\times10^{-16}$), which encodes a receptor for high-molecular-weight forms of adiponectin. Compared to *CDH13* variant rs7195409, which has been reported with nominal association evidence in a recent GWAS in Europeans but showed no association in CLHNS mother ($P = 0.16$), rs3865188 is located ~887 kb upstream ($D' = 0.06$, $r^2 = 0$, HapMap CHB+JPT) and represents a genome-wide significant and novel signal for adiponectin. Evidence for association was also detected near the *ADIPOQ* gene (rs864265, $P = 3.8\times10^{-9}$) and at a novel location 100 kb upstream of *ADIPOQ* near *KNG1* (rs11924390, $P = 7.6\times10^{-7}$). All three signals were subsequently observed in 1,774 young adult CLHNS offspring and in combined mixed model analyses that included all 3,550 mothers and offspring samples taking relatedness into consideration (all $P < 1.6\times10^{-9}$). Haplotype analysis performed using the haplo.glm function in the R haplo.stats package showed that an uncommon haplotype of rs11924390 and rs864265 (C-T haplotype frequency = 0.050) was strongly associated with lower adiponectin compared to the most common C-G haplotype in both CLHNS mothers ($P = 1.8\times10^{-25}$) and offspring ($P = 8.7\times10^{-32}$). Our findings provide convincing evidence that the *CDH13* locus is associated with plasma adiponectin. We also identify a novel uncommon haplotype strongly associated with adiponectin level in Filipinos, which may motivate future studies to investigate potential functional variants and to elucidate their molecular mechanisms.

1097/T

A genome-wide association scan identified sex-specific genes for obesity in Chinese. *T. Xu[1,2], Y. Cheng[1,2], YF. Pei[3], L. Zhang[3], N. Yu[1], TL. Yang[3], J. Chen[1,4], XH. Xu[3], SS. Dong[3], HB. Liu[1], J. Li[1], J. Hamilton[1], YJ. Liu[1], HW. Deng[1,2,3,4].* 1) School of Medicine, University of Missouri - Kansas City, Kansas City, MO 64108, USA; 2) Pharmacogenetics Research Institute, Institute of Clinical Pharmacology, Central South University, Changsha, Hunan, 410008, P. R. China; 3) The Key Laboratory of Biomedical Information Engineering, Ministry of Education and Institute of Molecular Genetics, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an 710049, P. R. China; 4) Laboratory of Molecular and Statistical Genetics, College of Life Sciences, Hunan Normal University, Changsha, Hunan 410081, P. R. China.

Obesity is a serious public health problem which increases the risk of some diseases such as cardiovascular disease and diabetes. The genetic determination of obesity is strong, but the majority of underlying genetic factors remain to be discovered. In this study, we performed association analyses in a sample of 1627 unrelated Chinese subjects (802 males and 825 females) genotyped for Affymetrix genome-wide human SNP array 6.0. We focused our analysis on 20131 mitochondria SNPs and 4974 SNPs at the CpG island due to the important role of mitochondria on energy metabolism and the importance of CpG island on gene transcription. Obesity phenotypes analyzed included trunk fat mass and waist/hip ratio. In the total sample analysis, none of the SNPs reached genome-wide significant level. However, in sex-stratified analysis, we identified two mitochondrial SNPs (rs9395490 and rs2182011) that were significantly associated with trunk fat mass in females only (P-values of 7.07×10^{-7} and 1.34×10^{-6} respectively). These two female-specific SNPs were located in a mitochondrial gene, MUT (methylmalonyl CoA mutase). In the analysis of SNPs at CpG island, we identified a SNP rs12371091 that was significantly associated with waist/hip ratio in males only (P-value of 8.81×10^{-7}). This male-specific SNP is located at the STX2 (syntaxin 2) gene. The MUT gene encodes Methylmalonyle-CoA mutase, which involves in the degradation of odd-chain fatty acids and cholesterol and tricarboxylic acid cycle in mitochondria. The STX2 gene encodes syntaxin-2, which regulates epithelial-mesenchymal interactions. In summary, our findings suggest two novel genes that have sex-specific effects on obesity in Chinese.

Key words: Genome-wide association analyses, MUT, STX2, Sex-specific, Obesity.

1098/T

The value of mapping genome-wide associations across other ethnic groups: An analysis of BMD in Mexican Americans. *L.M. Yerges-Armstrong[1], J.R. Shaffer[2], J.M. Bruder[3], T.D. Dyer[4], M.A. Carless[4], J.E. Curran[4], HHH. Göring[4], J. Blangero[4], C.M. Kammerer[2], B.D. Mitchell[1].* 1) University of Maryland, Baltimore, MD; 2) University of Pittsburgh, Pittsburgh, PA; 3) University of Texas Health Science Center, San Antonio, TX; 4) Southwest Foundation for Biomedical Research, San Antonio, TX.

Recent GWA studies carried out in European Caucasians have identified at least 25 SNPs associated with bone mineral density (BMD), including one on chromosome 7p14 upstream of STARD3NL, which encodes a membrane protein of unknown function. It is unclear whether the association with BMD is due to altered regulation or function of this gene or to other nearby genes that are in linkage disequilibrium (LD). For this reason there can be great value in carrying out association studies in other populations with differing LD patterns. We conducted a GWA study of BMD at the femoral neck and spine in 797 men and women in multi-generational Mexican American families as part of the San Antonio Family Osteoporosis Study using 531,800 SNPs genotyped on the Illumina 500K genotyping platform. Analyses were adjusted for relevant covariates (age, age2, sex, and weight) and allowed for residual familial relatedness using a variance components model. We examined GWA signals within 100kb of the previously reported SNPs in particular detail. Of 988 SNPs falling within 100kb of the 25 previously reported GWA association signals, 69 SNPs in 13 gene regions were nominally associated ($p<0.05$) with lumbar spine BMD and 79 SNPs in 12 gene regions were nominally associated with femoral neck BMD. After applying a conservative Bonferroni adjustment for multiple comparisons, three SNPs (two for lumbar spine and one for femoral neck) were associated ($p<5x10-5$) with BMD. All three fell within 75kb in the 7p14 region, ~50 kb telomeric to the previously associated SNP. The signal identified by our GWA analysis is closer to the EPDR1 and SFRP4 genes than it is to the previously reported association near the STARD3NL gene. Similar to our study, a GWA study in Asians also identified a SNP on 7p14 that mapped closer to SFRP4 than STARD3NL. In fact, the SNPs in highest LD with the SNP originally identified in Caucasians were associated less strongly with lumbar spine BMD ($p= 5x10-3$) and not associated with femoral neck BMD at all ($p=0.5$). Depending on criteria used to consider an association replicated, this region may have been considered a non-replicating region if only the primary association signal from the Caucasian meta-analysis was analyzed. While the location of the true causative SNP is not known, our results highlight the utility of expanding the scope of inquiry for replication to a larger window, particularly in replication cohorts comprising other ethnic groups.

1099/T

Genome-wide association analysis of Metabolic Syndrome and related quantitative traits in a Croatian island population. *G. Zhang[1,2], G. Sun[1], R. Karns[1], S. Indugula[1], N. Wen[1], H. Cheng[1], N. Narancic[3], N. Jeran[3], D. Havas[3], S. Missoni[3], Z. Durakovic[3], R. Chakraborty[4], P. Rudan[3], R. Deka[1].* 1) Center for Genome Information, Department of Environmental Health, University of Cincinnati, Cincinnati, Ohio, United States of America; 2) Department of Family and Community Medicine, University of Cincinnati, Cincinnati, Ohio, United States of America; 3) Institute for Anthropological Research, Zagreb, Croatia; 4) Center for Computational Genomics, University of North Texas Health Science Center, Fort Worth, Texas, United States of America.

Genome-wide association (GWA) studies represent a powerful approach to the identification of genes involved in common human diseases. In this report, we describe a GWA study (using Affymetrix Genome-Wide Human SNP Array 5.0) of Metabolic Syndrome (MetS) and related quantitative traits (including 15 anthropometric and 11 blood biochemical traits) in a sample of ~1,500 individuals drawn from a homogenous population of Hvar Island off the eastern Adriatic coast of Croatia. Association tests of quantitative traits identified a large number of independent association signals (with single-point P values less than 10^{-6}). These signals include many previously reported loci such as SLC2A9 (uric acid), TCF7L2 (FBG and HbA1c), CETP (HDL), HMGA2 and UQCC (body height) as well as some novel loci which may represent genuine effects. Some notable examples include: rs17154194 (intergenic SNP) was associated with FBG ($P = 2.8\times10^{-9}$) and triglyceride ($P = 2.4\times10^{-8}$); rs200116 (downstream of SEMA5A) and rs833820 (intron of ARF3) were associated with uric acid ($P = 3.7\times10^{-8}$ and 9.2×10^{-8}); rs11030796 (intron of STIM1) was associated with fibrinogen ($P = 1.8\times10^{-8}$); rs3746410 (FER1L4) was associated with body weight ($P = 1.2\times10^{-6}$). We also detected rs1521282 (downstream of CPNE4) as a susceptibility locus for MetS as a binary trait by either ATP ($P = 1.1\times10^{-7}$) or IDF ($P = 3.6\times10^{-6}$) definition. In addition, using a principle component analysis of the quantitative traits used in MetS diagnostic criteria (waist circumference, triglyceride, HDL, blood pressure and FBG), we observed several genes implicated in blood glucose homeostasis (KCNJ11, TCF7L2, SLC30A8 and FBP2) were associated independently with individual principle components of MetS traits. In summary, our study yield additional genetic loci that may influence MetS susceptibility or related quantitative traits. These findings were either supported by high-level of statistical significance and/or functional implications which make them highly interesting for further replication and follow-up. Our results also suggest that the genes involved in blood glucose homeostasis may play important role in the development of MetS.

1100/T

Targeted Genomic Enrichment and Next-Generation Sequencing of An Autosomal Dominant Trait Linked to Chromosome 5p13. *L. Racacho[1,2], S.M. Nikkel[3], J. MacKenzie[4], A. Hunter[3], D.E. Bulman[1,2].* 1) Dept. Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, ON; 2) Regenerative Medicine Program, Ottawa Hospital Research Institute, Ottawa, ON; 3) Dept. of Genetics, Children's Hospital of Eastern Ontario, Ottawa, ON; 4) Division of Genetics, Kingston General Hospital, Kingston, ON.

Targeted genomic enrichment, coupled with next-generation sequencing, represents a feasible resequencing strategy for monogenic loci in which no disease causing mutation has been identified. We tested this approach using brachydactyly A1 (BDA1) as a model. We previously identified a BDA1 locus on chromosome 5p13 segregating as an autosomal dominant trait in a single family. We hypothesize that a gene on chromosome 5p13 is necessary for proper bone development and a mutation in this gene will disrupt the Ihh-Pthlh signaling axis, causing the BDA1 phenotype. Two NimbleGen 385K arrays were designed to capture 3.2 Mb of the critical region from two affected individuals in our family. Next-generation sequencing of the enriched fragments was performed using Roche 454 Titanium pyrosequencing. A 30X coverage of the critical region yielded 205 novel and shared simple variants. More than 25 novel and shared breakpoints were identified on a separate genome analysis of single end reads. We are currently verifying and validating these sequence variants.

1101/T

A unified framework for testing multiple phenotypes for association with genetic variants. *M. Stephens.* Statistics & Human Gen, Univ Chicago, Chicago, IL.

In many ongoing genome-wide association studies, multiple related phenotypes are available for testing for association with genetic variants. In most cases, however, these related phenotypes are analysed independently from one another. For example, several studies have measured multiple lipid-related phenotypes, such as LDL-cholestrol, HDL-cholestrol, and Triglycerides, but in most cases the primary analysis has been a simple univariate scan for each phenotype. This type of univariate analysis fails to make full use of potentially rich phenotypic data. While this observation is in some sense obvious, much less obvious is the right way to go about examining associations with multiple phenotypes. Common existing approaches include the use of methods such as MANOVA, canonical correlations, or Principal Components Analysis, to identify linear combinations of outcome that are associated with genetic variants. However, if such methods give a significant result, these associations are not always easy to interpret. Indeed the usual approach to explaining observed multivariate associations is to revert to univariate tests, which seems far from ideal. In this work we outline an approach to dealing with multiple phenotypes based on Bayesian multivariate regression. The method attempts to identify which subset of phenotypes is associated with a given genotype. In this way it incorporates the null model (no phenotypes associated with genotype); the simple univariate alternative (only one phenotype associated with genotype) and the general alternative (all phenotypes associated with genotype) into a single unified framework. In particular our approach both tests for and explains multivariate associations within a single model, avoiding the need to resort to univariate tests when explaining and interpreting significant multivariate findings. We illustrate the approach on examples, and show how, when combined with multiple phenotype data, the method can improve both power and interpretation of association analyses.

1102/T

Celiac disease: can CNVs contribute to the genetic risk? *G. Trynka[1], PC. Dubois[2], L. Franke[1,2], A. Zhernakova[3,4], J. Romanos[1], R. McManus[5], D. Barisani[6], P. Saavalainen[7], DA. Van Heel[2], C. Wijmenga[1].* 1) Genetics, UMCG, Groningen, Netherlands; 2) Institute of Cell and Molecular Science, Barts and The London School of Medicine and Dentistry, London, UK; 3) Medical Genetics Dept, University Medical Center Utrecht, the Netherlands; 4) Dept of Rheumatology, University Medical Center Leiden, the Netherlands; 5) Departments of Clinical Medicine, Institute of Molecular Medicine, Trinity College Dublin, Dublin, Ireland; 6) Department of Experimental Medicine, Faculty of Medicine University of Milano-Bicocca, Monza, Italy; 7) Department of Medical Genetics, and Research Program of Molecular Medicine, University of Helsinki, Finland.

Celiac disease is an autoimmune disorder with prevalence of 1-3% in the Caucasian population and high heritability of 80%. HLA is the major genetic risk factor but accounts only for 35% of the genetic risk. Recent genome-wide association study (GWAS) of 4,533 cases and 10,750 controls and its follow-up in additional 4,918 cases and 5,684 matched controls have identified 26 non-HLA risk loci (p-value<5x10e-08) and suggested associations for a further 13 loci. Over 50% of the associated single nucleotide polymorphisms (SNPs) correlate with cis gene expression and for 70% the effect of the risk allele results in lower expression. Celiac disease has a complex genetic background, HLA and the 26 non-HLA loci account for 40% of the genetic risk. We sought to investigate the involvement of other genetic variations in the pathogenesis of celiac disease. Recent studies show that majority of copy number variations (CNVs) can be captured by tagging SNPs. There are over 30,000 SNPs reported to tag CNV changes and nearly 18,000 of these SNPs were present in our imputed GWAS data of 15,283 individuals. Excluding HLA region, 30 SNPs representing 29 loci were associated at p<10e-03. When investigating the association strength within the LD regions harbouring the CNV tag-SNPs, we observed that eleven tag-SNPs were the most associated among all the other tested SNPs. Additionally 23% of these associated tag-SNPs showed significant cis e-QTL effects. Furthermore, eight SNPs were perfectly tagging CNVs with R2>0.8. Because the remaining SNPs showed imperfect LD with the tagged CNVs, we hypothesised that the CNV variants may be more strongly associated than their tagging SNPs. Currently, we are genotyping 21 CNVs in ~3000 samples from the Netherlands and Italy. Our results indicate that CNVs, next to the SNP variants, may contribute to the genetic risk predisposing to celiac disease.

1103/T

Genome-Wide Association Study for Rheumatoid Arthritis in the Korean Population Points to Novel Candidate Loci and Indicates Overlap with European Susceptibility Loci. *J. Freudenberg[1], H.S. Lee[2], H.D. Shin[3], B.G. Han[4], Y.M. Kang[5], S.C. Shim[6], Y.K. Sung[2], C.B. Choi[2], A.T. Lee[1], P.K. Gregersen[1], S.C. Bae[2].* 1) Center for Genomics and Human Genetics Feinstein Institute for Medical Research North Shore LIJ Health System; 2) Hanyang University Hospital for Rheumatic Diseases, Seoul 133-792, Korea; 3) Sogang University, Seoul, Korea; 4) Korea National Institute of Health, Seoul, Korea; 5) Kyungpook National University School of Medicine, Daegu, Korea; 6) Eulji University Hospital, Daejeon, Korea.

To find novel susceptibility loci for Rheumatoid Arthritis (RA) and to compare the genetic basis of RA between populations, we performed a genome-wide association study (GWAS) in Korean individuals. We generated high quality genotypes for 441398 SNPs in 801 cases of RA and 757 controls. Genome-wide significance (P<5*e-08) was attained by markers from the MHC-region and at *PADI4*. We next partitioned SNPs into separate LD-blocks and quantified the observed excess of LD-blocks that contain associated SNPs by using permutation of the affection status (excluding the MHC-region). This showed an excess of 14 loci when calling SNPs associated using a threshold of P<1e-04 that increased to an excess of about 500 LD-blocks when calling SNPs associated with P<0.1. In the second stage, we genotyped 79 markers from 46 loci in a replication sample of 718 cases and 719 controls. The combined analysis of genotypes from both stages did not reveal any additional loci of genome-wide statistical significance. However, the replication data showed nominal association signals (P<5*e-02) for markers from 11 out of 46 loci. Genes that were most significant in the replication stage and in the combined analysis include the known European RA loci *BLK*, *AFF3* and *CCL21* as well as *PTPN2*, *FLI1*, *LCP2*, *GPR137B*, *TRHDE*, *CGA1* and *ARHGEF3*. Based on the P-value distribution of our replication genotypes, we estimate that at least four of these loci are genuine RA susceptibility genes. Finally, we systematically analyzed the overlap of association signals with previously established European RA loci. To this end we retrieved the set of LD-blocks that harbor associated SNPs in Europeans and estimated its odds ratio for showing a SNP-association in the Korean GWAS data. This odds ratio statistic was compared to its expected value as obtained from permutation of the affection status. This showed a significant enrichment of European RA loci, which became more pronounced with increasing stringency for calling loci associated with RA. We conclude that certain genetic RA susceptibility factors are shared between populations and that the use of independent knowledge provides a feasible way to exploit subthreshold association signals in GWAS datasets.

1104/T

Genome-wide meta-analysis of brachial circumference. *V. Boraska[1,2], A. Day-Williams[1], C. Beazley[1], E. Albrecht[3], L.J. Beilin[4], H. Campbell[5], L. Ferrucci[6], C. Franklin[1], C. Gieger[3], C. Hayward[7], C. Huth[3], N. Klopp[3], I. Kolcic[8], L.J. Palmer[9], M. Pehlic[2], C.E. Pennell[10], J.R.B. Perry[11], A. Peters[3], O. Polasek[8], I. Rudan[5,12], V. Vitart[7], N.M. Warrington[10], H.E. Wichmann[3,13,14], A.F. Wright[7], H. Yaghootkar[11], T. Zemunik[2], L. Zgaga[5,8], K. Elliott[15], E. Zeggini[1].* 1) Wellcome Trust Sanger Institute, The Morgan Building, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK; 2) Department of Medical Biology, School of Medicine, University of Split, Croatia; 3) Institute of Epidemiology, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Germany; 4) School of Medicine and Pharmacology, The University of Western Australia; 5) Centre for Population Health Sciences and Institute of Genetics and Molecular Medicine, College of Medicine and Veterinary Medicine, University of Edinburgh, UK; 6) Longitudinal Study Section, Clinical Research Branch, ASTRA Unit, Baltimore, USA; 7) MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine, Edinburgh, UK; 8) Andrija Stampar School of Public Health, School of Medicine, University of Zagreb, Croatia; 9) Centre for Genetic Epidemiology and Biostatistics, The University of Western Australia; 10) School of Women's and Infants' Health, The University of Western Australia; 11) Genetics of Complex Traits, Peninsula Medical School, University of Exeter, Exeter, UK; 12) Croatian Centre for Global Health, School of Medicine, University of Split, Croatia; 13) Institute of Medical Informatics, Biometry and Epidemiology, Chair of Epidemiology, Ludwig-Maximilians-Universität, Munich, Germany; 14) Klinikum Grosshadern, Munich, Germany; 15) Wellcome Trust Centre for Human Genetics, University of Oxford, UK.

Introduction: Brachial circumference (BC), also known as upper arm or mid arm circumference, can be used as an indicator of muscle mass and fat tissue, which are differently distributed in men and women. The purpose of this study is to identify genetic variants associated with BC through a large-scale genome-wide association scan (GWAS) meta-analysis. Methods: To identify gender-specific effects underlying BC we used fixed-effects meta-analysis to synthesise data from 7 GWAS on 3551 males, 4152 females and on the combined set of 7703 individuals. Association analyses were adjusted for age and BMI. Reported investigations have been carried out following the principles of the Declaration of Helsinki. Results and Discussion: The male-specific meta-analysis identified a signal between the nuclear receptor co-repressor 2 (NCOR2) and scavenger receptor class B, member 1 (SCARB1) genes on chr12 (e.g. rs275771, β= 2.824, 95%CI [1.842, 3.806], p-value=1.74E-08). The NCOR2 gene has been suggested to play a role in the regulating adipose tissue accumulation and NCOR2 +/- mice develop increased adiposity on a high-fat diet but have normal weight on a regular diet. The SCARB1 gene acts as a high-density lipoprotein (HDL) receptor and is involved in both HDL and low-density lipoprotein (LDL) metabolism. The female-specific meta-analysis identified several associated variants within the SEC14-like 5 (S. cerevisiae) (SEC14L5) gene on chr16 (e.g. intronic SNP rs12444698, β= -2.382, 95%CI [-3.306,-1.458], p-value= 4.44E-07). A conserved domain of SEC14L5 gene, Sec14p-like lipid-binding domain, is found in secretory proteins and in lipid regulated proteins. Meta-analysis across males and females identified a region on chr9 spreading across 120kb (e.g. rs2383455, β= -2.025, 95%CI [-2.814, -1.238], p-value= 4.78E-07), with the closest gene 700kb away. Conclusion: In this first GWAS meta-analysis for BC to date, we have identified promising signals (below or close to the genome-wide significance level), which will require replication in independent datasets. These studies are currently underway.

1105/T

Detection and Estimation of Identity by Descent with High Density Genotype Data. *L. Han, M. Abney.* Human genetics, University of Chicago, Chicago, IL.

Estimating identity by descent (IBD) sharing between individuals is of fundamental importance in genetics. It is important for a variety of problems including disease gene mapping, genotype imputation and haplotype inference, and detection of runs of shared genomic segments. We consider the problem of estimating IBD between pairs of individuals using dense SNP data. We describe a method that estimates IBD given a pedigree of arbitrary size and complexity. We extend the standard hidden Markov model using two different methods to incorporate linkage disequilibrium (LD). The first uses phased haplotypes and uses haplotype probabilities between pairs of SNPs. The second uses unphased genotypes and incorporates LD from multiple SNPs. We also describe an extension to our method that allows for IBD estimates when the pedigree is unknown. Our methods can estimate IBD at specific loci, chromosome-wide and genome-wide averages, and detect IBD segments shared between individuals. We demonstrate the accuracy of our methods using simulated data on pedigrees ranging from small (e.g. sib pairs) to large and complex. We consider both closely and distantly related pairs. We also estimate IBD for all pairs in a real data set consisting of 609 individuals (185,745 pairs) from a large and complex 13 generation pedigree, all of whom were genotyped on the 500k Affymetrix SNP chip. The software package IBDLD that implements our method is freely available.

1106/T

Association analyses of 249,796 individuals reveal eighteen new loci associated with body mass index. *C.J. Willer[1], E.K. Speliotes[2,3], S.I. Berndt[4], K.L. Monda[5], G. Thorleifsson[6], A.U. Jackson[1], H. Lango Allen[7], C.M. Lindgren[8,9], J. Luan[10], S. Vedantam[2,11], L. Qi[12,13], I.M. Heid[14,15], H.M. Stringham[1], A.R. Wood[7], M.N. Weedon[7], T. Frayling[7], U. Thorsteinsdottir[6,16], G.R. Abecasis[1], I. Barroso[17,18], M. Boehnke[1], K. Stefansson[6,16], K.E. North[5,19], M.I. McCarthy[8,9,20], J.N. Hirschhorn[2,11,21], E. Ingelsson[22], R.J.F. Loos[10] on behalf of the GIANT (Genetic Investigation of ANthropometric Traits) Consortium.* 1) Department of Biostatistics and Center for Statistical Genetics, University of Michigan, Ann Arbor, MI, 48103; 2) Metabolism Initiative and Program in Medical and Population Genetics, Broad Institute, Cambridge, Massachusetts 02142, USA; 3) Division of Gastroenterology, Massachusetts General Hospital, Boston, Massachusetts 02114, USA; 4) Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland 20892, USA; 5) Department of Epidemiology, School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514, USA; 6) deCODE Genetics, 101 Reykjavik, Iceland; 7) Genetics of Complex Traits, Peninsula College of Medicine and Dentistry, University of Exeter, Exeter, EX1 2LU, UK; 8) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, OX3 7BN, UK; 9) Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford, OX3 7LJ, UK; 10) MRC Epidemiology Unit, Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge, CB2 0QQ, UK; 11) Divisions of Genetics and Endocrinology and Program in Genomics, Children's Hospital, Boston, Massachusetts 02115, USA; 12) Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts 02115, USA; 13) Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts 02115, USA; 14) Regensburg University Medical Center, Department of Epidemiology and Preventive Medicine, 93053 Regensburg, Germany; 15) Institute of Epidemiology, Helmholtz Zentrum München - German Research Center for Environmental Health, 85764 Neuherberg, Germany; 16) Faculty of Medicine, University of Iceland, 101 Reykjavík, Iceland; 17) Wellcome Trust Sanger Institute, Hinxton, Cambridge, CB10 1SA, UK; 18) University of Cambridge Metabolic Research Labs, Institute of Metabolic Science Addenbrooke's Hospital, CB2 OQQ, Cambridge, UK; 19) Carolina Center for Genome Sciences, School of Public Health, University of North Carolina Chapel Hill, Chapel Hill, North Carolina 27514, USA; 20) NIHR Oxford Biomedical Research Centre, Churchill Hospital, Oxford, OX3 7LJ, UK; 21) Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115, USA; 22) Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, 171 77 Stockholm, Sweden.

Obesity is a well-established risk factor for a number of chronic diseases and presents a substantial public health challenge. While behavior and environment clearly play a role, strong evidence exists for a substantial genetic contribution to body mass index (BMI), a common measure of obesity. Previous genome-wide association (GWA) studies have identified a number of obesity-susceptibility loci. However, we expect that more loci, potentially leading to new biological mechanisms and therapeutic targets, can be identified from increased sample sizes. With this aim, we performed a meta-analysis of GWA data for BMI at 2.8 million SNPs in 123,865 individuals of European descent from 46 cohorts from the GIANT Consortium. We examined the most promising 42 SNPs in an additional set of follow-up samples (combined sample size up to 249,796 individuals). Combined analysis identified 18 new loci (p£5x10-8) that locate in or near *RBJ/ADCY3/POMC, MAP2K5/LBXCOR1, QPCTL/GIPR, FANCL, TNNI3K, LRRN6C, FLJ35779/HMGCR, SLC39A8, TMEM160/NDUFS3, CADM2, LRP1B, PRKD1, MTIF3/GTF3A, ZNF608, PTBP2, RPL27A/TUB, NUDT3/HMGA1,* and *GPRC5B*. We also confirmed 14 previously-identified obesity-related loci: *FTO, MC4R, TMEM18, GNPDA2, TFAP2B, BDNF, SH2B1, SEC16B, ETV5, NEGR1, MTCH2, FAIM2, NRXN3*, and *KCTD15*. Strongly associated SNPs near the novel locus GPRC5B are perfect proxies of a 21kb polymorphic deletion. Although these SNPs only explain ~1.5% of the variance in BMI, individuals with the highest genetic risk score (≥38 BMI-increasing alleles, 1.5% of individuals) had a mean BMI 2.7 kg/m^2 (typically corresponding to 15-19 lbs in weight) higher than individuals with the lowest genetic risk score (£21 BMI-increasing alleles, 2.2%). As previously, loci discovered indicate a CNS-mediated effect on obesity; however, the majority of new loci are as yet unannotated and likely represent novel biological mechanisms. Prediction modeling based on the observed effect sizes at these loci suggest that there are ~180 loci with similar effect sizes that have not yet been identified, and likely many more with smaller effect sizes. Ongoing and future research includes imputation based on the 1000 Genomes Project and follow-up of the top 5000 SNPs in hundreds of thousands of samples using a custom-designed Metabochip. These discoveries provide information about the allelic architecture and frequency spectrum of complex trait susceptibility variants and will guide the design of future sequencing studies.

1107/T

Strongly-correlated quantum-system approach in association study of rheumatoid arthritis. *I. Sandalov[1], L. Padyukov[2].* 1) Condensed Matter Physics, Royal Institute of Technology, S-164 40 Kista- Stockholm, Sweden; 2) Rheumatology Unit, Karolinska University Hospital Solna SE-171 76 Stockholm Sweden.

To identify putative relations between different genetic factors in human genome in development of common complex disease we applied strongly-correlated quantum systems approach to genetic data. To test applicability of approach, we analyzed 1820 rheumatoid arthritis (RA) cases and 947 healthy controls by correlation functions with data for 7 SNPs from HTR2A locus. Each SNP can be considered as a "spin", with three pairs (genotypes) represented in each "cell" of the genotype state vector (GV) characterizing each person. For our experimental data this ensemble can be separated into 151 GV subgroups g=(n,n_ctrl,n_case,v), n=n_ctrl+n_case, where n_ctrl,n_case are numbers of persons with several particular GV, which are specific only for controls (2.3%) or for cases (1.37%) GVs.GVs with large odds ratio (OR) assumed to contain SNPs in question. The characteristic matrix for each state is designed to compare many-spin states, introduce three population numbers of "single-particle" states |i >, and check for HWE for each SNP. The 3x3 matrices of pair correlation functions (CF) for those SNPs, which are suggested by GVs with high OR are calculated for control and case sets separately. The correlations between different components of different cells may strongly differ from each other, but all are relatively low, less than 5%. The strongest correlations are found between SNPs AA rs977003 and CC rs6314 (0.0554 in control and 0.0457 in case groups), however, the ctrl-case difference of CFs dK=K(ctrl)-K(case) in SNP-SNP correlators is larger for other SNPs, e.g., for CA rs977003 and CC rs6311 it is -0.0184, CA rs977003 and GG rs6313 combined it is -0.0181, etc.. Comparison of most contrast GVs reveals quite large shift of pair-SNP population of cells from controls to case, namely the heterozygotic state may increase a risk of disease. The whole picture, however, is complex and determined by more than one particular SNP shift. From our test we conclude that a reformulation of quantum theory methods for needs of genetics provide not only a cheap and efficient way for analysis, but, also, opens a somewhat new interesting perspectives for quantitative description and modeling of genomic structure of populations.

1108/T

Bayesian Methods to Overcome the Winner's Curse in Genetic Studies. *L. Xu[1], R. Craiu[1], L. Sun[1,2].* 1) Department of statistics, University of Toronto; 2) Division of Biostatistics, Dalla Lana School of Public Health , University of Toronto, Toronto, Ontario, Canada.

Introduction: Parameter estimates for associated SNPs, reported in the discovery samples, the so-called naïve estimates, are often grossly inflated compared to the values observed in the follow-up samples. This type of bias is a consequence of the sequential procedure, because a declared associated variant must first pass a stringent significance threshold. This phenomenon is also known as the Winner's curse. Both likelihood-based and bootstrap-based bias reduction methods have been proposed in the literature but the variation in the estimates could be very high for practical use in replication sample size calculations. Methodology: We propose a Bayesian hierarchical model that takes advantage of available prior information producing estimators with a smaller bias and variance especially in low power studies. We use a spike-and-slab prior to account for the possibility that the significant association may be a false positive. To robustify the method to prior specification, we adopt a Bayesian Model Averaging (BMA) strategy that averages over models with different prior belief in the discovery finding's authenticity. The proposed method requires only the summary statistics such as the reported naïve estimate of the odds ratio and the corresponding association p-value. Results: Extensive simulations confirmed that the BMA method performs well in a variety of settings and that it outperforms the likelihood-based approaches in low power studies. For example, the standard error in the sample size prediction for a replication study (80% power at the 0.05 level) using the BMA estimate is almost twice as small as the replication sample size calculated based on the likelihood estimate. We also applied the methods to four different association studies, including a candidate gene study and three genome-wide association studies of binary case-control or quantitative outcomes. Application results demonstrate the importance of utilizing known prior information such as the study design and putative power of detecting a SNP association.

1109/T

Validating and genotyping rare SNPs with Axiom™ microarray technology. *T.A. Webster, J. Gollub, E. Hubbell, Y. Zhan, Y. Lu, T. Asbury, L. Weng, M. Purdy, M. Shapiro.* Algorithms & DataAnalysis, Affymetrix, Santa Clara, CA.

Investigating the role of rare SNPs in common disease fuels the need for high-throughput, cost-effective, and accurate methods for validating and genotyping such SNPs. We present components of such a method that uses the Axiom™ Genotyping Solution, a high-performance microarray technology from Affymetrix. First, candidates from SNP discovery projects are screened by the Axiom system against samples from reference populations with enough diversity to elicit two to three examples of heterozygous genotype. Second, properties of the genotypes clusters produced by small number of heterozygous genotypes, along with the major homozygous genotypes, are used to validate that the SNP site is polymorphic. Third, the "Axiom GTv1" algorithm generates genotypes using a Bayesian procedure in which priors for the SNPs are combined with the data to obtain posterior estimates of genotype cluster centers and variance of the missing minor homozygous genotypes, as well as the heterozygous and major homozygous genotypes. This enables genotyping of rare SNPs in new data sets. Rare SNPs collected by this process fuel the Axiom database of validated SNPs. Genotypes from the reference populations in the screening data are used to estimate the contribution to coverage of rare SNPs in the genome. The current database covers more than 80% of rare CEU SNPs (MAF < 5%) in a reference set of HapMap plus validated SNPs from the 1000 Genomes Project. The resulting database is a resource from which multiple SNP panels can be derived, optimizing coverage of rare variants in studies seeking to understand the association of rare SNPs with common diseases.

1110/T

Replication of GWAS loci for fasting plasma glucose in African Americans. *E. Ramos[1], G. Chen[1], A. Doumatey[1], D. Shriner[1], N.P. Gerry[2], A. Herbert[3], H. Huang[1], J. Zhou[1], M.F. Christman[2], A. Adeyemo[1], C. Rotimi[1].* 1) Center for Research on Genomics and Global Health, NIH/NHGRI, Bethesda, MD; 2) Coriell Institute for Medical Research, Camden, NJ; 3) Department of Genetics and Genomics, Boston University School of Medicine, Boston, MA.

Chronically elevated blood glucose (hyperglycemia) is the primary indicator of type 2 diabetes, which has a prevalence that varies considerably by ethnicity in the United States with African Americans disproportionately affected. Genome-wide association studies (GWAS) have significantly enhanced our understanding of the genetic basis of diabetes and related traits including fasting plasma glucose (FPG). However, the majority of GWAS have been conducted in populations of European ancestry (EA), including a recent meta-analysis of multiple cohorts. Thus, it is important to conduct replication analyses in non-EA populations to both verify and identify shared loci associated with FPG across populations. We used data collected from nondiabetic individuals (n = 927) that participated in the Howard University Family Study, a cohort of African Americans, to replicate previously published GWAS of FPG. In addition to comparing SNPs directly, we queried a 500-kb window centered on each reported SNP for additional markers in linkage disequilibrium (LD). Using direct SNP and LD-based comparisons, we replicated multiple SNPs previously associated with FPG and strongly associated with type 2 diabetes in recently published meta-analyses and related GWAS in EA populations. The replicated SNPs included those in or near TCF7L2, SLC30A8, G6PC2, MTNR1B, DGKB-TMEM195, and GCKR. We also replicated additional variants in LD with the reported SNP in ZMAT4 and adjacent to IRS1. We replicated multiple GWAS variants for FPG in our cohort of African Americans. Using LD-based strategy, we also identified SNPs not previously reported demonstrating the utility of using diverse populations for replication analysis.

1111/T

COPACETIC, a genome-wide association study on chronic obstructive pulmonary disease (COPD). *J. Smolonska[1,2], P. Zanen[3], D.S. Postma[4], B. van Ginneken[5], B. de Hoop[6], A.E. Dijkstra[4], M. Platteel[1], M. Oudkerk[7], J-W. Lammers[3], H.J.M. Groen[4], A. Dirksen[8], J.H. Pedersen[9], K. Forsman-Semb[10], M. Dahlback[10], P. Nastalek[11], F. Mejza[11], E. Nizankowska[11], J. Vestbo[12,13], B.G. Nordestgaard[14,15], H.M. Boezen[2], C. Wijmenga[1], . COPACETIC[16].* 1) Department of Genetics, University Medical Center Groningen, the Netherlands; 2) Department of Epidemiology, University Medical Center Groningen, the Netherlands; 3) Department of Pulmonology University Medical Center, Utrecht, the Netherlands; 4) Department of Pulmonology University Medical Center Groningen, the Netherlands; 5) Image Sciences Institute, University Medical Center Utrecht, the Netherlands; 6) Department of Radiology, University Medical Center Utrecht, the Netherlands; 7) Department of Radiology University Medical Center Groningen, the Netherlands; 8) Department of Pulmonary Medicine, Gentofte University Hospital, Hellerup, Denmark; 9) 44Department of Cardiothoracic Surgery, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; 10) AstraZeneca R&D, Lund, Sweden; 11) Division of Pulmonary Diseases, Department of Internal Medicine, Jagiellonian University School of Medicine, Krakow, Poland; 12) Department Respiratory Medicine, Hvidovre Hospital, Denmark; 13) University of Manchester, Manchester, UK; 14) 14Department of Clinical Biochemistry, Herlev Hospital, Copenhagen University Hospital, Faculty of Health Sciences, University of Copenhagen, Denmark; 15) 15The Copenhagen City Heart Study, Bispebjerg Hospital, Copenhagen University Hospital, Faculty of Health Sciences, University of Copenhagen, Denmark; 16) European Union.

Chronic obstructive pulmonary disease (COPD) is characterized by persistent, progressive airway obstruction. COPD is a complex disease which develops due to interplay of environmental factors like tobacco smoking and genetic factors which are still largely unknown. The aim of our study is to identify novel genetic risk factors for COPD using a genome-wide association study (GWAS). Study participants were recruited from the Nelson study, a CT-based lung cancer screening trial in heavy smokers (>20 pack years). DNA samples were genotyped using Illumina Human610-Quad arrays. COPD was defined as obstruction (FEV1/FVC<70%) and controls as FEV1/FVC >70% and FEV1%pred >90. To gain statistical power we added blood bank controls with unknown smoking history which were genotyped using Illumina Human670-Quad arrays. Standard quality checks were performed to remove samples and SNPs performing less well. Ethnic outliers, duplicates and lung cancer cases were excluded from the analysis. Association tests were performed on 1030 COPD cases and 1799 controls. The genomic inflation factor showed no significant deviation indicating no population stratification. Top 312 SNPs were selected for replication in seven independent (population-based) cohorts comprising over 10,000 individuals. Replication set included Vlagtwedde/Vlaardingen, Doetinchem, GRIP, Glucold, BOLD, DCLST and CCHS cohorts of different European descents. Genotyping was performed using Illumina Golden Gate custom panels. All genotype clusters were inspected and adjusted manually if necessary. Each cohort was analyzed separately and results were combined in meta-analysis taking into account heterogeneity between studies. Meta-analysis of half of our replication set revealed several loci approaching genome-wide significance. Currently we are finalizing the analysis of the other half of the replication set and we expect to find more loci with more significant p values.

1112/T

An Efficient and Powerful Framework for eQTL Analysis. *E. Kostem[1], E. Eskin[1,2].* 1) Computer Sci Dept, UCLA, Los Angeles, CA; 2) Human Gen Dept, UCLA, Los Angeles, CA.

Most expression quantitative trait loci (eQTL) mapping studies collect gene expression data from tens of thousands of genes and genotype data on millions of single nucleotide polymorphisms (SNPs). For a study, this corresponds to computing billions of association statistics and these numbers will only increase as more gene isoforms and SNPs are being discovered by next-generation sequencing studies. However, only a small percentage of the SNPs are expected to be associated and there is a lack of a computationally efficient strategy to identify the associated SNPs without evaluating the association statistic at every gene/SNP pair. We introduce an efficient and powerful eQTL analysis framework that identifies all the associated SNPs with high probability using a two-stage method. In both stages our proposed method identifies a subset of the SNPs and evaluates the association statistic only at these SNPs. Our method uses the correlation structure between all the SNPs and, in the second stage, the observed association statistics from the first stage. In addition, it aims to minimize the total number of SNPs that will be observed such that a given percentage of all the associated SNPs is identified. We conduct simulation studies and use real eQTL data to show that our method can reduce the computational time significantly to identify nearly all of the associated SNPs.

1113/T

Variation in the HGF gene is associated with keratoconus (KC) in the Caucasian population. *Y. Bykhovskaya[1], X. Li[1,2], G. Tang[1], K.D. Taylor[2], E. Mengesha[2], T. Haritunians[2], D. Siscovick[3], J.I. Rotter[2], Y.S. Rabinowitz[1].* 1) Cornea Genetic Eye Institute, Cedars-Sinai Medical Center, Los Angeles, CA; 2) Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, CA; 3) Departments of Medicine and Epidemiology and Cardiovascular Health Research Unit, University of Washington, Seattle, WA.

Keratoconus (KC) is a bilateral non-inflammatory progressive corneal ectasia with an incidence of approximately 1 per 2,000 in the general population. It is a multi-factorial disease influenced by the complex genetic inheritance of multiple nuclear genes. To identify susceptibility loci for KC, we performed a genome-wide screen of 222 Caucasian patients with KC and 3324 Caucasian controls from the Cardiovascular Health Study, using the Illumina Human CNV370-Duo beadchip. Association was evaluated by the allelic test and by logistic regression. Population stratification was tested by EIGENSTRAT program and principal component variables were used as covariates. SNP rs3735520 in the regulatory region upstream of the HGF (Hepatocyte Growth Factor) gene was associated with KC (risk allele T, $p=6.05x10^{-7}$; corrected p_PCA=$4.66x10^{-5}$; corrected multiplicative per-allele odds ratio of 1.5; 95% confidence interval 1.2-1.9). Allele A of the nonsynonymous polymorphism rs5745687 in exon 8 of HGF (E304K) was also associated ($p=4.2x10^{-3}$). Predictive analysis of this amino acid substitution by PMUT software identified this amino acid change as potentially pathological. Population and family based genetic studies have previously identified genomic variants in the HGF gene in patients with refractive abnormalities of the eye. Allele T in the rs3735520 was associated with myopia in Chinese and Caucasian families (Han 2006; Yanovitch 2009), while other HGF gene polymorphisms were found to be significantly associated with hypermetropia (Veerappan 2010). A GWAS study recently presented at the ARVO meeting identified an association between non-coding variants in HGF gene, including the same polymorphism rs3735520, and KC in Caucasian individuals (Burdon 2010). Our study provides further evidence for an association between genomic variation in the HGF gene and various ocular pathologies in the patients of multiple ethnic backgrounds. This suggests that HGF is a potential major genetic determinant of corneal and refractive diseases of the eye. We are currently performing genotyping of the HGF polymorphisms in additional individuals with KC, as well as investigating potential functional consequences of HGF gene variation on the tissue-specific expression and function of the HGF gene.

1114/T

Genome-wide association study of refractive error in 1304 individuals. *S.M. Hosseini[1,2], D. Waggott[3], E. Shen[3], A.P. Boright[4], M.P. Sylvestre[3], W. Sun[5], P.A. Cleary[5], A.J. Canty[6], L. Sun[7], S.B. Bull[3,7], A.D. Paterson[1,2,7], the DCCT/EDIC Research Group.* 1) Genetics & Genome Biology Program, Hosp Sick Children, Toronto, Canada; 2) Inst. of Medical Science, University of Toronto, Toronto, Canada; 3) Prosserman Centre for Health Research, Samuel Lunenfeld Research Inst of Mount Sinai Hosp, Toronto, Canada; 4) Dept of Medicine and University Health Network, University of Toronto, Toronto, Canada; 5) The Biostatistics Center, The George Washington University, Rockville, MD, USA; 6) Dept of Mathematics and Statistics, McMaster University, Hamilton, Ontario, Canada; 7) Dalla Lana School of Public Health, University of Toronto, Toronto, Canada.

Background: Refractive errors, the most common cause of correctable visual impairment, affect one fifth of the population and impose a major economical burden worldwide. Twin and family studies support a genetic basis for refractive errors (estimated heritability 60-85%). Genome-wide linkage studies, mostly focused on myopia as a dichotomous trait, had some success in mapping loci. Quantitative trait linkage analyses have identified loci for the refraction continuum without fine-mapping them. **Purpose:** Identifying common alleles associated with refractive errors in the Diabetes Control and Complications Trial (DCCT) cohort **Methods:** DCCT was a randomized trial to compare the effects of intensive and conventional treatment on the development and progression of diabetes complications. Subjective refraction was measured annually in probands (average of 6 measures). Genotyping was performed using Illumina 1M beadchip. After removing poor performing SNPs and outliers based on population stratification, genotype data was available for ~841K SNPs with a minor allele frequency ≥1% for 1304 white probands. Genotypes for an additional 1.7M SNPs were imputed using HapMap data (CEU, r22). Association between spherical equivalent (after a normal score transformation) and each SNP was tested in linear mixed models assuming additive coding for genotypes. To increase the power, 5 repeated measures of refraction were used. Several sets of analyses were performed with the simplest modeling outcome as a function of SNP genotype alone and more complex models adjusting for important covariates (age, sex, education, near-work, glycemic exposure, etc.). **Results:** Several SNPs showed evidence of association with refractive error without reaching genome-wide significance (7 loci with P-value < 10^{-5}). Of these 5q14 locus (rs1352737, p=4.66E-6) coincides with a linkage peak for ocular axial length (Zhu et al, 2008). rs10260667 (p=2.95E-6) on 7p14 is located within a locus linked to myopia and ocular refraction (Wojciechowski et al, 2009; Stambolian et al, 2006). **Conclusion:** This genome-wide association study of ocular refraction identified a number of interesting loci, some of which within regions linked to myopia or refraction. Efforts are underway to prioritize association results based on linkage, gene-expression and proteomics data and to replicate the observed associations in independent cohorts.

1115/F

Identification of a novel gene influencing total antioxidant status using an integrative genomic approach. *S. Kumar, J.E. Curran, M.A. Carless, E.I. Drigalenko, T.D. Dyer, M.P. Johnson, L. Almasy, E.K. Moses, H.H. Goring, M.C. Mahaney, J. Blangero.* Southwest Foundation for Biomedical Research, San Antonio, TX.

Atherosclerosis, the leading cause of mortality in North America, is predicted to attain a similar distinction on a global scale within the next twenty years. A substantial portion of disease risk has been demonstrated to be due to genetic effects, although attribution of this risk to specific genes involved has been relatively slow. Recently, oxidative stress has been shown to be a significant contributor to atherosclerosis. Plasma total antioxidant status (TAS) is a summary measure of an individual's ability to accommodate oxidative stress that has been inversely related to atherosclerosis risk. While there is strong evidence that genetic factors play a major role in determination of plasma TAS (about 51% of the variation in plasma TAS is due to genes), specific genes for this underlying variation are yet to be identified. We report the comprehensive dissection of a novel candidate gene influencing quantitative variation in plasma TAS, in Mexican American individuals from the San Antonio Family Heart Study (SAFHS). Using genome-wide association data currently available on 1,159 SAFHS samples, we identified two SNPs exhibiting suggestive genome-wide evidence for associations with plasma TAS. One of these SNPs (rs2712104, $p = 1.7\times10^{-7}$) is located near the UBX domain protein 2A (*UBXN2A*) gene. In a separate analysis of whole transcriptome gene expression data from most of these same individuals, we also have determined that this SNP is correlated strongly with *UBXN2A* expression levels in lymphocytes (p-value $=1.0\times10^{-4}$). Further, we find that *UBXN2A* transcript abundance is positively correlated ($p = 4.5\times10^{-6}$) with plasma TAS. The wide distribution of the UBX domain protein families indicates that the UBX domain has a role in basic cellular processes conserved from yeast to man. However specific function for these proteins is not clearly known. Our empirical evidence (coming from two independent sources) strongly supports the hypothesis that *UBXN2A* represents a novel player in the causal pathway for antioxidant status. We are currently employing deep sequencing to identify sequence variants responsible for the variation observed in plasma TAS. Identifying genetic variants directly influencing plasma TAS should greatly advance our understanding of mechanisms of redox homeostasis and facilitate identification of additional contributors to variation in susceptibility to atherosclerosis and other oxidative stress-induced diseases.

1116/F

Inferring pathogenic mechanisms from genome-wide association studies in autoimmune disease. *C. Cotsapas[1,2,3], BF. Voight[2], K. Lage[1,2,4,5], ER. Rossin[1,2,3], SS. Rich[6], DA. Hafler[7], MJ. Daly[1,2,3], FoCIS Network of Consortia.* 1) Center for Human Genetics Research, Massachusetts General Hospital, Boston, MA, USA; 2) Broad Institute, Cambridge MA, USA; 3) Dept of Medicine, Harvard Medical School, Boston, MA, USA; 4) Pediatric Surgical Research Laboratories, MassGeneral Hospital for Children, Massachusetts General Hospital, Boston, Massachusetts, USA; 5) Center for Biological Sequence Analysis, Department of Systems Biology,Technical University of Denmark, Lyngby, Denmark; 6) Center for Public Health Genomics, University of Virginia, Charlottesville VA, USA; 7) Dept of Neurology, Yale University School of Medicine, New Haven CT, USA.

Recent genome-wide association (GWA) studies have identified approximately 140 replicable genetic associations influencing risk of common autoimmune and inflammatory diseases, with the number still rising. Moreover, several of these loci have been independently shown to influence risk to more than one such disease, suggesting overlap in genetic risk factors for disease.

Here, we show that at least 50% of autoimmune/inflammatory loci influence risk of multiple diseases. These observations allow us to cluster loci into small groups of interacting proteins present in the same tissues. In this way we are able to propose models of pathogenesis from association data. Importantly, we find that our models are often parts of larger pathways; each part appears to influence risk to distinct but overlapping sets of diseases. For example, subsets of proteins involved in the IL23R-mediated signaling pathway appear to predispose to different diseases. This process is thought to initiate activation of pathogenic IL-17 producing T helper cells (Th17) and has been implicated in pathogenesis of several autoimmune and inflammatory diseases including Crohn's disease and multiple sclerosis.

These mechanistic overlaps have prompted us to perform comparisons genome-wide between pairs of diseases to identify the complete sets of loci influencing risk to each pair. We are in the process of replicating some of these observations and will discuss the extent of sharing between diseases.

Our work cumulatively suggests that specific perturbations in cellular pathways may have different intermediate phenotype outcomes. Our mechanistic models will allow us to screen for these phenotypes and shed light on the biology of immune disease.

1117/F

Making Sense of Small Effects in GWAS: statistics for dealing with diversity by integrating information. *M.A. Reimers[1,2], K.S. Kendler[1,2].* 1) Biostatistics, Virginia Commonwealth University, Richmond, VA; 2) Virginia Institute for Psychiatric and Behavioral Genetics, Richmond, VA.

Recent studies of complex disease genetics have led many researchers to think that complex diseases are different from the Mendelian diseases, which have so far been the model for the development of statistical methodology in genetics. In particular it seems there are many possible causes of a single disorder, including common variants with small effects and rare variants with big effects. I describe several integrated approaches using bioinformatic data within a Bayesian framework. I will describe some novel work on detecting and estimating the size distribution of small effects. I will describe a novel method for tests of small effects organized by functional gene sets. I will describe an approach for selecting most probable pairs of variants to test for epistasis. Then I will review some recent work on tests for rare variants, and present some methods under development. Finally I will propose an empirical Bayesian framework for estimating effect sizes, which may distinguish among rare variants uniquely present in cases.

1118/F

Constructing Endophenotype of complex disease via Nonnegative Matrix Factorization. *H. Wang[1,3], C. Hsu[2], S. Chang[3], C. Fann[1,3].* 1) Division of Biostatistics, Institute of Public Health, National Yang-Ming University, Taiwan; 2) Institute of Information Science, Taipei, Taiwan; 3) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan.

To effectively identify susceptibility genes of complex disorders, the endophenotype approach has been advocated by many researches. In this study, we hypothesize the existence of molecular dissimilarity across patients, although they may have the same clinical phenotype and belong to the same disease status. We carried out two-stage approach to construct endophenotypes and to investigate the characteristics and biological interpretation of constructed endophenotypes by integrating transcriptional profiles and SNPs data. In the first stage, an unsupervised nonnegative matrix factorization (NMF) was adopted to explore correlations in the high dimensional transcriptional data space and to cluster similar molecular subtypes by cophenetic correlation coefficient criteria. We defined the molecular subtypes derived from transcriptional profiles as endophenotypes. In the second stage, we then used multivariate ANOVA (MANOVA) analysis to detect the significant SNPs among molecular subtypes and to determine the SNP-transcript pairs in which gene expression was correlated with genotype in molecular subtypes. To evaluate the applicability of our approach, we applied our method to a genome-wide late-onset Alzheimer disease (LOAD) case-control data. The results showed that NMF classified a rank-three molecular subtype model for patient group of LOAD. We then performed MANOVA analysis and obtained 88 statistically significant SNPs (. Of the three molecular subtypes, there were 10 and 19 genes which specific expressed in the subtype 1 and the subtype 3 respectively; the subtype 2 was found to have a similar expression pattern with the control group. In conclusion, our approach seems to be able to detect the potential difference in patient group and may further link transcripts of each endophenotype to known functional pathways.

1119/F

Expression and association analyses implicate genes of the epidermal growth factor family in Behçet's disease (BD) susceptibility. *J.M. Xavier[1,2], T. Krug[1,2], M. Shafiee[3], B.S. Abdollahi[3], B.V. Fonseca[1,2], G. Jesus[4], F. Barcelos[5], J. Vedes[6], M. Salgado[7], J. Vaz Patto[5], F. Davatchi[3], J. Crespo[8], S.A. Oliveira[1,2].* 1) UNIC - Genomics Disease Lab, Instituto de Medicina Molecular, Lisbon, Portugal; 2) Human Genetics Group, Instituto Gulbenkian de Ciência, Oeiras, Portugal; 3) Rheumatology Research Center, Tehran University of Medical Sciences, Tehran, Iran; 4) Hospital Infante D. Pedro, Aveiro, Portugal; 5) Instituto Português de Reumatologia, Lisbon, Portugal; 6) Hospital de Sousa Martins, Guarda, Portugal; 7) Hospital Pediátrico de Coimbra, Coimbra, Portugal; 8) Hospitais da Universidade de Coimbra, Coimbra, Portugal.

BD is a multisystemic immuno-inflammatory disorder characterized by a generalized vasculitis, particularly at oral and genital mucosa and eye. To better understand the pathogenesis of BD, we applied an integrative approach that combines expression profiling analysis with association studies. We first compared the gene expression profiles in PBMCs of 15 patients and 14 matched controls using Affymetrix microarrays. We identified 508 differentially expressed genes that allow a good separation between cases and controls in hierarchical clustering and principal component analysis. Pathway analysis revealed that several genes in the ErbB signalling pathway were significantly differentially expressed. The ErbB family of receptor tyrosine kinases couples binding of extracellular growth factor ligands to intracellular signalling pathways regulating diverse biologic responses, including proliferation, differentiation, cell motility, cell survival, and vascular permeability. Three differentially expressed genes (EREG, AREG, and NRG1) belonging to the epidermal growth factor family of ligands for ErbBs were tested for association with BD. Genotyping of 43 tagging SNPs was performed in 550 Iranian BD patients and 436 controls. Patients were selected as consecutive patients, according to ICBD criteria. To control for potential confounding due to population stratification, we genotyped 90 ancestry-informative markers in our dataset and assessed population structure using EIGENSOFT. Both principal component analysis and computation of the inflation factor lambda indicated the inexistence of population stratification and of outlier individuals. Association tests (unadjusted and adjusted for gender) were performed using the R software. We found an association ($p<0.05$) in all loci studied: two SNPs in EREG, one SNP in the EREG-AREG intergenic region, and four SNPs in NRG1. Analysis of imputation-based data revealed additional evidence of association with risk of BD for SNPs in the upstream region of EREG, which was confirmed by actual SNP genotyping ($p<0.01$). These association findings support a role for the ErbB signalling pathway in BD pathogenesis that warrants further investigation. This study highlights the importance of combining genetic and genomic approaches to dissect the genetic architecture of complex diseases.

1120/F

Large variation for ROS production among healthy individuals shows strong evidence for genetic control. *H. Attar[1], K. Bedard[2], E. Migliavacca[1], M. Gagnebin[1], Y. Dupré[1], P. Descombes[3], H. Prokisch[4], T. Meitinger[4], D. Mehta[4], E. Wichmann[4], E.T. Dermitzakis[1], KH. Krause[5], S.E. Antonarakis[1,6].* 1) Department of Genetic Medicine, University of Geneva Medical School, Geneva, Switzerland; 2) Pathology Department, Dalhousie University, Halifax NS B3H 4H7, Canada; 3) Genomics Platform, NCCR Frontiers in Genetics, University of Geneva Medical School, Geneva, Switzerland; 4) GSF Research Center, Human Genetics and Epidemiology, Neuherberg, Germany; 5) Department of Pathology and Immunology, University of Geneva Medical School and University Hospitals, Geneva, Switzerland; 6) Department of Genetic Medicine and Development University of Geneva Medical School, and University Hospitals of Geneva.

DNA sequence variation contributes to individual differences in quantitative traits. Several studies have shown the genetic control of gene expression variation, but few cellular traits have been investigated that might be more closely related to clinical phenotypes. Here, we investigate the production of reactive oxygen species (ROS), a complex cellular phenotype that is involved in a number of human disorders. We assessed individual variation of ROS production in EBV-transformed B-lymphoblastoid cell lines (LCL) with a fluorescent AmplexRed assay to identify potential regulatory loci. We found substantial individual variation in ROS production. Heritability estimates using 10 CEPH families were ~45%; linkage analysis using SNP markers identified 2 genome-wide significant and 2 suggestive linkage signals on loci of Hsa12 and Hsa15, and for Hsa1 and Hsa19 respectively. We next performed a genome-wide association analysis (GWA) for the combined cohorts KORA and GenCord (N=279), using enhanced marker resolution through imputation (>1.4 million SNPs). Results indicate significant associations for 53 SNPs with p-values of less than 5e-6, 2 of which mapped in the linked regions on Hsa15 and Hsa19. As a replication sample we used 58 HapMap individuals and > 2.2 million SNPs. A total of 197 SNPs with $p<1e-6$ were identified, and we confirmed hits on Hsa1, Hsa12, Hsa15 and Hsa19. When comparing top association hits among the two GWA analyses, we did not detect replicated SNPs, but only replication within the same region of 1 kb or 1-2 Mb, respectively. A link between Down syndrome and ROS production has been previously made in the literature; we detected a highly significant decrease in ROS production in DS LCLs (N=76; Wilcoxon rank sum test, $p<8.07e-13$). Interestingly, a significant association in HapMap on Hsa21 close to the gene SIK1 ($p=3.12e-8$) has been observed. Taken together, our results show strong evidence of genetic factors at the basis of ROS production variation in cell lines of healthy and in DS individuals. On the other hand cellular phenotypes appear to be more complex than gene expression phenotypes and validation of GWA signals also require considerable sample sizes.

1121/F

Fine mapping of Type 2 Diabetes and Myocardial Infarction associations on chromosome 9p21 by regional sequencing and imputation. *J. Shea[1, 2], A.A. Philipakkis[1, 3, 4], V. Agarwala[1, 3], B. Thompson[1], C. Guiducci[1], N.P. Burtt[1], L. Groop[5], D. Altshuler[1,2,6].* 1) Broad Institute, Cambridge, MA; 2) Harvard Medical School, Boston, MA; 3) Harvard-Massachusetts Institute of Technology Division of Health Sciences and Technology, Harvard Medical School, Boston, MA; 4) Brigham and Women's Hospital, Boston MA; 5) Department of Clinical Sciences, Diabetes and Endocrinology Research Unit, University Hospital Malmö, Lund University, Malmö, Sweden; 6) Massachusetts General Hospital, Boston, MA.

Genome-wide association studies (GWAS) have identified hundreds of loci associated with common diseases, but in only a few cases have specific causal mutations been identified. This is in part because many associations map to poorly annotated non-coding regions that we do not yet know how to interpret, as exemplified by chromosome9p21, a region with associations to Type 2 Diabetes (T2D), Myocardial Infarction (MI), and several cancers. A mouse model in which 70kb of non-coding DNA homologous to the MI-associated region were deleted has provided evidence for regulatory function in the region influencing expression of the nearby tumor suppressor genes CDKN2A and CDKN2B, but specific regulatory elements and variants have not yet been identified. A key step in identifying functional mutations on 9p21 is enumerating the complete set of variants that could explain each association signal. Using the T2D- and MI-associated regions as test cases, we compared several strategies currently available to researchers for assembling such lists, including methods for identifying the full set of variants in associated regions (performing high-coverage re-sequencing versus using emerging data from the 1000 Genomes (1kG) project), and methods for imputing identified variants into case-control samples so they can be tested for association to disease. We found that the 1kG Pilot 1 data contained 78% of all sites detected in high coverage sequencing and 97% of sites with a variant allele frequency greater than 10%. High coverage re-sequencing detected rare variants that were missed by 1kG but, because of heterogeneous coverage, missed some common variants identified by 1kG. We found that imputation directly from both high coverage re-sequencing and 1kG Pilot 1 data into disease samples performed comparably to imputation from a genotyped reference panel. However, in order to capture nearly 100% of common variation in imputation, it was necessary to genotype additional markers in our disease samples. Having tested the majority of common variation on 9p21 for association to T2D and MI, we did not find any evidence for signals stronger than the original GWAS signals. For each disease, we identify a set of potential causal variants with equivalent support for association, which can now be systematically assayed for function.

1122/F

Allelic expression in autism: A novel approach for gene discovery. *E. Ben-David, E. Granot-Hershkovitz, G. Monderer-Rothkoff, E. Lerer, S. Levi, M. Yaari, R. Ebstein, N. Yirmiya, S. Shifman.* The Hebrew University of Jerusalem, Jerusalem, Israel.

Autistic disorder is the most severe end of a group of neurodevelopmental disorders referred to as autism spectrum disorders (ASDs). Autism is a heterogeneous syndrome characterized by social deficits, language impairments and repetitive behaviors. Autism has a strong genetic basis, but despite that, most of the genetic factors that contribute to the disease risk are still unknown. Recent work has led to the identification of several autism susceptibility genes and an increased appreciation of the contribution of de novo and inherited copy number variations (CNVs). Most identified ASD associated mutations and chromosome abnormalities are believed to disrupt the expression of genes from a single parental chromosome. In this study, we present a novel approach for identification of genes implicated in ASD. This method is based on measurement of the relative expression of the two copies of a gene (the paternal and maternal copies) using genotyping arrays. Large allelic expression imbalance (AEI) could arise from a wide range of genetic and epigenetic abnormalities, including rare variants, newly arisen mutations and alteration of the methylation patterns when present in only one copy of a gene. Single nucleotide polymorphism (SNP) arrays provided us with the ability to screen for AEI across the genome, as well as the ability to find structural variations. We screened for genes showing large AEI in lymphoblastoid cell lines derived from an ASD cohort. We first identified around 700 regions in the genome of common AEI - regions showing AEI across multiple samples, including imprinted regions and random monoallelic expression. Additionally, we identified unique regions showing large AEI in specific ASD samples. Validation by quantitative sequencing demonstrated that the genes (or, in some cases, only parts of the genes) are monoallelic expressed. The genes include both known risk factors for ASD and novel candidates, and, in some, the genetic mutation causing the AEI was revealed. Our results demonstrate the ability to identify rare regulatory mutations using genome-wide AEI screens, capabilities which could be expanded to other complex diseases, especially those with suspected involvement of rare mutations.

1123/F

Genetics of sputum gene expression to identify genes for chronic obstructive pulmonary disease. *C.P. Hersh[1], W. Qiu[1], M.H. Cho[1], J.H. Riley[2], W.H. Anderson[3], D. Singh[4], P. Bakke[5], A. Gulsvik[5], A.A. Litonjua[1], D.A. Lomas[6], J.D. Crapo[7], T.H. Beaty[8], B.R. Celli[1], S. Rennard[9], R. Tal-Singer[10], S.M. Fox[2], E.K. Silverman[1], ECLIPSE Investigators.* 1) Brigham and Women's Hospital, Boston, MA; 2) GlaxoSmithKline, Uxbridge, UK; 3) GlaxoSmithKline, Research Triangle Park, NC; 4) University of Manchester, UK; 5) University of Bergen, Norway; 6) University of Cambridge, UK; 7) National Jewish Health, Denver, CO; 8) Johns Hopkins University, Baltimore, MD; 9) Nebraska Medical Center, Omaha, NE; 10) GlaxoSmithKline, King of Prussia, PA.

Background: Many studies have performed genomewide association studies for gene expression, but most studies have examined blood cells or cell lines from non-diseased individuals. Objective: To examine the genetics of gene expression in a relevant disease tissue from patients with chronic obstructive pulmonary disease (COPD) to find novel disease genes. Methods: The Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points (ECLIPSE) Study is a multi-center 3-year observational study. Genome-wide SNP genotyping has been performed on all ECLIPSE subjects using the Illumina HumanHap550 BeadChip. Gene expression profiling on induced sputum samples from 131 COPD cases has been performed using the Affymetrix Human U133 Plus2 array. Results: Examining all SNPs within 50kb of a transcript (562,787 tests), we found 4315 SNP-probe associations to be significant at FDR-adjusted $p<0.05$. These 4315 regulatory SNPs (rSNPs) were tested for association with COPD in a GWAS which included subjects from the ECLIPSE, Norway, and NETT-NAS studies (2940 cases, 1380 controls). Adjusted for 4315 tests ($p<1.16x10^{-5}$), the 2 SNPs which were significantly associated with COPD were located in 2 genes on chromosome 15 with known COPD associations: CHRNA5 and IREB2. 70 additional rSNPs which were associated with COPD at $p<0.01$ were genotyped in the International COPD Genetics Network and in the COPDGene Study, finding replicated associations with a SNP in PSORS1C1, in the HLA-C region on chromosome 6 (combined $p=2.2x10^{-4}$). Conclusion: Integrative analysis of GWAS and sputum gene expression data has located potential functional variants in two known COPD genes and has identified a novel COPD susceptibility gene. Funded by: GlaxoSmithKline (SCO104960, NCT00292552) and NIH/NHLBI (R01HL094635).

1124/F

A Novel Mutation in the Pre-mRNA Splicing Factor PRPF31 causes early-onset Autosomal Dominant Retinitis Pigmentosa. *D. Chung[1,2], E. Place[2,3], M. Falk[3], E. Pierce[1,2].* 1) F. M. Kirby Center for Molecular Ophthalmology, Scheie Eye Institute, Department of Ophthalmology, University of Pennsylvania School of Medicine, Philadelphia, PA; 2) The CHOP Pediatric Center for Inherited Retinal Degeneration, Division of Ophthalmology, The Children's Hospital of Philadelphia, Philadelphia, PA; 3) Division of Human Genetics, Department of Pediatrics, The Children's Hospital of Philadelphia and University of Pennsylvania School of Medicine, Philadelphia, PA.

Retinitis pigmentosa (RP) is a clinically and genetically heterogeneous group of inherited retinal dystrophies that are characterized by photoreceptor degeneration with associated visual impairment. It is the most common form of retinal dystrophy, affecting about 1in 3500 individuals world-wide. The autosomal dominant form of RP (adRP) is attributed to mutations in 20 different genes. PRPF31 is one of 4 adRP genes involved with pre-mRNA slicing activity. Here, we report the clinical and genetic characteristics of a large, multi-generational family with adRP caused by a novel nonsense mutation found in the PRPF31 gene. The proband's clinical course began with a 1-2 year history of progressive nyctalopia, and photophobia. Progressive visual field loss was subsequently noted with relatively stable central vision. The clinical diagnosis of RP was made at the age of 6 years. Currently age 14, the proband has had progressively decreased visual acuity (20/60 OD, 20/160 OS). Dilated fundus examination revealed mild optic nerve pallor, attenuated retinal vasculature, and bone-spicules in the mid-periphery. Electroretinogram exhibited decreased rod and cone function. Fundus imaging was performed by spectral domain optical coherence tomography (SD-OCT) illustrated significant photoreceptor loss in the mid-periphery with marked cystoid macular edema, bilaterally. The proband's mother and maternal grandmother were found to have similar clinical features and histories of onset. A 9 year old male sibling also shows early clinical signs of retinal degeneration. Whole blood was collected from the proband and mutational screening was performed on the 20 known adRP genes, SNRNP200, CA4, CRX, FSCN2, GUCA1B, IMPDH1, KLHL7, NRL, NR2E3, PRPF3, PRPF8, PRPF31, RDH12, RDS, RHO, ROM1, RP1, RP9, RP31 and SEMA4A. PCR amplification and direct DNA sequencing was performed in both directions of all coding exons and exon/intron borders. Sequencing revealed a novel nonsense mutation in the PRPF31 gene (p.R354X). This mutation appears to produce an earlier onset of disease (first decade) than typical of other PRPF31 mutations, which cause disease onset in the second to third decades. In conclusion, we identified a novel mutation in the PRPF31 gene as a cause for adRP that exhibits an early onset of nyctalopia and retinal degeneration. Correlation of retinal disease phenotypes with specific genetic mutations may improve insight into disease progression and prognostic outcome.

1125/F

Novel abdominal adiposity genes and their interactions with dietary factors in association with type 2 diabetes risk: findings from two cohorts. *E. Yeung[1], L. Qi[2], F. Hu[2], C. Zhang[1].* 1) Epidmeiology Branch, DESPR, NICHD, Bethesda, MD; 2) Departments of Nutrition & Epidemiology, Harvard School of Public Health, Boston, MA.

A recent genome-wide association study (GWAS) found three SNPs (LYPLAL1 (rs2605100), MSRA (rs545854), TFAP2B (rs987237)) for central obesity, a strong risk factor for type 2 diabetes (T2D). However, little is known about the role of these SNPs in the development of T2D. We investigated their associations with T2D in two large case control studies from the Nurses' Health Study and Health Professionals Follow-up Study. Self-reported T2D cases were confirmed by validated questionnaire. We examined associations with T2D and interactions with diet among incident cases (1245 women, 828 men) and controls (2149 women, 1326 men) with prospectively collected dietary data by food-frequency questionnaire. We used logistic regression to estimate the odds ratio (OR) and 95% confidence interval (CI). We compared plasma adipokine levels (i.e. total and high molecular weight (HMW) adiponectin, leptin, leptin receptor, and resistin) by genotype among a subgroup of female controls (n=945). The effective allele frequency was 72% for LYPLAL1, 17% for MSRA, and 18% for TFAP2B. LYPLAL1 was associated with T2D after adjustment for BMI in both cohorts (pooled allelic OR 1.12: 1.01-1.24). MSRA was related to slightly increased risk of T2D in men only (age-adjusted allelic OR of 1.24 (95% CI: 1.02-1.51). No significant associations were seen with TFAP2B. We observed significant interactions between B12 intake and LYPLAL1 genotype from both cohorts (p-interaction<0.05). B12 intake was protective of T2D risk only among those homozygous for the effective LYPLAL1 allele. For example, among women with GG, the highest tertile of B12 intake was associated with decreased T2D risk (OR 0.63: 0.47-0.85) compared to the lowest tertile. Similarly for men with GG, OR was 0.62 (p=0.01). However, no benefit of B12 was seen for the other genotypes (p>0.10). Carriers of the MSRA effective allele had higher leptin levels (20.7 vs. 18.8 ng/ml, p= 0.06) and lower percent HMW adiponectin (38.8% vs. 40.9%, p=0.04). The TFAP2B effective allele, however, was associated with lower leptin levels (17.4 vs. 20.1 ng/ml p=0.005). The LYPLAL1 SNP was not related to adipokine levels. Conclusion: These findings suggest potential associations of novel abdominal obesity genes with T2D risk and that LYPLAL1 may synergistically interact with dietary vitamin B in determining risk in both men and women. More research is needed to understand the molecular mechanisms for the interaction.

1126/F

Genetic variation in Mean Platelet Volume and platelet count and metabolic characterization. *R. Rawal[1], K. Suhre[2,7], T. Illig[1], J. Adamski[5,6], H.E. Wichmann[1,3,4], C. Gieger[1].* 1) Institute for Epidemiology, Helmholtz Center, Munich/Neuherberg, Germany; 2) Institute for Bioinformatics and Systems biology Helmholtz Center, Munich/Neuherberg, Germany; 3) Institute for Medical informatics biometry and Epidemiology, Ludwig-Maximilans-Universität, Munich, Germany; 4) Klinikum Grosshadern, Munich, Germany; 5) Institute of Experimental Genetics, Genome analysis Center, Helmholtz Center, Munich/Neuherberg, Germany; 6) Institute of Experimental Genetics, Life and Food Science Center, Weihenstephan, Technische Universität München, Munich, Germany; 7) Faculty of Biology, Ludwig-Maximilans-Universität, Munich, Germany.

Serum metabolite concentrations measured by mass spectrometry and nuclear magnetic resonance provide a direct readout of biological and physiological processes in the human body, and they are associated with disorders such as immunity and cancer. Our recent genome-wide association studies (GWA) have established association between single nucleotide polymorphism (SNP) and blood cell parameters such as Mena platelet volume (MPV) and platelet count. By simultaneously analyzing these phenotypes with genome-wide SNP data and metabolite concentrations (and their ratios), we aim to illustrate the genetic variation that is associated with both blood cell counts and a metabolite concentration, which would provide new information about the underlying etiological processes in disease causing mechanism for the diseases related with platelet counts and platelet volume such as immune responses to infections and cancers of the circulatory system. First we analyzed the association between the phenotype Mean platelet volume (MPV) and platelet count and 151 metabolites in 3044 participants from the KORA population (Germany). 151 metabolites were grouped in to 10 biochemical groups such as amino acids, sugars, acylcarnitines, and phospholipids. In the poster we will try to further elucidate the role of genetic loci from the pre-published genes (3 QTL in the loci near the regions of the gene TAOK1, ARHGEF3 and WDR66) reported for the platelet count and MPV taking in to account the metabolite ratios and associated genes for the enzymes in the metabolism.

1127/F

PhenX Measures: Enhancing Research Studies of Gene-Environment Interactions. *W. Huggins[1], L.C. Strader[1], D.C. Whitcomb[2], B. Entwisle[3], B. Pescosolido[4], L. Goldman[5], J.A. Hammond[1], T. Hendershot[1], R.K. Kwok[1], H. Junkins[6], E. Ramos[6].* 1) RTI International, Research Triangle Park, NC; 2) Gastroenterology, University of Pittsburgh School of Medicine, Pittsburgh, PA; 3) University of North Carolina at Chapel Hill Carolina Population Center, Chapel Hill, NC; 4) Indiana University at Bloomington, Bloomington, IN; 5) Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD; 6) National Human Genome Research Institute, Bethesda, MD.

Few Genome-Wide Association Studies (GWAS) have included robust environmental exposure measures even though gene-environment interactions modulate the severity and presentation of virtually all human disease. The PhenX (consensus measures for Phenotypes and eXposures) Toolkit offers high quality, well-established, standard measures of phenotypes and exposures from 21 domains for use in GWAS and other large-scale genomic research efforts. For each research domain, a Working Group (WG) comprised of academic and clinical researchers from diverse institutions and disciplines is assembled to prioritize a set of low burden, well established measures. Here, we demonstrate a possible scenario to examine gene-environment interactions by highlighting measures from four PhenX Domains: Gastrointestinal, Environmental Exposures, Social Environments, and Psychosocial. The Gastrointestinal WG identified a range of disorders that can be influenced by an individual's environment including abdominal pain, recurrent constipation, and gastrointestinal cancers. These modulating factors can be assessed by other standard measures in the Toolkit. For example, the Environmental Exposures measures include water source, occupation history, and contact with common chemicals and solvents. The Social Environments WG identified a range of relevant exposures that address aspects of work, family, and neighborhood/community. The Psychosocial WG selected measures related to stress, coping, wellbeing, and social connectedness. Researchers can visit the web-based PhenX Toolkit (https://www.phenxtoolkit.org/) to review and select measures. For each measure, the Toolkit provides a description, selection rationale, protocol(s) for collecting the measure, and references. Users can browse, select and download the protocols for measures that are relevant for their research. The Toolkit provides a common currency for investigators who wish to add measures that are outside their primary research focus or to extend their research through the inclusion of salient PhenX measures to go beyond to explore the physical and social environments to determine the etiology of complex diseases. As investigators incorporate PhenX measures into their studies, there will be new opportunities for cross-study analyses to identify loci with small effect sizes and to discover gene-gene and gene-environment associations. Supported by: NHGRI, Award No. 1U01 HG004597-01.

1128/F

Investigation of *FTO* variants and interactions with physical activity among 3,484 Hispanic postmenopausal women in the Women's Health Initiative Study. *E. Rampersaud[1], T. Edwards[2], K. Monda[3], K. North[3], C. Carlson[4], S. Wassertheil-Smoller[5], M. Neuhouser[4], D. Crawford[6], S. Liu[7], M. O'Sullivan[8], DRV. Edwards[2], A. Naj[1].* 1) Hussman Institute for Human Genomics, University of Miami, Miami, FL, USA; 2) Vanderbilt Epidemiology Center, Vanderbilt University Medical Center, Nashville, TN, USA; 3) University of North Carolina at Chapel Hill Department of Epidemiology, Chapel Hill, NC; 4) Fred Hutchinson Cancer Research Center; Seattle WA; 5) Yale University, New Haven CT; 6) Center for Human Genetics Research, Vanderbilt, Nashville TN; 7) Program on Genomics and Nutrition Department of Epidemiology, UCLA; 8) Department of Obstetrics and Gynecology, University of Miami, Miami, FL, USA.

The fat mass and obesity associated (FTO) gene has consistently been associated with obesity and other measures of adiposity in multiple cohorts. Genetic interactions with physical activity have also been identified for FTO variants in some studies. In the Women's Health Initiative Observational Study (WHI-OS), associations of rs9939609 and rs8050136 with obesity were validated in an ethnically diverse subset of 3,600 women, which included 415 Hispanics (Song et al. 2008). We performed an expanded analysis of the complete post-QC set of FTO SNPs (n=184) in all 3,484 Hispanic samples genotyped in the WHI SHARe cohort GWAS (Affymetrix Human SNP Array 6.0) and examined their interactions with physical activity (self-reported total MET-hours/week. Linear regression was used to test for main effects of BMI, hip and waist circumference, and waist-hip ratio and logistic regression of obesity, and for interactions with physical activity. Genotype was modeled using an additive term (0, 1, or 2). All analyses were adjusted for smoking, alcohol, dietary energy, and axes of ancestral variation. Both rs9939609 and rs8050136 were significantly associated with all traits (p£0.01) in the complete Hispanic cohort but were not consistently the most highly associated SNPs overall. We did not detect a main effect for physical activity or interaction for either SNP with physical activity in this cohort (p>0.10). However, significant adiposity-physical activity interactions were found for an uncorrelated SNP rs9934504 (p=0.05 for main effect, p= 0.02 for the interaction term) with BMI, waist circumference (p=0.05 for main effect, p=0.008 for interaction term) and obesity (p=0.07 for main effect, p=0.03 for interaction term. Based on our preliminary analysis, we have confirmed the association of several SNPs in FTO with adiposity traits. However our analyses thus far incorporating self-reported physical activity collected at baseline in the WHI-OS study shows limited evidence of FTO x physical activity interactions. Analyses are ongoing in the African American cohort of the WHI-OS.

1129/F

Lifestyle interactions of FTO: Exercise a serious player. *P.J. Wagner[1,2], K. Silander[1,2], S. Männistö[6], S. Ripatti[1,2], T. Mäkinen[11], S. Keinänen-Kiukaanniemi[7,8,9], H. Oksa[10], L. Peltonen[1,2,3,4,5].* 1) Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Helsinki, Finland; 2) National Institute for Health and Welfare, Unit of Public Health Genomics, Helsinki, Finland; 3) Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK; 4) The Broad Institute, Cambridge, MA; 5) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 6) National Institute for Health and Welfare, Unit of Chronic Disease Epidemiology and Prevention, Helsinki, Finland; 7) University of Oulu, Department of Public Health Science and General Practice, Oulu, Finland; 8) Oulu University Hospital, Unit of General Practice, Oulu, Finland; 9) Oulu Deaconess Institute, Department of Sport Medicine, Oulu, Finland; 10) Tampere University Hospital, Tampere, Finland; 11) National Institute for Health and Welfare, Unit of Health and Welfare Inequalities, Helsinki, FInland.

Variants in and around the FTO gene are consistently strongly associated with BMI and waist circumference. Speculations on the variants' mechanisms causing the physical changes related to obesity include effects on satiety, exercise habits and energy expenditure. We tested the hypotheses behind FTO's effects using the SNP rs8050136 and a study set of 25-65 year-old adults from the Finnish population-based cohorts Health 2000 (H2000) and FINRISK-2007/DILGOM (contributing n=4176 and n=3407, respectively, after exclusions based on missing data and age >65). rs8050136 was associated in additive linear regression models to BMI in both H2000 (p-value 0.013) and DILGOM (p-value $6.49x10^{-10}$). The obesity risk allele (A) is not associated with elevated total energy intake or total dietary fat as a percentage of energy intake in either data set (p-values 0.349-0.994), suggesting that FTO does not affect overall appetite or the preference of a high-fat diet. FTO also did not have an effect on individuals' level of leisure exercise (p-values 0.844-0.998). On the other hand, leisure exercise attenuates FTO's effect on BMI in the DILGOM study (interaction p-value of 6.51e-06). The effect of the FTO risk allele is greater among the inactive group (1.89 kg/m^2 per A allele, standard error of 0.36) compared to the active group (0.26 kg/m^2 per A allele, standard error of 0.11). The interaction was similar among women in the H2000, but was not significant using the same additive model. The women of the H2000 cohort showed a significant interaction under a model comparing AA homozygotes to C-allele carriers (interaction p-value 0.0164). In the inactive group, women homozygous for the risk allele (AA) had average BMI of 28.77 kg/m^2, 1.7 kg/m^2 greater than that among the C-allele group; while in the most active group, AA-homozygotes had a lower average BMI compared to that of C-allele carriers (24.48 vs 24.98 kg/m^2). The interaction among men in the DILGOM study was weaker than that among women, and thus the allele's effect may differ between men and women. We are currently testing the interaction in the following cohorts that have already been genotyped for the same FTO SNP: FinRisk 1992 (n=6000), FinRisk 1997 (n=8000) and FinRisk 2002 (n=8000; approximate sample sizes include people over 65). In conclusion, the effect of FTO at SNP rs8050136 on BMI appears to be dependent on an individual's level of physical activity, and this relationship may differ by gender.

1130/F

Lack of evidence for gene-environment interactions in a population-based sample of isolated CL/P from Norway: A combined case-control and offspring-parent triad study-design. *A. Jugessur[1,2], O. Skare[1], HK. Gjessing[1,3], AJ. Wilcox[4], T. Trung Nguyen[5], RT. Lie[3,5], JC. Murray[6].* 1) Division of Epidemiology, Norwegian Institute of Public Health, N-0403 Oslo, Norway; 2) Craniofacial Research, Musculoskeletal Disorders, Murdoch Childrens Research Institute, Royal Children's Hospital, 3052 Parkville, Australia; 3) Section for Epidemiology and Medical Statistics, Department of Public Health and Primary Health Care, University of Bergen, N-5020 Bergen, Norway; 4) Epidemiology Branch, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC 27709, USA; 5) Medical Birth Registry of Norway, Norwegian Institute of Public Health, N-5020 Bergen, Norway; 6) Departments of Pediatrics, Epidemiology and Biological Sciences, University of Iowa, Iowa City, IA 52242, USA.

Orofacial clefts are complex birth defects, with strong evidence for both genetic and environmental causal factors. In this study, we looked for evidence of gene-environment (GxE) interactions using data from a nationwide study of orofacial clefts in Norway. Genotypes for 1315 SNPs in 334 autosomal candidate genes were available from a previous analysis of 562 case-parent triads and 592 control-parent triads, in which we looked for the effects of fetal genes on the risk of orofacial clefts. For the current study, data were available for the following exposures during the first trimester of pregnancy: cigarette smoking, alcohol, coffee, folic acid and vitamin A supplementation, and dietary folic acid. We used a combined case-control and offspring-parent triad study-design to look for GxE effects in 311 isolated cleft lip with or without cleft palate (CL/P) triads. Prior to analysis, each exposure was treated as a binary variable (exposed vs. non-exposed). Single-marker and haplotype-based analyses were performed using the statistical software package HAPLIN (http://www.uib.no/smis/gjessing/genetics/software/haplin/). Gene effects were estimated for each stratum of the exposure variable and a Wald test statistic, consisting of a weighted sum of differences of the stratified effect-estimates, was used to screen for evidence of GxE interactions. Finally, separate pathway-wide analyses were done for genes involved in folic acid metabolism and genes involved in the detoxification of xenobiotic compounds such as cigarette smoke and alcohol. To ensure that the Wald test was valid for the sample sizes available for analysis, we generated QQ plots to check whether the distribution of the GxE p-values after permutation of the exposures was consistent with the null hypothesis. Overall, however, none of the p-values were statistically significant in either single-marker or haplotype-based analyses. Furthermore, pathway-wide analyses of genes involved in folic acid metabolism and detoxification of xenobiotics compounds did not reveal any statistically significant interaction. Despite strong evidence for genetic causes of CL/P and previously reported GxE interactions with maternal vitamins and smoking, we found no convincing indication that these exposures influence the risk of isolated CL/P in our data.

1131/F

GENTES, an ensemble approach for feature selection in complex diseases. *M. Pinelli[1,5], D. D'andrea[2,5], R. Amato[2,4,5], R. Tagliaferri[3,5], S. Cocozza[1,5], G. Miele[2,4,5].* 1) Dipartimento di Biologia e Patologia Cellulare e Molecolare "L. Califano", Universitá degli Studi di Napoli "Federico II", Napoli, NA, Italy; 2) Dipartimento di Scienze Fisiche, Universitá degli Studi di Napoli "Federico II", Napoli, NA, Italy; 3) Dipartimento di Matematica e Informatica, Universitá di Salerno, Fisciano, SA, Italy; 4) INFN, Italy; 5) Gruppo Interdipartimentale di Bioinformatica e Biologia Computazionale, Universitá di Napoli ``Federico II'' - Universitá di Salerno, Italy.

Complex diseases are multifactorial phenotypes which are caused by the genetic and environmental factors. As we described in a previous work, genetic and environmental factors can interact in additive or non-additive way (Amato et al, BMC Informatics 2010). The identification of the relevant features among a large number of possible candidates and inside typically small sized datasets hardly challenges any applicable Feature Selection Method (FSM). Especially, if genetic and environmental features interact in a non-additive way. As it is well known, each FSM better performs in particular conditions. In this concern, an ensemble approach combining several FSMs succeeds in adding the positive characteristics of each FSM, diluting at the same time the weakness points. Ensemble application to the features selection task have been explored, however they usually put together several instances of the same methods on permuted datasets. We present new software that implements an ensemble of features selection methods aimed to identify relevant genetic and non-genetic features involved in a given complex disease. The ensemble can be composed by any type of features selection method, as well we present the implementation of four of them, namely the Binary Logistic Regression, the Linear Discriminant Analysis, the Multifactor Dimensionality Reduction and an univariate χ^2 or t-test. We optimized the performances of the ensemble in identifying gene-environment interactions by a large set of simulated datasets as provided by the challenGENS website (http://www.challengens.org/). Once challenged against simulated datasets, the ensemble showed generally better or comparable performances than each one of its components. Moreover it behavior resulted more stable, namely less affected by the reduction of the size of dataset.

1132/F

Cognitive Flexibility is Influenced by N-Methyl-D-Aspartate Receptor Gene Variants and Modulated by Substance Dependence. *H. Zhang[1], H.R. Kranzler[2], N. Li[1], J. Poling[1], J.H. Krystal[1], J. Gelernter[1].* 1) Department of Psychiatry, Yale University School of Medicine, New Haven, CT; 2) Department of Psychiatry, University of Connecticut Health Center, Farmington, CT.

The N-methyl-D-aspartate receptors (NMDARs) are glutamate-gated ion channels, mediating excitatory neurotransmission in the brain. They play a critical role in synaptic plasticity, a cellular mechanism for learning and memory. This study investigated whether NMDAR subunit genes and their interaction with substance (alcohol or drug) dependence (SD) influenced cognitive flexibility. Five *GRIN1*, 18 *GRIN2A*, 25 *GRIN2B* and three *GRIN2C* SNPs were genotyped in 391 European Americans [EAs; 337 were affected with SD and 54 were controls] and 519 African Americans [AAs; 443 were affected SD and 76 were controls]. Their cognitive flexibility was assessed by the Wisconsin Card Sorting Test (WCST) and represented by three major indexes [perseverative responses (PR), perseverative errors (PE), and non-perseverative errors (N-PE)]. The main effects of variants and haplotypes and their interaction with SD on cognitive flexibility were examined. Among the four subunit genes, only *GRIN2B* variants exerted a main effect on cognitive flexibility. In EAs, subjects homozygous for the *GRIN2B* rs11055682 minor T-allele made significantly more PRs and PEs than those with the rs11055682 major C-allele [%PRs: 23.75±2.23 *vs*. 14.69±1.08, *P*= 0.003; %PEs: 20.18±1.71 *vs*. 13.01±0.83 , *P*=0.0002]. However, *GRIN2B*-SD interaction improved the cognitive flexibility in EAs with rs11055682 T/T genotype [%PRs: 16.39±1.71 (with SD) vs. 31.11±4.12 (without SD), *P*= 0.0003; PEs: 14.52±1.31 (with SD) vs. 25.84±3.18 (without SD), *P*=0.0002]. Two haplotypes (T-G-T and C-A-T, comprised alleles of three *GRIN2B* SNPs (rs10492141, rs7314376 and rs11055682, in one haplotype block) and both with the rs11055682 minor T-allele, were weakly associated with worse WCST performance, i.e., more PRs (*P*=0.056 and 0.044 for T-G-T and C-A-T, respectively) and PEs (*P*=0.055 and 0.036 for T-G-T and C-A-T, respectively). Similarly, the unfavorable effect of these two haplotypes was reversed by SD, leading to fewer PRs (P=0.094 and 0.071 for T-G-T and C-A-T, respectively) and PEs (P=0.052 and 0.034 for T-G-T and C-A-T, respectively). In AAs, a weak genetic effect of *GRIN2B* rs741327 on cognitive flexibility was observed (PR: P=0.039; PE: P=0.041), but there was no interactive effect of rs741327 with SD on cognitive flexibility. In summary, we found that cognitive flexibility may be influenced by GRIN2B variants and modulated by SD, with a greater genetic effect of GRIN2B on cognitive flexibility in EAs than in AAs.

1133/F

HLA-DRB1*14 allele confers a protective role to Multiple Sclerosis in a Colombian population. *M. Lattig[1], CS. Perea[1], C. Guio[2], A. Porras[4], H. Groot[1], JM. Gonzalez[3], J. Toro[1,2].* 1) Laboratorio de Genetica Humana Biological Sciences, Universidad de los Andes Bogota, Colombia; 2) Grupo Esclerosis Multiple Unidad de Neurologia Hospital Universitario Fundacion Santa Fe de Bogota Bogotá - Colombia; 3) Facultad de Medicina Universidad de los Andes Bogotá - Colombia; 4) Centro de Estudios e Investigación en Salud Hospital Universitario Fundación Santa Fe de Bogotá Bogotá-Colombia.

Multiple Sclerosis (MS [MIM 126200]), the most common demyelinating disease of the central nervous system has a prevalence characterized by geographic variation. According to Kurtzkee the prevalence of MS can be divided into three risk areas based on the disease frequency: low (<5/100.000 inhabitants), medium (5-30/100.000) and high (>30/100.000). In Bogota, an area of defined low risk for the disease, an MS prevalence rate of 4,4/100.000 was previously reported by our group. In this study we evaluated 56 MS patients and 51 controls with a female to male ratio of approximately 4:1 and a mean age of 32.2 years (s.d. 8.3 years) in patients and 35.1 years in controls (s.d. 14.1 years). Clinical evaluations were performed according to McDonald criteria and only those individuals with definite MS were included in the study. Since the HLA -DRB1*15 allele, located within the major histocompatibility complex (MHC) superlocus on chromosome 6p21, is the strongest genetic factor influencing MS susceptibility we performed a molecular analysis of the 16 HLA DRB1 alleles to determine if there was any association with any of these alleles and the disease. We also looked for associations among the alleles and clinical variables such as disease course, mean age of onset, and disability measured by the EDSS. We found no indication of association to the DRB1 alleles and MS or any of the clinical variables, but instead we found association to the HLA -DRB1*14 allele (Fisher 10.7, p=0.001) that suggests a probable protective effect of the allele and the development of MS. Since MS is a multifactorial disease where genes and environment both play important roles in the development of the disease, we propose that in Colombia, specifically in Bogota (a low prevalence zone), the exposure to high UV radiation, due to the high altitude of Bogota (2,600 meters above sea level), could directly influence Vitamin D synthesis which combined with the HLA-DRB1*14 allele may be acting as environmental protective factors of MS.

1134/F

Analysis of nuclear bioavailability of GR in response to glucocorticoid in a pediatric asthma cohort and in the Brown Norway rat model of atopy. *S. Cornejo[1,2], I. Mandeville[2], K. Tantisira[3,4], S. Weiss[3,4], B. Raby[3,4], F. Kaplan[1,2,5].* 1) Human Genetics, McGill University, Montreal, QC, Canada; 2) Montreal Children's Hospital Research Institute, Montreal, QC, Canada; 3) Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Boston, MA, USA; 4) Harvard Medical School, Boston, MA, USA; 5) Pediatrics and Biology, McGill University, Montreal, QC, Canada.

Rationale: There is considerable variation in therapeutic response to glucocorticoids (GCs) in asthma. This variation is multifactorial, but it is clear that it has a genetic component. Defective nuclear import of the GC receptor (GR) is believed to contribute to GC insensitivity. Importin 13 (IPO13), a nuclear transport receptor cloned by us, mediates nuclear entry of GR in airway epithelial cells. We showed previously that IPO13 variation associates with airway hyperresponsiveness in a pediatric asthma cohort. **Objective:** The objective of the present study was to investigate the effect of GCs on nuclear translocation of GR in lymphoblasts derived from children in the Childhood Asthma Management Program (CAMP) (with a range of GC sensitivity) and in isolated lung cell cultures from the Brown Norway (BN) rat model of atopy. **Methods:** Lymphoblasts from patients at two ends of the spectrum of GC responsiveness and lung fibroblasts or epithelial cells from postnatal BN rats were grown in culture and treated with GC (hydrocortisone 10^{-6}M) for 15, 30, 120, 240 min. and 24 hr. GR and IPO13 mRNA levels were assessed by quantitative real-time PCR. Nuclear localization of GR and IPO13 was assessed by western blot analysis and immunofluorescence. **Results:** GC caused a mild-upregulation of GR mRNA in "good responders" which peaked at 120 min. and was followed by a dramatic down-regulation of GR mRNA expression. By contrast, in lymphoblasts from "poor responders" a very brief stimulation of GR (at 15-30min.) was followed by down-regulation of GR mRNA to baseline levels. Whereas GC addition caused a transient increase in the proportion of GR that localized to the nucleus of "poor responders", in "good responders" the proportion of nuclear GR rapidly increased and reached a plateau that was still maintained at 120 min. following GC treatment. GC had no effect on IPO13 mRNA expression in either group. However, the proportion of IPO13 protein that localized to the nucleus was significantly reduced 15 min. after GC addition and was recovered very quickly in the "good responders" but not in "poor responders". In GC-treated lung fibroblasts isolated from BN rat, nuclear import of GR was significantly greater than that observed in control Lewis rats suggesting that the BN rat modeled a GC responsive phenotype. **Conclusion:** GC insensitivity may be linked to impaired synthesis and/or nuclear translocation of GR and IPO13 in a subset of CAMP patients.

1135/F

Network analysis defines the impact of gene-physical activity interactions. *L. Parnell, C.Q. Lai, Y.C. Lee, J.M. Ordovas.* Nutrition & Genomics, JM-USDA Human Nutrition Research Center on Aging, Boston, MA.

Many genetic variants associating with clinical measures of metabolic diseases have been uncovered by genome-wide association studies. Often, those associations fail to replicate in other populations, thought largely due to genetic and environmental differences. However, a substantial number of gene-environment interactions have been described from approaches examining specific genes where environment equals diet, physical activity (PA), tobacco/alcohol use and sleep. In a gene-environment interaction, the allele-phenotype association is valid only when a lifestyle or dietary factor passes some threshold. Constructing a database of 320 such interactions involving obesity and blood lipids from disparate research reports, we combined those with 318 of our unpublished observations. Placing these triangular interactions (gene/genetic variant, phenotypic measure of obesity or dyslipidemia, and environmental modulator) into a network has revealed some startling connections. For example, extensive relationships exist between genetic factors and both HDL- and LDL-cholesterol levels that are modified by PA to the exclusion of triglycerides. Combining output from bioprocess pathway analysis, using Ingenuity®, g:Profiler and protein-protein interaction (PPI) data, with graph theory on connectivity, we identify in the physical activity-gene network key interacting modules and the nodes that bridge those modules. This network of genes harboring PA-sensitive variants and their PPIs shows an enrichment of functions: transcription, insulin signaling, appetite and response to stress. The derived network allows us to traverse from traditional biochemical pathways to specific variants of genes encoding pathway constituents to disease phenotypes and dietary/environmental factors. This will facilitate management of genetic risk of disease onset and progression by specific intervention.

1136/F

Glaucoma cell line collection in the NIGMS Human Genetic Cell Repository. *L. Toji[1], B. Frederick[1], G. Spaeth[2], P. Bender[3], T. Bosley[4].* 1) Cell Repositories, Coriell Inst Med Res, Camden, NJ; 2) Wills Eye Institute, Philadelphia, PA; 3) Division of Neuroscience and Basic Behavioral Science (DNBBS), NIH, Bethesda, MD; 4) Department of Opththalmology, College of Medicine, King Saud University, Riyadh, Saudi Arabia.

Glaucoma is one of the leading causes of blindness world-wide, occurring in about 2% of individuals older than 40 years of age. A small percentage of cases have been found to be associated with mutations in the MYOC, OPTN, CYP1B1, WDR36, and OPA1 genes. About half of the individuals diagnosed with the most common type of glaucoma, primary open angle glaucoma (POAG), have a family history of glaucoma. As part of a Wills Eye Institute (WEI) study of molecular genetics of glaucoma risk factors, submission of samples from patients with POAG and age-matched spousal controls to the NIGMS Human Genetic Cell Repository for establishing lymphoblastoid cell lines (LCLs) was incorporated into the informed consent document approved by the WEI Institutional Review Board for the study. Samples were submitted to the NIGMS Repository with only a WEI code on the sample and the medical record associated with it. The medical record included a questionnaire developed at WEI and the results of an ophthalmologic exam and Humphrey visual field assessment administered to both patients and controls by a glaucoma specialist. No identifiers were provided. The Repository assigned a unique reference number for each sample, established and stored LCLs, and put the clinical data elements in the questionnaire into the database. The collection of cell cultures in the NIGMS Repository includes: 51 primary open-angle glaucoma LCLs, one primary closed-angle glaucoma LCL and one exfoliative glaucoma LCL, along with 32 controls. Samples are from African-Americans and Caucasians. Although no significant copy number variation was detected in the glaucoma group compared to the control group (Abu-Amero et al., Molecular Vision, 15:1594-98, 2009), additional molecular studies are continuing at WEI and King Saud University in Riyadh, Saudi Arabia. The NIGMS Repository database will be updated with results from future studies as they become available. This glaucoma collection and accompanying clinical data are available to investigators through the NIGMS Human Genetic Cell Repository: http://ccr@coriell.org.

1137/F

Identification of Type 2 Diabetes-associated combination of SNPs using Support Vector Machine. *H.J. Ban, J.Y. Heo, K.J. Park, K.J. Park.* Bio-Medical Informatics, Korea Center for Disease Control and Prevention, Seoul, Korea.

Type 2 diabetes mellitus (T2D), a metabolic disorder characterized by insulin resistance and relative insulin deficiency, is a complex disease of major public health importance. Its incidence is rapidly increasing in the developed countries. Complex diseases are caused by interactions between multiple genes and environmental factors. Most association studies aim to identify individual susceptibility single markers using a simple disease model. Recent studies are trying to estimate the effects of multiple genes and multi-locus in genome-wide association. However, estimating the effects of association is very difficult. We aim to assess the rules for classifying diseased and normal subjects by evaluating potential gene-gene interactions in the same or distinct biological pathways. We analyzed the importance of gene-gene interactions in T2D susceptibility by investigating 408 single nucleotide polymorphisms (SNPs) in 87 genes involved in major T2D-related pathways in 462 T2D patients and 456 healthy controls from the Korean cohort studies. We evaluated the support vector machine (SVM) method to differentiate between cases and controls using SNP information in a 10-fold cross-validation test. We achieved a 65.3% prediction rate with a combination of 14 SNPs in 12 genes by using the radial basis function (RBF)-kernel SVM. Similarly, we investigated subpopulation data sets of men and women and identified different SNP combinations with the prediction rates of 70.9% and 70.6%, respectively. As the high-throughput technology for genome-wide SNPs improves, it is likely that a much higher prediction rate with biologically more interesting combination of SNPs can be acquired by using this method. Support Vector Machine based feature selection method in this research found novel association between combinations of SNPs and T2D in a Korean population.

1138/F

Back to family values: Replacing population based association analysis for mapping menopause genes by a novel family approach. *P.L Pearson, S. Costa, A.M. Vianna-Morgante.* Dept of Genetics and Evolutionary Biology, University of Sao Paulo SP, Brazil.

Introduction: Association studies to detect menopause genes have been singularly unsuccessful on financial cost, number of genes found and low proportion of explained variance. An alternative based on association analyses in pooled data from small families is proposed. This approach, called Intra-Familial Association Analysis, reduces the genetic noise found in population samples by the power of family kinship combined with the simplicity of association analysis. ***Materials and methods:*** The idea emerged from studying normal menopausal age (NMA) in FMR1 pre-mutation carriers. From ~300 Fragile-X families in Sao Paulo 18 families were identified in which both menopausal pre-mutation carriers and menopausal normal sisters were present. NMA comparisons between normal and carrier sisters showed a positive correlation (r=0.63, p= 0.03), and that, while the FMR1 pre-mutation clearly reduced NMA relative to the normal siblings, the familial genetic background (FGB) was a much stronger determinant of NMA variance with relative contributions of respectively 40% and 20%. The true contribution to NMA variance by the FMR1 premutation only emerged when NMA of carriers was compared to that of their normal siblings. The idea was extended to detecting association between SNPs and NMA within families by summing NMA difference between sisters with a SNP difference between them for a given marker and between families. A computer model was developed to compare the population with the family association approach by creating a virtual population and 400 virtual families. ***Results:*** The NMA variance within families was 2-3 times smaller than the population, mainly due to ~66% SNPs becoming monomorphic and there-fore making no contribution to NMA variance within families. Further, it appeared that the family approach required far fewer data points than population analyses to achieve significant association. Arguably the most important advantage of the family approach is that it corrects for huge differences in genetic background between families. ***Conclusions:*** The method permits use of 2 or 3 child families and no parents are required. It exhibits far more power in detecting association than population analyses for mild affect alleles, which will help map genes that currently remain undiscovered using population samples. In principle, the approach can also be adapted for analysis of other quantitative traits such as height and intelligence. ***Support: FAPESP and CNPq***.

1139/F

Comparison of pooled allelic ratios (CoPAR) analysis: an efficient method for mapping genetic traits in extended pedigrees. *S.B. Bleyl[1,2], N. Matsunami[3], J. Stevens[3], C.B. Arrington[1], N.E. Bowles[1], T. Tuohy[4], M. Jahromi[4], D.W. Neklason[4], R.W. Burt[5], M.F. Leppert[3], J.D. Schiffman[4].* 1) Dept Pediatrics; 2) Dept Neurobiol Anat; 3) Dept Human Genetics; 4) Dept Oncological Sci; 5) Dept Medicine. University of Utah, Salt Lake City, UT.

Among various hurdles to overcome when mapping genes for complex disorders (including many birth defects), one of the most significant is genetic heterogeneity, or the existence of multiple susceptibility loci that contribute to the phenotype in unpredictable combinations. Typical approaches to locate genes for complex disorders, such as genome wide association studies (GWAS), must utilize large cohorts of affected and control individuals to outweigh the negative effects of this problem. An alternative approach takes advantage of large pedigrees in which multiple individuals affected with a complex trait share a common ancestor. Extended kindreds for a wide variety of multifactorial or low penetrance conditions can be ascertained using family history databases like the Utah Population database (UPDB). In such large families, any single occurrence of the disease is likely due to multiple disparate factors. However one of these factors, a genetic susceptibility, can be assumed to segregate to all affected individuals within a block haplotype that is identical by descent (IBD). We describe an efficient and economical method for identifying disease loci in large kindreds segregating susceptibility genes for complex genetic disorders by comparison of pooled allelic ratios (CoPAR) between affected and control groups. This technique is based on the concept that the allele frequencies of SNPs within haplotype blocks inherited from a common ancestor (IBD haplotype blocks) shared among all affected individuals in an extended pedigree (i.e., a 'linked' haplotype) will deviate from allele frequencies in a control population with a similar ethnic background. This excess of linked alleles in the affected group can be identified using either individual genotypes and calculating allele ratios or using pooled DNA in a single sample and directly measuring allele ratios for each SNP. The latter approach offers a dramatic cost-savings in the number of SNP chips needed to perform a genome-wide survey, which could be as few as two: one for the affected pool and one for the control pool. We demonstrate proof of principle for this method in several disorders, highlight its strengths and weaknesses and discuss possible future uses.

1140/F

Genetic contributions to BMI and related conditions. *J. Derringer[1], R.F. Krueger[1], R.A. Grucza[2].* 1) Psychology, University of Minnesota, Minneapolis, MN; 2) Psychiatry, Washington University, St. Louis, MO.

Obesity, as typically measured by body mass index (BMI), is a major current public health concern. A recent report in the Journal of the American Medical Association shows rates of obesity in the United States to be currently relatively stable in adults, at 32.2% in men and 35.5% in women. In addition, BMI is one of the most robust predictors of increased morbidity and mortality. BMI is itself highly heritable, with approximately 50-70% of its variance accounted for by additive genetic factors. Previous BMI GWAS and meta-analytic efforts have identified several reliable, replicable variants associated with BMI, such as those located in the genes FTO and MC4R. However, these specific variants explain a very small proportion of variance in BMI, and the extent to which BMI-related variants are associated with BMI specifically, or with other commonly co-morbid conditions in general, is currently largely unknown. The goal of our study was to examine the overlap among genetic risk factors for BMI and other related conditions, using a recently described genetic scoring approach (e.g. Purcell et al., 2009, Nature). We split an initial sample of over 9,000 individuals, all of whom had been genotyped on the Affymetrix 6.0 platform, into "discovery" (90% of the sample) and "testing" (10% of the sample) sub-samples. A GWAS was initially run in the discovery sample. Aggregating across SNPs whose GWAS p-value in the discovery sample fell below increasing thresholds created genetic "risk" scores. For each individual in the testing sample, SNPs were weighted according to their discovery sample regression weights and summed into a single summary score (for each p-value threshold). This score was then correlated with BMI within the testing sample, to estimate the proportion of variance explainable by this aggregate genetic risk score. The BMI-derived risk scores in the testing sample were then correlated with BMI-related conditions, including diabetes, hypertension, high cholesterol, and alcohol use, to examine the extent to which this genetic score accounted for BMI-specific variance, or variance common across multiple related phenotypes.

1141/F

Protein tyrosine phosphatase receptor type G (PTPRG) influences the correlated architecture of hepatic steatosis and metabolic phenotypes in the NHLBI Family Heart Study (FamHS). *M.F. Feitosa[1], M.K. Wojczynski[1], K.E. North[2, 3], J. Wu[1], M.A Province[1], J.J. Carr[4], I.B. Borecki[1].* 1) Division of Statistical Genomics, Washington University, Saint Louis, MO; 2) Department of Epidemiology, University of North Carolina, Chapel Hill, NC; 3) Carolina Center for Genome Sciences, University of North Carolina, Chapel Hill, NC; 4) University Health Sciences Image Lab, Wake Forest University, Winston Salem, NC.

Non-alcoholic fatty liver disease (NAFLD) is a metabolic disorder characterized by fatty infiltration of the liver in the absence of alcohol consumption, and it presents as a disease spectrum from fatty liver (steatosis), to nonalcoholic steatohepatitis, to fibrosis. Liver attenuation, measured using computed tomography (CT), is a quantitative measure that is inversely related to the amount of fat in the liver and is highly correlated (r^2= 0.92) with the macrovesicular hepatic steatosis in humans. Features of metabolic syndrome, including obesity, insulin resistance, and hypertriglyceridemia are associated with NAFLD; LA is inversely correlated with insulin resistance (HOMA-IR): r^2= -0.38, triglycerides (TG): r^2= -0.25, hypertension (HBP: systolic ≥ 140 mmHg or diastolic ≥ 90 mm Hg blood pressure or taking antihypertensive medication): r^2= -0.14, and percent body fat (PBF): r^2= -0.32, suggesting shared underlying determinants. A search for genetic variants that account for some of the correlated architecture was undertaken by considering LA with each of these risk factor variables using a correlated meta-analysis of 2,679 subjects of European descent in the FamHS. Phenotypes were adjusted by age, sex, clinical centers, and principal components for stratification (EIGENSTRAT); the LA was also adjusted for CT phantom measurements and alcohol consumption. A genomewide (GW) scan of ~2.5 million imputed SNPs was undertaken, correcting for the non-independence of family members and of the paired scans. Variants of PTPRG (3p14-21) were implicated in LA with several phenotypes including HOMA-IR (rs349174: meta-p=4.8E-08), TG (rs1848417: meta-p=4.9E-08), HBP (rs1848417: meta-p=2.0E-08) and PBF (rs6764654: meta-p=8.9E-10), meeting a GW significance criterion. PTPRG encodes a member of the protein tyrosine phosphatase (PTP) family and is known to be signaling molecules that regulate a variety of cellular processes including cell growth, differentiation, mitotic cycle, oncogenic transformation. Some members of PTP have been previously suggested to have a role in insulin and leptin signaling.

1142/F

Genome-wide meta analysis of gender heterogeneity identifies sexually dimorphic variants associated with human anthropometric traits. *J.C. Randall on behalf of the GIANT (Genetic Investigation of ANthropmetric Traits) Consortium.* Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom.

Genome-wide association studies (GWAS) of complex traits such as bone density, Schizophrenia, and body shape have previously reported gender-specific variants, but these were typically identified in overall analyses of men and women together, and gender-specific analyses were performed only after replication of the overall signal. Though these studies establish the existence of sexually dimorphic genetic variants affecting complex traits, they were not well-powered to detect variants with the most significant between-gender heterogeneity.

To increase power to detect sexually dimorphic variants influencing human anthropometric traits, we conducted gender-specific meta-analyses of 58 GWAS comprising 133,723 individuals (60,586 men & 73,137 women). Each study stratified by gender and used the additive model to test ~2.8M imputed SNPs for association with 9 anthropometric measures: height, weight, body mass index (BMI), waist circumference (WC), hip circumference (HC), waist-hip ratio (WHR), WC adjusted for BMI (WCadjBMI), HC adjusted for BMI (HCadjBMI), and WHR adjusted for BMI (WHRadjBMI). We performed fixed-effects inverse-variance meta-analyses and pruned the resulting lists of markers using the fine-scale genetic map data from HapMap to define loci (SNPs within 0.2cM are considered part of the same locus).

To maximize power, we pursued two parallel strategies to scan for sexually dimorphic variants of two classes: those with concordant effect direction (CED) and those with opposite effect direction (OED). For CED, we controlled for false discovery rate (FDR) in the association signal of men or women at 5%, filtered out previously reported loci, and took the rest forward into additional samples for replication of the gender-specific association signal. For OED, we performed genome-wide gender heterogeneity testing, comparing the effect estimates in men and women across all 9 traits and controlling for FDR at 20%, taking all loci forward into additional samples for replication of the gender heterogeneity signal.

Work continues on completing our replication effort, but preliminary results from the discovery phase and taken forward into replication include promising candidates, such as those implicated in developmental signaling (*ASXL2*, *DAAM1*, *IGFL2*, & *SOX21*), the control of fat metabolism and insulin sensitivity (*ADIPOQ*), regulation of adipocyte differentiation (*PPARG*), and Bardet-Biedl syndrome (*BBS7*), whose symptoms include short stature and obesity.

1143/F

Molecular reclassification of Crohn's disease by cluster analysis of genetic variants. *I. Cleynen[1], J.M. Mahachie John[2,3], L. Henckaerts[4], W. Van Moerkercke[1], P. Rutgeerts[1], K. Van Steen[2,3], S. Vermeire[1].* 1) Department of Gastroenterology, KU Leuven, Leuven, Belgium; 2) Systems and Modeling Unit, Department of Electrical Engineering and Computer Science, University of Liège, Liège, Belgium; 3) Bioinformatics and Modeling, GIGA-R, University of Liège, Liège, Belgium; 4) Department of Medicine, UZ Leuven, Leuven, Belgium.

Crohn's Disease (CD) has a heterogeneous presentation, and is typically being classified according to extent and location of disease. The genetic susceptibility to CD is well known and genome-wide association scans (GWAS) and meta-analysis thereof have identified over 30 susceptibility loci. Except for the correlation between ileal CD and NOD2 mutations, efforts in trying to link CD genetics to clinical subphenotypes have not been very successful. We hypothesized that the large number of confirmed genetic variants enables (better) classification of CD patients. Patient and control genotypes of 46 SNPs identified from CD GWAS were analyzed separately by Latent Class Analysis (LCA) to look for molecular subgroups. Six molecular subgroups were identified in CD patients, which were significantly different from the five subgroups found in healthy controls. The identified CD clusters are therefore likely to contribute to disease pathogenesis. The SNPs determining the molecular subgroups do not cluster in specific pathways, pointing to the existence of disease susceptibility pathways leading to CD overall (autophagy, IL23/Th17), as opposed to single disease modifying genes that define the specific disease subtype. Although differences in prevalence of disease location and behavior could be observed among the CD clusters, Random Forest analysis showed that patients could not be allocated to one of the 6 molecular subgroups, based on the typically used clinical parameters alone. This suggests that the currently used clinical subphenotypes are imperfect, and need to be supplemented or amended. This approach serves as a first step to reclassify Crohn's disease. The used technique can be applied to other common complex diseases as well, and will help to complete patient characterization, in order to evolve towards personalized medicine.

1144/F

Genome scan for spelling deficits: effects of verbal IQ on models of transmission and trait gene localization. *W.H. Raskind[1,2], K. Rubenstein[3], M. Matsushita[1], V.W. Berninger[4], E.M. Wijsman[1,3].* 1) Departments of Medicine; 2) Psychiatry and Behavioral Sciences; 3) Biostatistics; 4) and Educational Psychology, Univ of Washington, Seattle, WA.

Dyslexia is a complex learning disability with strong evidence for a genetic basis. Deficits in fluent oral reading and spelling persist into adulthood for many individuals with a childhood diagnosis, even after other manifestations have been corrected. Thus spelling deficits are a key aspect of dyslexia and are useful as phenotypes in both children and adults. Strategies that may be useful for dissecting the genetic basis of dyslexia include study of component phenotypes, which may simplify the underlying genetic complexity, and use of an analytic approach that accounts for the multilocus nature of the trait to guide the investigation and increase power to detect individual loci. Here we present results of a genetic analysis of spelling disability as a component phenotype on a sample of 260 2-4 generation pedigrees (2040 individuals) selected through a dyslexic proband. Total spelling scores from the WIAT and WRAT3 spelling tests were used, after adjustment for age, cohort, and gender. We also evaluated scores with and without adjustment for verbal IQ (VIQ). We used an MCMC-based oligogenic trait model for both an initial segregation analysis, and a subsequent joint segregation and linkage analysis in a marker-based genome scan. Bayes' factors (BF) for linkage and marker-simulation under the hypothesis of no linkage were used for evaluation of evidence for linkage. Oligogenic segregation analysis identified a small number of quantitative trait locus models (QTLs) that are sufficient to explain the inheritance of the VIQ-adjusted and unadjusted traits in our sample, with one prominent QTL for the VIQ-unadjusted, and two prominent QTLs for the VIQ-adjusted trait. Each of these QTLs was recessive for low spelling scores. In the genome scans, each of these QTLs mapped to one of four genomic regions: the VIQ-unadjusted trait to chromosome (chr) 6 (max BF=35, p<0.005, 167 cM), and the VIQ-adjusted trait to chr 2, 9, and 15 (max BF=22, 19, and 25, at 145, 155, and 23 cM, respectively). For the VIQ-adjusted trait, each of the 2 QTLs from the segregation analyses mapped separately to each of chr 9 and 15, and both QTLs mapped to the region on chr 2. Because the genomic locations of the mapped QTLs vary as a function of whether a VIQ adjustment is included, these results suggest mediation of a genetic basis for spelling deficits through both VIQ-related and unrelated pathways.

1145/F

miRNA biomarkers for detecting lung diseases. *A.C. Keller[1], P. Leidinger[2], E. Meese[2].* 1) Biomarker Discovery Center Heidelberg, Heidelberg, Germany; 2) Deparmtne of Human Genetics, Saarland University, Homburg, Germany.

Purpose Perturbed expression of non-coding RNAs is associated with various pathological cellular processes. The miRNome of both, tissues and body fluids promises to contribute to better diagnosis and prognosis of diseases. Aim of this study is to evaluate blood and serum as source of specific biomarkers for detecting lung diseases including lung cancer and chronic obsessive pulmonary diseases (COPD). Methods We profiled the miRNome, containing about 900 miRNAs, from blood and serum samples of individuals using a microarray platform and validated the results using qRT-PCR. Altogether, the cohort contains over 800 samples including a variety of cancer and non-cancer diseases as well as unaffected controls. Results The detected profiles in lung cancer and COPD could be separated from healthy controls and non-lung diseased samples with high accuracy. It was even possible to separate lung cancer from COPD as a non-cancerous lung disease with an accuracy, specificity and sensitivity of 90.4%, 89.2%, and 91.7%, respectively. By using a restricted subset of six highly relevant markers, accuracy still was as high as 86.3%. Moreover, the comparison of serum profiles to autologous blood profiles revealed significantly different profiles. Serum of lung cancer patients, collected in a retrospective study indicated a pre-symptomatic profile of lung cancer. Conclusion Taken together, our comprehensive results on miRNome wide profiling of serum and blood samples impressively outlines the potential of these small and stable molecules for non-invasive detection of lung cancer samples from unaffected controls and COPD patients and furthermore indicates pre-symptomatic serum profiles in lung cancer patients.

1146/F

Finding the Missing Heritability of a Common Disease: Multi-Allelic Copy-Number Variation of Complement C4A, and HLA-DRB1 alleles DR3 and DR2 Are Common, Medium-to-High, and Additive Risk Factors for Human Systemic Lupus Erythematosus (SLE) of European Ancestry. *Y.L. Wu[1], E. Lundström[2], C.C. Liu[3], Y. Yang[1], B.P. Tsao[4], E.K. Chung[1], B. Zhou[1], K.N. Jones[1], H.N. Nagaraja[5], G.C. Higgins[1], C.H. Spencer[1], H.I. Brunner[6], D.J. Birmingham[7], B.H. Rovin[7], I. Gunnarsson[2], E. Svenungsson[2], J.M. Ahearn[3], L.A. Hebert[7], L. Padyukov[2], C.Y. Yu[1].* 1) The Research Institute at Nationwide Children's Hospital, Columbus, OH; 2) Department of Medicine, Karolinska Institutet, Karolinska Hospital, SE-171 76, Stockholm, Sweden; 3) Lupus Center of Excellence, University of Pittsburgh School of the Health Sciences, Pittsburgh, PA; 4) Division of Rheumatology, Department of Medicine, University of California, Los Angeles, CA; 5) Department of Statistics, The Ohio State University, Columbus, OH; 6) Division of Rheumatology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 7) Division of Nephrology, Department of Internal Medicine, The Ohio State University, Columbus, OH.

The MHC at chromosome 6p21.3 has the strongest association with human SLE. Because of complex polymorphisms, multi-allelic copy-number variations (CNV) and strong linkage disequilibrium, identifying SLE disease causal variant(s) in the MHC has been difficult. This study aims to dissect the roles of HLA-DRB1 polymorphisms and complement C4 CNVs, separated by 544 kb in the MHC, in genetic risks of SLE. The study population included 744 SLE patients and 760 unrelated healthy controls of European ancestry, recruited mainly from mid-western US (patients/controls: 476/461) and Sweden (268/299). Copy-numbers of total C4, C4A and C4B were determined by genomic Southern blot analyses and real-time qPCR with double standard-curve calibrations. Two-digit genotypings for HLA-DRB1 were performed by 20 independent SSP-PCRs for each sample. χ^2 analyses, 2-tailed t-tests, and multiple logistic regression analyses (MLRA) were performed to calculate p-values, odds ratios (OR) and identify independent risk factors for SLE. The gene copy-number (GCN) of C4A in a diploid genome varies from 0 to 6, with the norm at 2 copies. Low C4A GCN (0 or 1 copy), the presence of HLA-DRB1 alleles DR3 and DR2 together occurred in 71.2% of SLE patients, compared to 53.2% of controls ($p=7.1x10^{-13}$). MLRA revealed that each of these three parameters can be independent risk factors for SLE in the US and Swedish study cohorts, separately and combined. Using subjects with non-low C4A, non-DR3 and non-DR2 as the reference group, the OR for SLE with low C4A only was 2.22 (p=0.031), DR3+ only was 1.81 (p=0.004), and DR2+ only was 1.65 (p=0.0002). Notably, the ORs increased to 4.16 ($p=2.5x10^{-8}$) for subjects with all three parameters, 2.80 ($p=3.6x10^{-12}$) with low C4A and DR3, and 2.71 (p=0.037) with low C4A and DR2. In the US cohort, a clear gene-dosage effect was present for association of C4A-CNV with SLE from high to moderate risk, to neutrality and then protection when copy-numbers increased from 0 to 1, 2, and 3, respectively (OR: 6.07®0.41). In the Swedish cohort, an autosomal dominant effect was observable: subjects with 0 and 1 copy were at risk (OR: 2.3-2.4), and with 2 and 3 copies were protected (OR: 0.61-0.66). In conclusion, C4A-CNV, HLA-DR3 and HLA-DR2 are common variants with medium to high effects for SLE, which fills in part of the missing link of heritability between common variants with low effects and rare variants with high effects for a common disease.

1147/F

Overlap between loci influencing risk of type 2 diabetes and fasting glucose through large-scale replication using "Metabochip": preliminary results. *I. Prokopenko[1,2], A. Mahajan[1,3], V. Lagou[1], A. Kumar[1,4], N. Robertson[1], N.W. Rayner[1], R. Mägi[1], C. Groves[2], A.J. Bennett[2], S. Wiltshire[1], J. Trakalo[1], S.E. Hunt[7], K. Stirrups[7], G. Mirza[1], D. Buck[1], K. Zhou[5], C.N.A. Palmer[5], A.D. Morris[5], T. Frayling[6], A. Hattersley[6], M. Weedon[6], J. Perry[6], C. Langford[7], S. Potter[7], P. Deloukas[7], P.J. Donnelly[1], A.P. Morris[1], C.M. Lindgren[1,2], M.I. McCarthy[1,2], Wellcome Trust Case Control Consortium, the DIAGRAM consortium, MAGIC.* 1) WTCHG, Univ Oxford, Oxford, United Kingdom; 2) OCDEM, Univ Oxford, Oxford, United Kingdom; 3) Institute of Genomics and Integrative Biology, CSIR, Delhi, India; 4) Swiss Tropical and Public Health Institute, University of Basel, Switzerland; 5) Biomedical Research Institute, University of Dundee, Ninewells Hospital & Medical School, Dundee, United Kingdom; 6) Institute of Biomedical and Clinical Science, Penninsular Medical School, Exeter, United Kingdom; 7) Wellcome Trust Sanger Institute, Hinxton, United Kingdom.

Complex relationships exist between disease traits and related continuous endophenotypes. Among 16 loci influencing fasting glucose (FG) identified in healthy non-diabetic individuals by the MAGIC meta-analysis, eight were shown to be associated with type 2 diabetes (T2D) at genome wide significance levels ($P<5x10^{-8}$). However, the extent to which other common variants with a putative role on FG levels have an impact on T2D risk has not been determined. The "Metabochip", a custom-made iSELECT array (~195,000 SNPs), supports large-scale follow-up of putative associations for metabolic (including FG), cardiovascular traits and T2D. This analysis is based on genotype data, called using GenCallv1.1, from 4,434 T2D cases and 7,932 controls from the UK T2D Genetics Consortium, the Warren 2 collection and 1958 British Birth Cohort. Additional T2D *in silico* GWA results were available from the DIAGRAM consortium (12,057 cases, 56,071 controls of European-descent). After excluding known T2D loci, we analysed 4,621 high-quality, top-ranked ($10^{-75}<P<0.02$) independent SNPs selected for the Metabochip from the stage 1 discovery meta-analysis for FG performed by MAGIC investigators. Association analyses to determine the effects of these variants on T2D-risk were performed within each sample under an additive model assuming allelic effects and combined using fixed effects meta-analysis in up to 16,491 T2D cases and 64,003 controls. Overall 2,657 of 4,621 SNPs (57.5%) showed directional consistency of association between increased FG levels and higher T2D risk (binomial $p<10^{-24}$). Of these 2,657, 308 showed nominal association with T2D ($p<0.05$, same effect direction) whereas only 90 of the total of 4,621 SNPs showed $p<0.05$ in the opposite direction (binomial $p<10^{-43}$). Only 2 Metabochip loci prioritised for FG showed suggestive association with T2D: at *CCDC88C* (rs17803791, $P=4x10^{-7}$, MAGIC FG $P=8x10^{-3}$) and near *TOP1* (rs6072299, $P=1x10^{-6}$, MAGIC FG $P=3x10^{-4}$). For both, the glucose raising allele contributed to an increased risk of T2D. In this study, we examined SNPs showing putative associations with FG in healthy non-diabetic individuals and tested them for their impact on T2D risk, demonstrating a substantial degree of concordant evidence for association. Joint modelling of FG and T2D may help to uncover novel loci contributing common effects to both traits.

1148/F

Explaining the "missing heritability" of osteoporosis: contributions of ESR1 and LRP4 polymorphisms to fracture susceptibity in families. *B.N.H. Tran[1], S.C. Nguyen[1], N.D. Nguyen[1], J.R. Center[1,2], J.A. Eisman[1,3], T.V. Nguyen[1,2,3].* 1) Osteoporosis and Bone Biology, Garvan Institute of Medical Research, Darlinghurst, NSW, Australia; 2) School of Public Health and Community Medicine, University of New South Wales, Kensington, NSW, Australia; 3) St Vincent's Hospital and St Vincent's Clinical School, Sydney, NSW, Australia.

Genetic factors could account for up to 50% of fracture liability, but single nucleotide polymorphisms (SNPs) identified by genomewide association studies (GWAS) have explained less than 5% of the genetic variance in fracture, which raises the question of "missing heritability". We hypothesize that the part of the "missing heritability" can be explained by considering the effects of these SNPs within families. This study was designed to test the hypothesis by examining the contribution of SNPs to fracture susceptibility in within families.

The study was undertaken as part of the on-going Dubbo Osteoporosis Genetics Study (DOGS), which involved 53 families, with 147 individuals aged 18 and 80 years. Seventy-four SNPs in 34 genes were genotyped. Bone mineral density (BMD, GE-Lunar Corp, WI, USA) at the femoral neck and lumbar spine was measured prior to the fracture event. Fracture was ascertained by X ray report during 1990 and 2009. The association between fracture risk and SNPs was analysed by the Genomewide Association Analyses with Family (GWAF) program.

Four genes, including SPTBN1, PRDM12, RPS6K5, and CHD20 genes, were found to be associated with variation in femoral neck BMD. The 4 genes explained 6% variance in femoral neck BMD. During the follow-up period, 13 individuals (9.7%) had sustained fracture. Two SNPs in the ESR1 gene (rs4870044, rs712219) and 3 SNPs in the LRP4 gene (rs10838635, rs7935346, and rs7121418) were independently associated with fracture risk under the dominant genetic model. The relative risk of fracture associated with these variants ranged between 1.73 and 1.87 ($P < 0.001$). The association was independent of age, BMD, and gender. The area under the receiver operating characteristic curve (AUC) was 0.77 for a model with age, BMD and gender as predictors; incorporation of the 4 variants into the model increased the AUC value to 0.93 ($P < 0.001$).

The results are consistent with the hypothesis that a large part of "missing heritability" can be explained by analysing the effect of genes within families rather than in unrelated individuals. These results also indicate that genetic factors can enhance the prognosis of fracture over and above traditional clinical risk factors.

1149/F

The missing genetic variation: beyond the additive model. *L. Atwood, M. Phillips, N. Heard-Costa.* Framingham Heart Study, Boston Univ Sch Med, Boston, MA.

The additive model is ubiquitous in genome wide association analysis (GWAS) of quantitative traits. Here we compare the additive model to a more general model which assumes the entire trait sample is distributed as a mixture of normal distributions (a distribution for each genotype). We compare the performance of these two models on body mass index (BMI) measured in 1114 participants in the Framingham Heart Study by performing a GWAS using 547,554 SNPs. To remove any concern about inflation of the genetic signal we computed the null distribution empirically (an empirical null distribution is considered the gold standard of statistical testing). The null distribution (1000 replicates) showed that the genome wide p=0.05 significance level was at a nominal p=0.000000126 (-log(p)=6.9). In order to insure stable parameter estimates, we only analyzed those SNPs with at least 30 genotypes in each genotypic group.

The largest -log(p) from the additive model was 5.5. The mixture model found 207 SNPs with -log(p) > 6.9, representing 122 independent loci. The largest -log(p) was 12.7 and 65 SNPs showed -log(p) > 8.0. The additive model did find an average -log(p) of 1.6 for these 207 SNPs, thus showing a small degree of robustness. Parameter estimates for all significant SNPs showed a large degree of non-additive effects with 69% (142/207) being either dominant or recessive.

A major difference between the mixture approach and additive approach is that the mixture approach models residual variation in a genotype specific manner whereas the additive approach models it as a single error variance. Theoretically, modeling genotype specific variation allows the effect of the SNP to show some dependence on its genetic and environmental context (the additive approach can only model independence). To explore this difference, we repeated the mixture GWAS with the genotype specific variation removed. The genetic signal was greatly reduced; the largest -log(p) was 6.2. We infer that the apparent added power comes from modeling the genotype specific variation, i.e. relaxing the independence assumption.

1150/F

An uncommon SNP strongly associated with adiponectin levels in Filipinos is indirectly associated with a GWA signal 800 kb away at the ADIPOQ gene. *D.C. Croteau-Chonka[1], Y. Wu[1], Y. Li[1,2], L.A. Lange[1], C.W. Kuzawa[3,4], T.W. McDade[3,4], M. Laakso[5], J.B. Borja[6], L.S. Adair[7], E.M. Lange[1,2], K.L. Mohlke[1].* 1) Department of Genetics, University of North Carolina, Chapel Hill, NC; 2) Department of Biostatistics, University of North Carolina, Chapel Hill, NC; 3) Department of Anthropology, Northwestern University, Evanston, IL; 4) Cells 2 Society: The Center for Social Disparities and Health at the Institute for Policy Research, Northwestern University, Evanston, IL; 5) Department of Medicine, University of Eastern Finland, Kuopio, Finland; 6) Office of Population Studies, Inc., University of San Carlos, Cebu City, Philippines; 7) Department of Nutrition, University of North Carolina, Chapel Hill, NC.

Adiponectin is a protein hormone responsible for regulating glucose and fat metabolism. Multiple common genetic variants in the *ADIPOQ* gene region have been associated with adiponectin, but few causal variants have been suggested. In a cohort of 1,776 Filipino mothers from the Cebu Longitudinal Health and Nutrition Survey (CLHNS), we found a common SNP in *ADIPOQ* (rs864265, minor allele frequency [MAF] = 0.12) to be associated with adiponectin levels in genome-wide analysis ($P = 3.7 \times 10^{-9}$). Further analysis identified evidence for an uncommon haplotype at rs11924390-rs864265 (C-T frequency = 0.05) that showed markedly stronger evidence of association with lower adiponectin ($P < 1 \times 10^{-24}$). We now detect that a single uncommon SNP (MAF = 0.03) located nearly 800 kb from rs864265, downstream of the body mass index-associated *ETV5* gene, was strongly associated with adiponectin in the 1,725 young adult offspring of the CLHNS mothers ($P = 2.9 \times 10^{-15}$) and in a combined analysis of mothers and offspring accounting for relatedness ($P = 1.1 \times 10^{-15}$). This *ETV5* SNP is in low linkage disequilibrium with rs864265 ($D' = 0.37$) in the CLHNS offspring and was monomorphic in >2,000 samples of northern European ancestry. Conditional analyses using phased haplotypes indicated that neither the rs11924390-rs864265 haplotype nor the *ETV5* variant fully explained the observed association between adiponectin and this locus. Comprehensive imputation of >2,800 SNPs in a 2 Mb region encompassing *ADIPOQ* using 1000 Genomes Project reference haplotypes from CHB, JPT, and CEU revealed no variants that were more strongly associated with adiponectin in the mothers, suggesting that known variants do not completely explain the observed association. Ongoing sequencing of the *ADIPOQ* gene region may detect additional candidate functional variants. These data suggest that the *ETV5* SNP is present on a long haplotype (>800 kb) that contains one or more unknown rare SNPs that may be responsible for some of the observed evidence of association at the *ADIPOQ* locus.

1151/F

Predicting Progression to Advanced Age-Related Macular Degeneration Using a Polygenic Score. *V. Gateva[1], T. Bhangale[2], X. Sun[1], W. Ortmann[1], L.A. Damico-Beyer[3], E. Strauss[3], M. Friesenhahn[4], T.W. Behrens[1], R.R. Graham[1].* 1) Department of Human Genetics, Genentech, Inc, San Francisco, CA; 2) Department of Bioinformatics, Genentech, Inc, San Francisco, CA; 3) Early Development, Genentech, Inc, San Francisco, CA; 4) Department of Biostatistics, Genentech, Inc, San Francisco, CA.

Age-Related Macular Degeneration (AMD) is a slowly progressive degenerative disease that culminates in loss of central vision. Only 18% of patients with intermediate AMD (Category 3) will progress to advanced AMD over 5 years. Identifying individuals at higher risk of rapid progression would enable clinical trials to test novel AMD therapies, identify a patient population amenable to treatment with intravitreal therapeutics, and provide insight into pathogenic pathways. We first confirmed the ability of 7 known AMD genes to enrich for progression to advanced AMD in 764 individuals with Intermediate AMD (category 3) from the Age-Related Eye Disease natural history study. Using a composite score of the 7 known AMD risk alleles, we identified a population (14% of the intermediate AMD population) with a progression rate of 31% at 5 yrs, a 1.6 fold increase over the unselected population. We next tested the hypothesis that a polygenic score computed using thousands of common variants could be predictive of progression to advanced AMD. We conducted a genome-wide association study on 925 advanced AMD cases and 7,863 healthy controls of European descent. We created a polygenic score composed of 10,616 independent loci with p-value < 0.10 from the genome-wide association scan. Using 10,616 independent SNPs with p-value <0.1 from this study we computed polygenic score for each of 764 individuals with Intermediate AMD (category 3) as the average sum of the number of risk alleles (0, 1 or 2) at the SNP weighted by the log odds ratio for that SNP. Individuals with high polygenic score (14% of the intermediate AMD population) have a 47% risk of progression at 5 yrs compared to only 13 % risk for the rest of the intermediate AMD population. This represents a 2.6 fold increase over the unselected population, and a significant improvement in predictive power to a score calculayed using 7 confirmed AMD loci. Our results demonstrate that thousands of common variants can be predictive of AMD progression, and suggests that hundreds of AMD risk loci of modest individual effects contribute to the heritability of AMD.

1152/F

Validity of the "missing heritability" concept. *F. Clerget-Darpoux.* INSERM UMRS 669, Univ Paris-Sud, Villejuif, France.

Over the last three years Genome Wide Association Studies (GWAS) identified many SNPs associated with multifactorial diseases. The information provided by these SNPs was quantified as a proportion of explained heritability and, in line with this terminology, the information yet to be identified was termed "missing heritability". These measurements presuppose that the genetic factors are numerous, each with minor, independent effects, and that no interaction with environmental factors is involved. In order to correctly estimate missing heritability these assumptions must be correct. However, interaction between genes involved in disease susceptibility is very likely and, in our view, demonstrating its existence statistically is complicated due to the fact that the SNPs identified through GWAS poorly represent the gene effects in terms of differential risks. This is illustrated in Multiple Sclerosis where the joint information from two SNPs of IL2RA corresponds to an almost 4-fold differential risk between the least and most at-risk genotypes, whereas it is less than 1.6 for the SNP reported in the literature as the most associated. Similarly for Rheumatoid Arthritis, the differential risk between the least and most at-risk genotype is 2.7 for the PTPN22 SNP most strongly associated, but reached 4.7 for a combination of 3 SNPs in this gene. In both cases, the linkage information provided by the Identity By Descent allele shared between affected sibs within the family is compatible with our gene model but not with the risk hierarchy corresponding to the single SNPs reported in the literature. A good hierarchy of risk for a gene must be established to test the potential interaction between two genes and to reconstruct the entire physio-pathological pathway. In addition, in contrast to monogenic diseases or monogenic sub-entities of a disease, the primary cause(s) of a multifactorial disease may be non-genetic. This is clear for some diseases for which the environmental factor is well known. Thus, for leprosy, the genetic differences described for a predisposition to develop this disease only come into play when the Hansen bacterium is encountered. For most multifactorial diseases, although the environmental factors are unknown, exposure to them may be essential in disease initiation. Thus, the missing information is not generally measurable although it may be the key to disease etiology.

1153/F

Sequencing and fine-mapping within known loci identify new associated variants, implicates causal genes, and increases the explained heritable variation in fetal hemoglobin levels. *G. Galarneau[1], C. Palmer[2,3], V. Sankaran[4,5], S. Orkin[4,5], J. Hirschhorn[2,3], G. Lettre[1,6].* 1) Montreal Heart Institute, Montréal, Québec, Canada; 2) Divisions of Genetics and Endocrinology and Program in Genomics, Children's Hospital Boston, Boston, MA, USA; 3) Program in Medical and Population Genetics, Broad Institute, Cambridge, MA, USA; 4) Division of Hematology/Oncology, Children's Hospital Boston, Boston, MA, USA; 5) Department of Pediatric Oncology, Dana-Farber Cancer Institute, Boston, MA, USA; 6) Département de Médecine, Université de Montréal, Montréal, Québec, Canada.

Fetal hemoglobin (HbF) is a strong and heritable modifier of disease severity for patients with sickle cell disease (SCD) and β-thalassemia: patients with high HbF levels have less severe complications and a longer life expectancy. Three loci - BCL11A on chromosome 2, HBS1L-MYB on chromosome 6, and the β-globin locus on chromosome 11 - carry common DNA sequence variants that modulate HbF levels in population of European and African ancestry. To identify additional variants, fine-map association signals, and potentially pinpoint causal genes at these loci, we re-sequenced 175.2 kb from these three loci in 190 individuals, including 70 African-American SCD patients. We discovered 1,489 DNA sequence variants, of which 910 were previously unreported. We genotyped 88 common SNPs and nine rare missense mutations in 1,100 African-American SCD patients from the Cooperative Study of Sickle Cell Disease (CSSCD). We identified one additional independent HbF signal at BCL11A, refined the HbF signals at HBS1L-MYB and the β-globin locus, and ruled out the previously proposed rs7482144-XmnI polymorphism upstream of HBG2 as causal for HbF regulation. We also found rare missense mutations in MYB that strongly influence HbF levels, suggesting that MYB, rather than the neighboring HBS1L gene, plays a causal role in influencing HbF levels in SCD patients. Our results provide important insights into the regulation of a clinically important trait for diseases that affect millions of individuals worldwide. Moreover, they provide a proof-of-principle of the utility of fine-mapping known associated loci to address the "missing heritability" question: our results increase the HbF phenotypic variance explained in African-American SCD patients from 21.8% to 27.8%, with heritable variation explained increasing from 24.5-36.3% to 31.2-46.3%.

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Epidemiologic Architecture for Genes Linked to Environment (EAGLE): Association of ABCG2 Q141K with Uric Acid Concentration and Gout in the National Health and Nutrition Examination Surveys. *K.L. Spencer, K. Glenn, K. Brown-Gentry, D. Murdock, J.L. Haines, M.D. Ritchie, D.C. Crawford.* Ctr Human Genetics Res, Vanderbilt Univ, Nashville, TN.

Uric acid levels are highly heritable, and increased levels are strongly associated with gout, a common form of arthritis affecting nearly 3 million Americans. A recent meta-analysis of more than 28,000 people has firmly established that the Q141K rs2231142 polymorphism in the urate transporter ABCG2 is associated with increased uric acid levels and increased risk of gout in individuals of European descent. However, to our knowledge, only 1 study has examined this variant in African Americans and there have been no studies in Mexican-Americans. We, as part of the Population Architecture using Genomics and Epidemiology (PAGE) Study, are genotyping DNA samples collected for the National Health and Nutrition Examination Surveys (NHANES), a cross-sectional survey of Americans representing 3 major groups: non-Hispanic Whites (n=6605), non-Hispanic Blacks (n=3442), and Mexican-Americans (n=3949). We tested Q141K for association with serum uric acid levels and self-reported gout in unadjusted regression models and in models adjusted for age, sex, body mass index, glomerular filtration rate, systolic blood pressure, and type II diabetes status. Based on previous positive reports, we also investigated the potential for gene*sex interaction on uric acid level. The frequency of the T allele (encoding 141K) varied across the 3 groups: 0.20 in W, 0.03 in B, and 0.11 in MA, though the mean uric acid levels were similar (5.4 mg/dL W, 5.4 B, 5.2 MA). However, in all 3 groups, the T allele was strongly associated with increasing uric acid level (in adjusted models β=0.32, p<10-10 in W, β=0.28, p=0.003 in B, β=0.25, p<10-10 in MA). Self-report of gout was available in only a subset of samples from NHANES III (n=87 W cases, 2315 W controls, 36 B cases, 1718 B controls, 16 MA cases, 1754 controls). Despite being underpowered, ABCG2 Q141K was significantly associated with gout in MA (odds ratio (OR)=2.22, 95% confidence interval (CI)=1.08 to 4.52, p=0.03), though not in W (OR= 1.22, 95% CI=0.78 to 1.89, p=0.38) or B (OR=2.22, 95% CI=0.90 to 5.46, p=0.08). We did not detect evidence for a gene*sex interaction on uric acid level (p>0.09 in all groups), despite being adequately powered. In conclusion, the association of ABCG2 Q141K with uric acid level was common to all 3 groups, though the frequency of this polymorphism varied widely across race-ethnicities. Thus, this association first observed in European-descent populations can be generalized to other race-ethnicities.

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Fine mapping of the NRG1 Hirschsprung's-associated gene. *M. Garcia-Barcelo[1], C. Tang[1,2], W.K. Tang[1], M.T. So[1], P.C Sham[2], S.S. Cherny[2], P.H. Tam[1].* 1) Dept Surgery, Univ Hong Kong, Hong Kong, NA, Hong Kong; 2) Dept Psychiatry, Univ Hong Kong, Hong Kong.

Hirschsprung's disease (HSCR, aganglionic megacolon), is a congenital disorder characterized by the absence of enteric ganglia in variable portions of the distal intestine. HSCR has a complex pattern of inheritance and presents mainly sporadically. Besides the major HSCR gene, RET, there is evidence that other loci contribute to HSCR. Through a genome-wide association study (GWAS) on Chinese individuals we identified the association of a 350kb genomic region encompassing the NRG1 gene with HSCR. Within the region, the strongest associations were found for two physically close SNPs, rs16879552 and rs7835688 with association values of p= 1.80x10-8 and p=1.12x10-9, respectively. These NRG1 HSCR-associated SNPs are not predicted to functionally affect the gene. Thus, we hypothesized that a common causative/functional variant must lie within the 350kb region and was not revealed by the 500K Affymetrix chips used in the GWAS. To identify the functional variant/s, we resorted to increase the marker density within the region by genotyping 325 SNPs in 380 HSCR Chinese HSCR patients and 380 Chinese controls and test for association. Genotype imputation was also used to increase the SNPs density. We identified a SNPs (rs10088313T/G) highly associated with HSCR, with an EIGENSTRAT corrected p-association value of 6.71x10-5, which is lower than those obtained for the NRG1 intron 1 SNPs identified through the GWAS after correction. Importantly, rs10088313 maps »20kb upstream the NRG1 transcription start site. Since rs10088313 could be either functional or/and be in perfect LD with other functionally relevant markers, we focused on the region encompassed by Chinese Han from Beijing (CHB) HapMap markers whose r2 with rs10088313 equals 1 (rs7830563; rs10113578; rs10107065; rs10113593; rs10094655). This delineated a 10.2 kb region. As a) further genotyping of these 5 SNPs in an expanded sample would not help discern among these SNPs in perfect LD and, b) the finding of association across an intron implies involvement in regulation, we used comparative genomics to investigate the 10.2kb region. rs7830563, rs10088313 and rs10113593 create/disrupt binding sites of transcription factors with a prominent in the development of the enteric nervous system. The predicted changes caused by the SNP HSCR-associated alleles are bound to alter the regulation of NRG1 transcription and could possibly affect the NRG1 signalling during ENS development.

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Association of endothelin receptor type A and migraine without aura in a group of Portuguese patients. *C. Lemos[1], J.L. Neto[1,2], J. Pereira-Monteiro[2,3], D. Mendonça[2,4], J. Barros[2,3], J. Sequeiros[1,2], I. Alonso[1], A. Sousa[1,2].* 1) UnIGENe, IBMC, Porto, Portugal; 2) ICBAS, Univ. Porto, Portugal; 3) Serv. Neurologia,CHP- HSA, Porto, Portugal; 4) Instituto de Saúde Pública, Univ. Porto, Porto, Portugal.

Migraine is a highly prevalent disorder, affecting about 15% of the general population. Different mechanisms have been proposed as contributing for migraine. However, some results are contradictory and replication is needed to confirm the observed association in other populations. Anomalies of vascular function, with dilatation of cerebral blood vessels and release of vasoactive neuropeptides have also been implied in migraine pathophysiology. Endothelin type A receptor (EDNRA) mediates the biological effects of endothelin-1 (ET-1), leading to vasoconstriction. Some authors described an association between a polymorphism of the ENDRA gene and migraine. All these findings point to a possible role of EDNRA in migraine. Our aim was to assess the involvement EDNRA in susceptibility to migraine in a sample of Portuguese migraineurs, by a case-control study. Three tagging SNPs (rs702757, rs5333 and rs5335) were analyzed in 188 cases - 111 without (MO) and 77 with aura (MA) - and 287 controls. A multivariable logistic regression included the three SNPs, adjusted for gender. Allelic and haplotypic frequencies were compared between cases and controls. We found a borderline increased risk for the rs702757 T-allele (OR=1.44, 95% CI: 1.05-1.99) and for the TT genotype (OR=2.34, 95% CI: 1.12-4.90) for MO. A trend towards an increased risk for MA regarding the C-allele of rs5333 was also found. The T-C-G haplotype was found to be significantly overrepresented in the MO subgroup. Our results reinforce EDNRA as a susceptibility factor for MO, although we cannot exclude the involvement of this gene in MA susceptibility in our population. Dissecting migraine genetic susceptibility will be crucial to develop better therapeutic strategies, as the risk variants implied and their effects may vary in different populations.

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Genetic variants of the FAS gene are associated with vitiligo in a brazilian, family-based population sample. *L.M. Nascimento[1], C.C.S. Castro[1,2], R.I. Werneck[1], M. Olandoski[1], M.T. Mira[1].* 1) Graduate Program in Health Sciences, Pontifical Catholic University of Paraná, Curitiba, Parana, Brazil; 2) Department of Dermatology, Santa Casa de Misericordia Hospital, Pontifical Catholic University of Parana, Curitiba, Brazil.

Vitiligo is an acquired systemic, chronic disease characterized by macules devoid of melanic pigment and identifiable melanocytes. Previous findings have suggested that polymorphisms of the FAS gene are associated with an increased risk of autoimmune diseases, including vitiligo. Moreover, an abnormal expression of FAS has been observed in biopsy samples from vitiligo-affected skin. We hypothesized that genetic variants of FAS might be risk factors for vilitigo in a family-based population sample from south of Brazil. To test this hypothesis, we genotyped 5 tag SNPs capturing the information of the entire FAS gene in 600 individuals distributed in 212 trios composed by an affected child and both parents. Markers were genotyped using the SEQUENOM MassARRAY platform, which uses the iPLEX assay to incorporate mass-modified terminal nucleotides that are then detected by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry. Family-based association analysis was performed as implemented in the software FBAT. Association analyses identified at least two variants that contribute independently to increased risk of disease in our population sample: the allele "A" of synonymous tag SNP rs2234978 and allele "A" of intronic tag SNP rs4406738 (P = 0.001, OR = 1.65, 95% IC = 1.20-2.29; P=0.01, OR = 2.02, 95% IC = 1.14 - 3.58, respectively). These results are the first independent replication of association between vitiligo and variants of the FAS gene of the apoptosis regulatory pathway.

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Analysis of polymorphisms in 4 complement related genes, *CFH*, *C2*, *CFB* and *C3*, and in *ARMS2* in Mexican Mestizo patients with advanced age-related macular degeneration. *A. Ramos-Perez[1], J.C. Canseco-Mendez[1], U. Rodríguez-Corona[1], F. Morales-Mandujano[1], L. Sebastian-Medina[1], J.C. Zenteno-Ruiz[2], D. Ochoa Contreras[3], S. March[1], E. Graue[2,4], A.V. Contreras[1], G. Jimenez-Sanchez[1], I. Silva-Zolezzi[1].* 1) National Institute of Genomic Medicine, Mexico DF, Mexico; 2) Conde de Valenciana Hospital, IAP, Mexico DF, Mexico; 3) School of Medicine, UNAM. Mexico DF, Mexico; 4) Asociacion para Evitar la Ceguera en Mexico, AC.

Age-related macular degeneration (AMD) is the most common cause of central blindness in the elderly population. This disease is characterized by poor vision in the central field due to a progressive destruction of the macular area. Recently, several studies in different populations have clearly identified five genes associated to the disease with independent contributions: Complement Factor H (*CFH*), complement component 2 and 3 (*C2* and *C3*), complement factor B (*CFB*) and age-related maculopathy susceptibility (*ARMS2*). To evaluate the participation of these five loci in the Mexican Mestizo population we genotyped six SNPs in these genes previously associated with AMD in 278 unrelated Mexican patients with the two forms of advanced AMD and 204 healthy controls and 300 population controls from the Mexican Genome Diversity Project. We confirmed a significant association to AMD in Mestizo patients from Mexico City of *ARMS2* rs10490924 (OR=3.09, 95%CI [2.47-3.85], p-value=5.7E-24), *CFH* rs1061170(C) (OR 1.85, 95%CI [1.30-2.65], p-value=7.4E-04, *C2* rs547154(A) (OR=0.177, 95%CI [0.06-0.53], p-value=5.2E-4), *CFB* rs4152667(A) (OR=0.503, 95%CI [0.29-0.89], p-value=1.6E-2) and *C3* rs1047286(A) (OR=2.16, 95%CI [1.42-3.27], p-value=2.2E-4), and rs2230199(C) (OR=2.15, 95%CI [1.48-3.12], p-value=4E-5). Our results show that *ARMS2* and *C3* are major contributors to advanced AMD in Mexicans as previously found in other populations. Interestingly, the contributions of *CFH*, *C2* and *CFB* in Mexicans are minor to those of other populations due to the low frequency of the associated variants in Mexican Mestizos: 15% for *CFH* rs1061170(C), 6% *C2* for rs547154(A) and 4% for *CFB* rs4152667(A). Currently, we are analyzing these data together with other variables such as smoker status in a multivariate model.

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Resequencing analysis of the complement Factor H gene (*CFH*) in Mexican Mestizo patients with advanced age-related macular degeneration. *U. Rodriguez-Corona[1], J.C. Canseco-Mendez[1], A. Ramos-Perez[1], F. Morales-Mandujano[1], L. Sebastian-Medina[1], J.C. Zenteno-Ruiz[2], D. Ochoa-Contreras[3], S. March[1], E. Graue[2,4], A.V. Contreras[1], G. Jimenez-Sanchez[1], I. Silva-Zolezzi[1].* 1) National Institute of Genomic Medicine, Mexico DF, Mexico; 2) Conde de Valenciana, Hospital, IAP, Mexico DF, Mexico; 3) School of Medicine, UNAM, Mexico DF, Mexico; 4) Asociación para evitar la Ceguera en México, AC.

Age-related macular degeneration (AMD) is the most common cause of central blindness in the elderly population. This disease is characterized by poor vision in the central field due to a progressive destruction of the macular area. Recently, several studies in different populations have clearly identified five genes associated to the disease with independent contributions: Complement Factor H (*CFH*), complement component 2 and 3 (*C2* and *C3*), complement factor B (*CFB*) and age-related maculopathy susceptibility (*ARMS2*). We have confirmed the association of 6 different SNPs in these five loci in the Mexican Mestizo population: *ARMS2* rs10490924 (p-value= 5.7E-24), *C3* rs2230199 and rs1047286 (p-value=4.0E-5 and 2.2E-4 repectively), *CFH* rs1061170 (p-value=7.4E-4), *C2* (p-value=5.2E-04) and *CFB* (1.6E-2). Due to its low frequency (frequency in Mestizos), the contribution of *CFH*(rs1061170) in Mexicans is minor to that in Caucasian groups. An important proportion (57%) of our advanced AMD patients do not have this mutation. Considering these data and due to the relevance of *CFH* in the genetic contribution to AMD in other population groups, we have define it as a candidate gene for medical resequencing to search for additional variants associated to this disease in Mexicans. To characterize genetic variants of potential functional relevance we have designed a resequencing strategy using amplification primers with M13 adaptor sequences at the 5' end for the 23 exons of the *CFH* gene. Our approach is based in resequencing DNA samples of 48 advanced AMD patients (CARMS grade 4 and 5) and 48 healthy controls to have >85% power to detect variants with allele frequencies above 1%, and >99% power for those above 5%. To date we have successfully amplified the 23 exons and are in the process of analyzing the resequencing data. We have identified an insertion/deletion tetranucleotide variant in exon 23 in our cases group. In addition to the generation of variants potentially associated to AMD, in this study, we will generate the first catalog in the Mexican population of genetic variants with potential functional relevance in *CFH*, a highly relevant gene in different medical conditions (AMD and renal diseases).

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A 3' UTR polymorphism in *ANXA11* is associated with pulmonary tuberculosis in the South African Coloured Population. *M. Salie[1], M. Möller[1], P.D. van Helden[1], S. Schreiber[2], E.G. Hoal[1], A. Nebel[2], S. Hofmann[2].* 1) Division of Molecular Biology and Human Genetics, MRC Centre for Molecular and Cellular Biology and the DST/NRF Centre of Excellence for Biomedical TB Research, Faculty of Health Sciences, Stellenbosch University, Tygerberg 7505, South Africa; 2) Institute of Clinical Molecular Biology, Christian-Albrechts-University, Schittenhelmstraβe 12, 24105 Kiel, Germany.

Tuberculosis (TB) remains one of the leading causes of death by an infectious agent. Annexin A11 (ANXA11) has recently been shown to be associated with sarcoidosis, a disease with clinical and pathophysiological similarities to TB. ANXA11 belongs to the annexin superfamily of calcium/phospholipid binding proteins and is involved in apoptosis, calcium signalling, cell division and vessicle trafficking. A population-based case-control study with 417 cases and 410 controls from the South African Coloured population was done to investigate the role of polymorphisms in the *ANXA11* gene and susceptibility to TB using the TaqMan® genotyping system. Of the six polymorphisms investigated, we observed a nominal association with the intronic polymorphism, rs2784773 (P_{gen}=0.048 and P_{allele}=0.03; OR[95%CI]=0.80[0.66-0.98]) and a strong association with the 3' UTR polymorphism, rs7071579 (P_{gen}=0.01 and P_{allele}=0.0017; OR[95%CI]= 1.38[1.13-1.68]). Three estimated haplotypes were also associated with TB, where two haplotypes carrying the A-allele of rs7071579 were found more frequently in cases (*P*=0.047 and *P*=0.00037) and the remaining haplotype carrying the G-allele of rs7071579 was found more frequently in controls (*P*=0.01). Haplotype analysis between the South African and German populations show differences in their linkage disequilibrium structure, which indicated that the variants identified in the German population as susceptibility variants may not hold true for the South African population. Alternatively, the susceptibility variant for sarcoidosis may not be the susceptibility variant for TB. This study suggests a possible role of *ANXA11* polymorphisms in the outcome of developing TB in the South African Coloured population. This could be due to the apoptotic and vessicle trafficking properties of the ANXA11 protein or its potential role in the endocytic-autophagic pathway.

1161/F

Replication study of genetic variants affecting glucose and insulin levels in the Chinese. *C. Hu, R. Zhang, C. Wang, W. Yu, J. Lu, F. Jiang, K. Xiang, W. Jia.* Shanghai Diabetes Inst, Shanghai, Shanghai, China.

Recent studies reported 19 loci influencing glucose and insulin related traits including G6PC2, GCK, GCKR, MTNR1B, DGKB, ADCY5, MADD, ADRA2A, CRY2, FADS1, GLIS3, SLC2A2, PROX1, C2CD4B, GIPR, VPS13C, IGF1, SLC30A8 and TCF7L2. To test for the association of SNPs from these loci and traits related to glucose metabolism and type 2 diabetes, we genotyped 22 SNPs from these loci in 6822 Shanghai Chinese Hans comprising 3410 cases and 3412 controls. We found SNPs from GCK, G6PC2, MTNR1B, DGKB, MADD and SLC30A8 showed association to fasting plasma glucose; SNPs from GCKR, TCF7L2, PROX1 showed association to OGTT 2h plasma glucose; SNPs from GCKR, GIPR, GLIS3 and SCL30A8 showed association to type 2 diabetes risk; IGF1 rs35767 showed association to fasting insulin levels and index of insulin resistance; and SNPs from DGKB and PROX1 showed association to index of insulin secretion.

1162/F

Association study of common type 2 diabetes risk gene polymorphisms with disease in Latvian population. *J. Klovins[1], I. Kalnina[1], L. Tarasova[1], K. Geltnere[2], V. Pirags[2].* 1) Latvian Genome Ctr, Latvian Biomedical Research and Study Ctr, Riga, Latvia; 2) University of Latvia and Department of Endocrinology, Pauls Stradins Clinical University Hospital , Riga, Latvia.

A number of new common genetic variants associated with type 2 diabetes (T2D) have been recently discovered on the basis of genome-wide screening and confirmed in different populations. The present study was conducted to confirm the possible association between 11 candidate genes in T2D patients selected from the Genome Data Base of Latvian Population, the population based biobank. A total of 16 previously reported single- nucleotide polymorphisms (SNPs) from the KCNJ11, SLC2A2, ABCC8, HHEX, PPARG, IGF2BP2, CDKAL1, TCF7L2, FTO, CDKN2A&CDKN2B AND SLC30A8 were analyzed. Candidate SNPs were genotyped in 645 T2D patients and 900 control subjects. In our case-control group the association of four SNPs, rs4402960 (IGF2BP2), rs10811662 (CDKN2A&CDKN2B), rs8050136 and rs9939609 (FTO), were replicated with statistical significance (adjusted p value <0.05). The odds ratio (OR) was significantly increased in patients with a higher total number of risk alleles ranging from OR 1.08 to OR 2.02 for 2 risk alleles versus 6 risk alleles, respectively. The presence of risk alleles was significantly associated with age of disease onset in the group of T2D patients for SNP located in the FTO gene locus. rs4402960 located in IGF2BP2 gene showed strong interaction with BMI being associated with increased risk of T2D only in obese individuals. Interestingly, the SNP rs7903146 located in the TCF7L2 gene whose association with T2D has been replicated in almost all populations studed was not associated with T2D in our cohort (p=0.923). Three additional SNPs in TCF7L2 gene were selected to study LD architecture of this locus in Latvian population. It should be noted that this is the first replication study of T2D candidate genes in the Baltic countries and further studies in these populations are required to confirm our findings. In conclusion, T2D susceptibility of three candidate genes was confirmed in Latvians.

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The search for early-onset type 2 diabetes loci using Metabochip. *A. Mahajan[1,2], A. Kumar[1], N.W. Rayner[1], N. Robertson[1], V. Lagou[1], S. Wiltshire[1], R. Magi[1], I. Prokopenko[1], C.M. Lindgren[1], C. Groves[3], J. Trakalo[1], G. Mirza[1], D. Buck[1], K. Stirrups[4], C. Langford[4], S.E. Hunt[4], S. Potter[4], P. Deloukas[4], A. Doney[5], C.N.A. Palmer[6], A.D. Morris[6], J. Perry[7], T.M. Frayling[7], A.T. Hattersley[7], M.I. McCarthy[1,3], A.P. Morris[1], The Wellcome Trust Case Control Consortium, The DIAGRAM Consortium.* 1) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom; 2) Institute of Genomics and Integrative Biology, CSIR, Delhi, India; 3) Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford, United Kingdom; 4) Wellcome Trust Sanger Institute, Hinxton, United Kingdom; 5) Department of Medicine and Therapeutics, Ninewells Hospital and Medical School, Dundee, United Kingdom; 6) Biomedical Research Institute, University of Dundee, Ninewells Hospital and Medical School, Dundee, United Kingdom; 7) Institute of Biomedical and Clinical Science, Penninsular Medical School, Exeter, United Kingdom.

Despite the success of genome-wide association studies (GWAS) of type 2 diabetes (T2D), the more than 30 established loci account for only ~10% of the genetic component of the disease. One approach that may aid the identification of novel T2D loci is to focus on early-onset cases, defined here to have age of diagnosis <45 years, who are less likely to be affected by exposure to environmental risk factors, and thus may have greater genetic loading.

The Metabochip is a custom iSELECT array, designed to support follow-up of putative associations for metabolic and cardiovascular traits, including 1040 SNPs for early-onset T2D. These SNPs were chosen from the strongest signals of early-onset T2D association from a meta-analysis of 1317 cases and 29078 controls from five GWAS undertaken by the DIAGRAM consortium. We have analysed these Metabochip SNPs for association with early-onset T2D under an additive model in an additional 886 cases and 7915 controls from the UK T2D Genetics Consortium, the Warren 2 Collection and the 1958 British Birth Cohort.

We compared patterns of replication between the “Stage 1” DIAGRAM and “Stage 2” Metabochip data at 947 independent SNPs passing quality control protocols. We observed directional consistency (i.e. the same allele at high risk in both stages) at 557 of these SNPs (binomial test p=3.2x10^{-8}). After removal of SNPs within established T2D loci, the evidence for directional consistency between stages was diminished, but still highly significant (507 of 889 SNPs, binomial test p=1.6x10^{-5}). These preliminary results are consistent with a long tail of common, modest effect variants contributing to early-onset T2D.

In an attempt to discover novel loci contributing effects to early-onset T2D, we performed meta-analysis of the two stages of our study. No novel loci achieved a genome-wide level of significance (p<5x10^{-8}). However, after removal of SNPs within established T2D loci, there is still a strong enrichment of nominal associations (p<10^{-5}) with early-onset disease (13 of 889 independent SNPs, binomial test p<10^{-16}). The strongest novel signal of association is in *ANXA11* (p=8.8x10^{-7}, allelic OR 1.25 [1.14-1.37]). This locus has previously failed in DIAGRAM replication efforts for overall T2D, but has not been tested for early-onset disease.

1164/F

The association analysis of susceptibility loci for ulcerative colitis in Japanese Crohn's disease. *K. Yamazaki[1], M. Takazoe[2], Y. Nakamura[3], N. Kamatani[4], M. Kubo[1].* 1) Laboratory for Genotyping Development, Center for Genomic Medicine, RIKEN, Kanagawa, Japan; 2) Department of Medicine, Division of Gastroenterology, Social Insurance Chuo General Hospital, Tokyo, Japan; 3) Laboratory of Molecular Medicine, Institute of Medical Science, The University of Tokyo, Japan; 4) Laboratory for Statistical Analysis, Center for Genomic Medicine, RIKEN, Kanagawa, Japan.

Inflammatory bowel disease (IBD) represents the two common types of chronic intestinal disorders, Crohn's disease (CD) and ulcerative colitis (UC). Genetic epidemiological studies suggest UC and CD share many common genetic factors and resulted in the similarity of mechanistic features. Recent genome-wide association studies (GWAS) of CD and UC supported this hypothesis and it have been uncovering some shared factors in Caucasian population. To investigate the common susceptible loci between CD and UC across populations, we have examined 32 SNPs which identified by GWAS of UC. These markers were including 5 SNPs identified in Japanese UC patients and others were in Europeans. We genotyped them in a total of 484 CD patients and 992 controls and detected 5 SNPs with positive association. Two SNPs in MHC region showed strong association; rs9263739 in *CCHCR1* reported in Japanese population (p=5.79x10-5, OR= 1.67 (1.29-2.19)) and rs660895 in *HLA-DRB1* (p=4.57x10-10, OR=1.69 (1.43-1.99)). Other three SNPs showed marginal association; rs9268858 in *HLA-DRA* (p=0.0020,OR=1.28 (1.09-1.50)), rs2395185 on 6p21 (p=0.0026, OR=1.28 (1.09-1.50)) and rs1558744 near *IFNG* (p=0.045, OR=1.29 (1.00-1.66)). Among five variants with association in this study, the *IFNG* gene was reported that are secreted by abundant lamina propria lymphocytes in the mucosa of patients with CD. Other associated SNPs with Japanese CD patients were located in MHC region. These results indicate that the MHC region and IFNG will be common genetic factors for CD and UC in populations from various ethnics. Identifying shared and disease-specific susceptibility loci for CD and UC would help define the biologic relationship of IBD. However, our study is small to detect modest genetic effect. Further studies with larger sample sizes are necessary to clarify the common susceptibility loci between CD and UC in various populations.

1165/F

Interaction analysis for the *HLA-DRB1* shared epitope alleles and the MHC class II transactivator *CIITA* gene variant in the risk of rheumatoid arthritis. *M. Ronninger[1], M. Seddighzadeh[1], D. Plant[3], N.A. Daha[4], R.E.M. Toes[4], J. Worthington[3], L. Alfredsson[2], L. Padyukov[1].* 1) Dept Medicine, Karolinska Inst & Hosp, Stockholm, Sweden; 2) Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden; 3) 1arc-Epidemiology Unit, University of Manchester, Manchester, UK; 4) Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands.

Several risk factors for rheumatoid arthritis (RA) have been found to interact with *HLA-DRB1* shared epitope (SE) alleles. Recently we found a variant of the MHC class II transactivator (*CIITA*) gene, which associates with inflammatory diseases. This association was not consistently replicated in different populations. *CIITA* is known to be the key regulator of MHC2 expression and may be involved in development of RA in relation with SE alleles. We hypothesize that the risk variant rs3087456 in the *CIITA* gene may interact with the *HLA-DRB1* SE in development of rheumatoid arthritis. Interaction between *HLA-DRB1* SE alleles and rs3087456 in *CIITA* was first investigated in a cohort of 2519 RA cases and 1347 matched controls from Swedish EIRA study and later repeated in two cohorts from UK (1916 cases and 1270 controls) and The Netherlands (486 cases and 283 controls). Genotyping was performed by Taqman genotyping assay (*CIITA*) and by SSP-PCR (HLA). The ACPA (anti-citrullinated protein antibody) status was identified for all RA patients by anti-CCP ELISA. We have used departure from additivity of effects as a measure of interaction between rs3087456 (homozygous for G allele) and *HLA-DRB1* shared epitope alleles defined as any of *DRB1*01*, *DRB1*04* or *DRB1*10*. This measurement gives the proportion of risk among those carrying both genetic risk factors that is attributed only to the interaction (attributable proportion due to interaction, AP). In the analysis of the 3866 individuals from Swedish population for interaction between rs3087456 and SE we found no significant interaction (AP=0.15, 95%CI: -0.24-0.53). Since SE is primarily a risk factor for ACPA positive disease we stratified data according to positive (AP=0.27, 95%CI: -0.05-0.59) and negative (AP=-0.19, 95%CI: -1.0- 0.62) autoantibody status. We further found no significant interaction between the subgroups of SE (*DRB1*01*, *DRB1*04* or *DRB1*10*) and *CIITA*. Similar analysis of two independent RA cohorts from two European populations confirmed an absence of interaction between genetic marker in *CIITA* and SE alleles in development of RA. We did not observe a significant interaction between rs3087456 and SE alleles with regard to risk of RA. Since a biological link between products of these genes is evident, this finding may point towards a more complex relation between *CIITA* and class 2 antigens in the autoimmune process.

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Replication study of leprosy susceptibility genes PARK2/PACRG and LTA. *A.S. Francio[1], R.I. Werneck[1], C.C. Mackert[1], F.P. Lazaro[1], F.C.G Fernandes[1], M.B. Xavier[2], A. Alter[4], A. Alcais[3], E. Schurr[4], M.T. Mira[1].* 1) Graduate Program in Health Sciences, Pontifical Catholic University of Paraná, Curitiba, Paraná, Brazil; 2) Department of Tropical Medicine, Federal University of Pará, Belém, Brazil; 3) Laboratory of Human Genetics of Infectious Diseases, University of Paris René Descartes, Paris, France; 4) McGill University and Genome Quebec Innovation Centre, Montreal, Canadá.

Leprosy is a chronic infectious disease caused by Mycobacterium leprae that affects mainly the skin and the peripheral nerves. Previous studies have shown a major contribution of genetic variants controlling host susceptibility to leprosy. For example, positional cloning has led to the identification of variants of the regulatory region shared by PARK2 and PACRG as common risk factors for leprosy. The same strategy, followed by stepwise replication, revealed the low-producing LTA+80 allele as a risk factor for early-onset disease. In order to independently replicate the association signals observed between leprosy and both PARK2/PACRG and LTA, we enrolled a study population of families composed of leprosy patients and their parents recruited at the former leprosy colony of Santo Antonio do Prata, located at the amazonic state of Pará, north of Brazil. The colony has been geographically and socially isolated for almost a century, with the population being continuously exposed to high prevalence and incidence rates of the disease. We genotyped 20 tag SNPs capturing the information of the PARK2/PACRG regulatory region and the LTA gene in 154 individuals distributed in 48 trios composed by an affected child and both parents. Genotyping was performed using the SEQUENOM platform. Family-based association analysis was performed as implemented in the software FBAT. Linkage disequilibrium estimations were carried out using the Haploview software. Association analyses identified at least six variants of PARK2/PACRG that contribute to increased risk of disease in our population sample: the allele "C" of tag SNP rs1040079 (P = 0.01), allele "T" of tag SNP rs10945859 (P = 0.006), allele "G" of tag SNP rs2276201 (P = 0.005), allele "T" of tag SNP rs2803073 (P = 0.04), "C" of tag SNP rs6930532 (P = 0.01). Of note, the risk allele of marker rs1040079 is the same as observed previously. Allele "T" of LTA tag SNP rs2239704, also known as LTA+80, was borderline associated with leprosy in our population sample (P = 0.09). Here we present results of a perfect replication of association between leprosy and variants of the PARK2/PACRG genes in a hyper-endemic leprosy population sample.

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Replication of a GWAS for Behçet's disease confirms the association of the KIAA1529 gene. *S.A. Oliveira[1,2], N.M. Shafiee[3], J.M. Xavier[1,2], F. Ghaderi[3], B.S. Abdollahi[3], F. Shahram[3], F. Davatchi[3].* 1) Instituto de Medicina Molecular, Lisbon, Portugal; 2) Instituto Gulbenkian de Ciência, Oeiras, Portugal; 3) Rheumatology Research Center, Tehran University of Medical Sciences, Tehran, Iran.

A recent genomewide association study for Behçet's disease (BD) demonstrated an association of KIAA1529, LOC100129342, CPVL and BASH3B genes with BD (Fei et al. 2009). Since replication in independent samples remains the gold-standard in association findings, we investigated the reported associations with BD of single nucleotide polymorphisms (SNPs) in LOC100129342, CPVL and BASH3B, and of all tagging SNPs in KIAA1529. A total of nine SNPs were genotyped in 550 Iranian BD patients and 436 controls. Patients were selected as consecutive patients, according to ICBD criteria. We tested the association of alleles, genotypes and haplotypes with BD, unadjusted and adjusted for sex and ethnicity. SNP rs7038496 in KIAA1529 showed a significant association with BD (p=0.040). This SNP belongs to a 20kb haplotype block (from rs7038496 to rs725229) that also shows a significant association (p=5.6x1E-3). We found a different polymorphism in KIAA1529 associated to BD that the one previously described (rs2061634). This finding further supports the involvement of KIAA1529 in BD susceptibility, but the exact marker within this genes remains to be pinpointed.

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Study of systemic lupus erythematosus susceptibility genes in a Hispanic population: Identification of six new candidate susceptibility loci. *E. Sanchez-Rodriguez[1], K.M. Kaufman[1, 2], J.A. Kelly[1], A.H. Williams[3], P.S. Ramos[3], A. Rasmussen[1], C.O. Jacob[4], P.M. Gaffney[1], K.L. Moser[1], B.P. Tsao[5], L.A. Criswell[6], R.P. Kimberly[7], T.J. Vyse[8], C.D. Langefeld[3], J.B. Harley[2,9], B. Pons-Estel[10], M.E. Alarcon-Riquelme[1,11], SLEGEN.* 1) Arthritis and Immunology, Oklahoma Medical Research Foundation, Oklahoma City, OK, USA; 2) US Department of Veterans Affairs Medical Center, Oklahoma City, Oklahoma, USA; 3) Wake Forest University Health Sciences, North Carolina, USA; 4) University of Southern California, Los Angeles, California, USA; 5) University of California, Los Angeles, California, USA; 6) University of California, San Francisco, California, USA; 7) University of Alabama, Birmingham, Alabama, USA; 8) Imperial College London, Hammersmith Hospital, London, UK; 9) University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, USA; 10) Sanatorio Parque, Rosario, Argentina; 11) Center for Genomics and Oncological Research (GENyO), Granada, Spain.

Background: Genome-wide association studies (GWAS) have proven highly effective for identifying hundreds of associations across numerous complex diseases. Four GWAS for systemic lupus erythematosus (SLE) in European populations and two in Asian populations have identified more than 20 robustly associated susceptibility genes. Differences in the prevalence and severity of SLE between various ethnicities are well documented, showing the need for further genetics studies in non-European populations. Objective: The aim of this study was to evaluate and replicate all genes previously associated with SLE with p values <0.05 in the SLEGEN(1) study through fine mapping in Hispanic SLE cases and controls. Material and methods: Here we evaluated 7069 single nucleotide polymorphisms (SNPs) in a set of 1510 Hispanic SLE patients and 825 Hispanic healthy controls. Tests of association were under a logistic regression model (SNPGWA), adjusting for ancestry proportions (ADMIXMAP) as covariates. Results: We confirmed 17 previously reported loci in European and Asian populations (BLK, IRF5, TNPO3, STAT4, ITGAM, ITGAX, 1q25, TNIP1, MSH5, HLA-DRA, XKR6, CFB, C2, MICB, PRDM1, ATG5 and LYN). In addition, we identified six new susceptibility loci in Hispanic SLE with a P< 0.0001 (C6orf10, MSRA, SLC44A4, ZBTB12, EHMT2 and CLIC1,). Conclusion: While we replicated the majority of the previous GWAS genes, new candidate genes have been discovered. These findings support the need to perform GWAS and additional fine mapping in Hispanics to locate additional susceptibility loci. (1)Harley JB, Alarcon-Riquelme ME, Criswell LA, et al. Genome-wide association scan in women with systemic lupus erythematosus identifies susceptibility variants in ITGAM, PXK, KIAA1542 and other loci. Nat Genet 2008;40:204-10.

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Electronic Medical Records for genetic research in a multi-ethnic Rheumatoid Arthritis cohort. *F.A.S. Kurreeman[1,2], K.P. Liao[1], T. Cai[1], V. Gainer[1], B. Thomson[2], K. Ardlie[2], S. Mahan[2], S. Raychaudhuri[1,2], E. Stahl[1,2], L.B. Chibnik[1,2], L. Bry[1], G. Li[1], P. Szolovits[1], S. Churchill[1], S. Murphy[1], I. Kohane[1], E.W. Karlson[1], R.M. Plenge[1,2].* 1) Brigham and Women's Hospital, Boston, MA; 2) Broad Institute, Cambridge, MA.

Introduction Electronic medical records (EMR) represent a rich source of clinical data, but are often considered inaccurate for research purposes. Linking EMR with biospecimens (DNA and plasma) can also be challenging, partially due to the cost of enrolling patients in traditional registries. Here, we demonstrate proof of concept that we can link EMR data and discarded blood samples to establish a case-control cohort with highly accurate clinical data for genomic research of rheumatoid arthritis (RA). **Methods** We used clinical data from the EMR to define a case-control cohort of 4,500 RA cases and 13,500 matched controls. We collected discarded blood samples on a subset of 1500 RA cases and 1500 controls that presented to the clinic for routine laboratory testing. We measured RA disease-specific autoantibodies against citrullinated peptide antigens (ACPA). We compared the odds ratios (ORs) of 29 SNPs genotyped in our EMR cohort to the ORs from a GWAS of >25,000 case-control samples collected as part of traditional registries. We calculated a cumulative genetic risk score (GRS) using a weighted log-additive model of all SNPs, and used area under the ROC curve (AUC) to estimate the effect size of the GRS. All analyses were subset by ACPA status and genetically assigned ethnicity [defined using a panel of 190 ancestry informative markers (AIMs)]. **Results** After controlling for population ancestry with AIMs, a panel of 29 RA risk variants demonstrated ORs largely consistent with studies using traditional patient cohorts. A GRS derived from these variants differentiates RA cases from controls with high statistical significance across all RA cases vs controls, ACPA+ (n=1051) vs controls, and ACPA- (n=446) vs controls ($P_{ALL}=10^{-43}$, $P_{ACPA}+=10^{-58}$, and $P_{ACPA-}=10^{-4}$). Analyses restricted to the ACPA+ subgroup across the three major continental populations of European, African and Asian descent showed similar results (AUC = 0.70, 0.61 and 0.67, respectively), implying that despite some non-overlapping loci, a common effect exists across these continental populations. **Conclusion** The approach outlined here has broad implications in diseases that seek to use vast amount of clinical data available in EMRs in a largescale manner that matches the capacity to generate genetic and genomic data. For diseases with complex phenotypic diversity, this method allows the interrogation of specific disease subsets and ethnic groups in an unbiased fashion.

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Exploration of molecular mechanisms for tuberculosis susceptibility by genetic association study. *HQ. Qu[1], Q. Li[2], B.I. Restrepo[1], S.P. Fisher-Hoch[1], J.B. McCormick[1].* 1) University of Texas Health Science Center at Houst, School of Public Health, Brownsville campus, Brownsville, TX; 2) Endocrine Genetics Lab, The McGill University Health Center (Montreal Children's Hospital), Montréal, Québec, Canada.

Genome-wide association studies (GWAS) are a unique approach to understand the molecular mechanism of many human complex diseases. The Wellcome Trust Case-Control Consortium (WTCCC) genome-wide case-control association study for tuberculosis in a population from The Gambia is one of the largest case-control cohorts and is the only GWAS on tuberculosis to date (WTCCC, 2007). However, in contrast to studies of other human complex diseases, particularly non-infectious diseases, the GWAS on tuberculosis is more complicated. The Gambia has multiple ethnic populations. The population structure in The Gambia and the nongenetic confounding factors, e.g. the intensity of contact with infectious tuberculosis patients, AIDS, diabetes, aging, and socioeconomic condition increase the difficulty of identifying genetic loci of tuberculosis susceptibility. For our analysis we removed 341 ethnic outliers, and we used the Eigenstrat algorithm (Price et al. 2006) with the application of genomic control (Devlin and Roeder, 1999) for the association test in 2,653 individuals. Genetic associations were suggested by both the Q-Q plot and the individual tests corrected for multiple comparisons. A number of candidate genes that may be involved in tuberculosis susceptibility, i.e. *SLC30A2*, *TNFRSF1B*, and *FAM19A1*, were highly statistically associated with TB (cleared the strict Bonferroni correction). We screened the tuberculosis-associated SNPs that cleared false discovery rate (FDR) corrections for association with gene expression levels (GENEVAR data, Stranger et al. 2005; 2007). The TB-associated SNP, rs4875958, was identified in association with the CLN8 gene expression in all three HapMap Phase 2 population samples, i.e. African, European, and East Asian. Higher *CLN8* expression is associated with increased tuberculosis susceptibility. This SNP could affect altered oxidative stress reaction, host cholesterol metabolism, or lysosome function. Validation of the tuberculosis associations in an independent case-control cohort is warranted. To address this issue, we are actively recruiting a case-control cohort of Pakistan population, including 600 cases and 1200 controls. Acknowledgments. This study makes use of data generated by the Wellcome Trust Case-Control Consortium. A full list of the investigators who contributed to the generation of the data is available from www.wtccc.org.uk. Funding for the project was provided by the Wellcome Trust under award 076113 and 085475.

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Polymorphisms in *MSX1*, *IRF6* and *MTHFR* genes are associated with isolated cleft lip with or without cleft palate in a Mexican population. *J.A. Velazquez[1,3], M.A. Alcantara[1], B. Estandia[1], C. Cruz[2], S. Villagomez[1], M. Diaz[1], A. Gonzalez[1].* 1) Genetics, Instituto Nacional de Pediatria, Mexico, Distrito Federal, Mexico; 2) Genetics, Instituto Nacional de Psiquiatria, Mexico, Distrito Federal, Mexico; 3) Posgrado Ciencias Biologicas, Universidad Nacional Autonoma de Mexico, Distrito Federal, Mexico.

Introduction. Isolated cleft lip with or without cleft palate (CL/P) is a common birth defect, prevalence vary between 0.5-1.7/1000(Mexico 1/1000).Several genes have been associated with CL/P but results are contradictory.The aim of our work was to identify association between 12 polymorphisms in *TGFA*, *TGFB3*, *MSX1*, *PVRL1*, *MTHFR*,*IRF6* candidate genes and isolated CL/P.Methods. A Case control study was carried out. Genotyping was performed by PCR-Restriction assay or allelic discrimination RT-PCR. Chi square test was performed to identify differences in allelic frequencies.Results. From 12 variants analyzed 4 showed association. On the other eigth polymorphisms no significant differences on allelic frequencies were observed.

Polymorphism	Controls/ Cases	Alelle freq Control	Allele freq Cases	Odds Ratio
MTHFR 677 C>T	342/122	0.38/0.62	0.50/0.50	0.61 C.I.= 0.45-0.82
IRF6 V274I	255/122	0.64/0.36	0.75/0.25	0.57 C.I.= 0.41-0.81
MSX1 1170A>G	179/85	0.40/0.60	0.31/0.69	1.52 C.I.= 1.03-2.25
MSX1 X1.3	269/127	0.84/0.16	0.63/0.37	3.14 C.I.= 2.22-4.42

Discussion. In variant *MTHFR* 677C>T we observed an elevated proportion of the allele T in controls compared with Europeans (0.62 vs 0.25 HapMap-CEU), allele T has been associated with CL/P but in our population seems to be the opposite, it may be explained if it does not have functional effect on protein.*IRF6* V274I showed association with CL/P. Two variants in *MSX1* were associated with CL/P, since Mexican population is heterogeneous we expected differences in polymorphisms associated with CL/P.

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Large population risk of a common variant on chromosome 11q13 (rs7927894) on eczema, asthma, and hay fever. *Y.A. Lee[1,2], A. Bauerfeind[1], J. Esparza-Gordillo[1], T. Kerscher[1,2], R. Granell[3], J. Henderson[3], I. Marenholz[1,2].* 1) Max-Delbruck-Centrum for Molecular Medicine, Berlin, Germany; 2) Pediatric Pneumology and Immunology, Charité Universitätsmedizin Berlin, Berlin, Germany; 3) University of Bristol, Department of Community-based Medicine, Bristol, United Kingdom.

Eczema (atopic dermatitis) is a chronic inflammatory skin disorder and a major manifestation of allergic disease. In a genome-wide association study for eczema, a common variant on chromosome 11q13.5 (rs7927894) has been identified as a novel susceptibility locus in four European study populations. The aim of this study was to evaluate the effect of this risk variant on eczema and on other allergic phenotypes such as asthma, hay fever, and elevated specific IgE on the population level. To this end, we performed an association study of rs7927894 in more than 9300 individuals from the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort, a large, well-characterized, population-based cohort of children born in 1991 and 1992 in the Avon area, UK.

Beyond replicating the association with eczema (OR 1.19; 95% CI 1.09-1.30; P =$4.9x10^{-5}$), we found that the effect of this risk variant was restricted to individuals with the atopic subtype of eczema (OR 1.21; 95% CI 1.04-1.40; P =0.013). In contrast, no association of rs7927894 with non-atopic eczema was observed. Moreover, we detected an association of rs7927894 with concomitant allergic asthma and hay fever, yielding the strongest effects on the combined phenotypes allergic asthma and eczema (OR 1.48; 95% CI 1.19-1.85; P =$4.0x10^{-4}$) and on hay fever and eczema (OR 1.40; 95% CI 1.19-1.65; P =$7.56x10^{-5}$). Finally, the ALSPAC cohort enabled us to estimate the population attributable risk fraction which indicates the proportion of cases in the population attributable to the rs7927894 risk allele. The population attributable risk fractions for eczema, concomitant asthma, and concomitant hay fever were estimated to be 9.3%, 24.9%, and 23.5% respectively.

We conclude that rs7927894 is a common variant that confers moderate individual risk for allergic disease, but carries a substantial risk at the population level. We demonstrate that rs7927894 is not only a susceptibility factor for eczema, but also for concomitant asthma and hay fever. The association of this variant with allergic skin and airways disease may point to a shared molecular mechanism underlying different allergic disorders.

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Replication of 39 celiac disease risk loci in the North-African Saharawi population. *A. Szperl[1], J. Romanos[1], G. Trynka[1], C.C. van Diemen[1], J. Fu[1], L. Franke[1], A. Zhernakova[2,3], R. Kanninga[1], S. Teresi[4], G. Iacono[5], R. Francavilla[6], S. Castellaneta[6], A. Ravelli[7], M.D Cantarero[8], C. Catassi[9], C. Wijmenga[1].* 1) Genetics Department , UMC Groningen, Groningen, Netherlands; 2) Medical Genetics Department , University Medical Center Utrecht, the Netherlands; 3) Department of Rheumatology, University Medical Center Leiden, the Netherlands; 4) Clinical Chemistry Department , Children's Hospital "G. Di Cristina"; 5) Pediatric Gastroenterology Department , Children's Hospital, Palermo, Italy; 6) Pediatrics Department , University of Bari, Italy; 7) Pediatrics Department , University of Brescia, Italy; 8) Centro de Especialidades de Illescas, Toledo, Spain; 9) Pediatrics Department , Università Politecnica delle Marche, Ancona, Italy.

Celiac disease (CD) is a complex, chronic inflammatory disorder of the intestine affecting as much as 1% of the Caucasian population. The disease is caused by a strong immune reaction towards gluten in genetically predisposed individuals. All CD patients carry HLA DQ2 and/or DQ8 risk variants which accounts for around 35% of the heritability. Two genome-wide association studies (GWAS) in CD and their follow-up have identified 39 non-HLA loci that contribute to CD risk. The highest prevalence of CD in the world (5.6%) has been described in the Saharawi, an African population of Arab-Berber origin living in the Western Sahara. Our aim was to investigate the association of previously described CD loci in this exotic population. We have conducted a genome-wide scan in a total of 384 individuals from 143 Saharawi families with CD (168 affected and 216 non-affected family members). For this study, we tested 1.601 SNPs from the 39 previously identified risk loci for association using family based association tests (FASTA; GenABLE). After correcting for multiple testing per locus, we replicated association with CD for the ETS1 locus on 11q23.3 (P corrected= 0.0007), but due to the small sample size we did not observe significant association for the other loci. This is supported by a separate analysis on the different Caucasian populations from the original GWAS (British, Dutch, Italian and Finnish) where we observed that for only two out of the four populations there was at least one SNP among the 1.601 SNPs that was significant. To increase statistical power, we therefore conducted a meta-analysis of the Caucasian and Saharawi cohorts. In this analysis we observed 24 loci for which association signal did not decrease, indicating that by increasing the sample size for the Saharawi population we will likely to identify more non-HLA CD risk loci in this population.

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Efficient Replication of Genetic Associations with Medical Phenotypes Using Self-Reported Data. *J.Y. Tung, N. Eriksson, C.B. Do, B.T. Naughton, J.M. Macpherson, A.K. Kiefer, J.L. Mountain.* 23andMe, Inc, Mountain View, CA.

While the pace of advances in genetics has increased in recent years, the high cost of collecting phenotype data in large cohorts of patients still hampers genomic research. This can make the replication of associations difficult, leaving many initial discoveries in a limbo of uncertain validity. Here we sought to determine whether collecting self-reported information via the web is an effective phenotyping method for large genome-wide association studies on medical conditions by assessing our ability to replicate genetic associations with health-related phenotypes in our genotyped customer database. We gathered self-report data using web-based questionnaires on approximately 65 medical phenotypes in a generally unselected cohort of over 12,000 individuals and replicated over 100 previously reported associations, including many in type 2 diabetes, prostate cancer, and multiple sclerosis. Of a list of genome-wide association hits curated by the National Human Genome Research Institute's Office of Population Genomics, we successfully replicated ~70% that we expected to detect. There was significant variation across conditions in the percentage of expected associations that we observed, which may reflect differences between our population and the initial study populations, or differences across diseases in the difficulty of diagnosis or communication to patients. These data demonstrate that collecting self-reported information can be a very efficient method for medical phenotyping in large genome-wide studies and can significantly reduce the overall resources required to validate an association.

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Coding Variant R32W in Complement Factor B and Risk of Age-Related Macular Degeneration. *A. Hughes, G. Mullan, D. Bradley.* Centre for Public Health, Queen's Univ Belfast, Belfast, United Kingdom.

We investigated the association between rs12614 (R32W) in the complement factor B (*CFB*) gene and risk of age-related macular degeneration (AMD) in a case-control association study of 224 patients with neovascular AMD and 181 disease-free controls. The minor allele of the *CFB* rs12614 (R32W) polymorphism was associated with protection from AMD on simple allele count (odds ratio 0.56 (95%CI 0.32-0.97); p=0.028) and in a logistic regression model (odds ratio 0.39 (95%CI 0.20-0.75); p=0.005). Complement factor B has an important role in amplification of the alternative complement pathway. We report that the minor alleles of rs12614 (R32W) and rs641153 (R32Q) are associated with protection from AMD. The size of each effect is consistent with previously reported functional effects of these polymorphisms on alternative complement pathway activation in vitro.

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Genome-wide loci of serum bilirubin levels as predictors of gallstone composition and gallstone risk. *S. Buch[1], C. Schafmayer[2], H. Völzke[5], B. Timm[4], M. Seeger[1], O. von Kampen[1], S. Schreiber[1,3], J.H. Egberts[2], C.D. Bröring[2], J. Hampe[1].* 1) Department of General Internal Medicine, Kiel, University Hospital Schleswig-Holstein-Kiel, Germany; 2) Department of General and Thoracic Surgery;University Hospital Schleswig-Holstein-Kiel, Germany; 3) Institute for Clinical Molecular Biology; University Hospital Schleswig-Holstein-Kiel, Germany; 4) POPGEN Biobank; University Hospital Schleswig-Holstein-Kiel, Germany; 5) Dept. of Community Medicine; University of Greifswald, Germany.

Background: Genome wide association studies have established a map of loci associated with serum bilirubin levels. Because bilirubin forms one of the major components of gallstones, these variants are likely candidates for gallstone bilirubin content and possibly also for overall gallstone risk. Aim: To investigate loci associated with serum bilirubin levels as predictors of gallstone composition and gallstone risk. Methods: Loci from a recent meta-analysis meeting a genome-wide significance level of p<1.0x10-7 (UGT1A1, SCLO1B1, LST-3TM12, SLCO1A2) were analyzed in over 1000 individuals with known gallstone composition as measured by FTIR spectroscopy. The likely mode of inheritance was extracted for each locus from the meta-analysis. Gallstone risk was analyzed in 2606 German individuals (71% females) with operated gallstone disease and 1121 matched stone-free controls. Results: Using the presence of bilirubin as a phenotype in comparison to gallstones without bilirubin (<=5%), variants rs6742078 (UGT1A1, p=0.003,recessive;OR 1.73), rs4149056 (SLCO1B1, p=0.005,dominant;OR 1.57) and rs4149000 (SLCO1A2, p=0.045,dom.;OR 1.43) yielded significant nominal association with gallstone composition. Only UGT1A1,(rs6742078,p=0.018,rec.;OR 1.3 [1.04-1.64]) was also associated with overall gallstone risk in the total cohort. In a gender-stratified post-hoc analysis the rs6742078 gallstone disease risk was confined to the male sex (males:p=2.1x10-7,rec.;OR 2.34 [1.68-3.26];females:p=0.47, rec.;OR 1.10[0.84-1.45]). Discussion: This study shows how genome wide association data of a laboratory value, i.e. bilirubin levels, can be translated into predictors for stone composition and gallstone risk. We evaluated the four most strongly associated loci for serum bilirubin levels for their relevance to stone composition and tested for disease association. The tested UGT1A1 variant was previously show to be in high LD with a common UGT1A1*28 promoter polymorphism that underlies Gilbert's syndrome and that was reported to increase the susceptibility to gallstone formation in patients with hereditary hemolytic anemias, and recently in a small study of healthy Greek adults. We confirm the association of the UGT1A1 locus with gallstone disease and show that the overall disease association is primarily confined to the male sex. As it is known that the Gilbert syndrome is 4 times more common in men a more complete etiological picture of gallstone pathogenesis is emerging.

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Association of *ARMS2* with Exudative Age-related Macular Degeneration. *P.O.S. Tam[1], T.Y.Y. Lai[1], D.T.L. Liu[2], Y.Q. Zheng[1,3], S.W.Y. Chiang[1], X.Y. Liang[1], L.J. Chen[1], C.P. Pang[1].* 1) Department of Ophthalmology & Visual Sciences, The Chinese University of Hong Kong, HKSAR, China; 2) Hong Kong Eye Hospital, HKSAR, China; 3) Joint Shantou International Eye Center of Shantou University and the Chinese University of Hong Kong, Shantou, China.

Purpose: The mapping of 10q26 chromosomal locus in age-related macular degeneration (AMD) had shed light on its genetic etiology, mainly *HTRA1* and *ARMS2*. But the contribution of *ARMS2* remains controversial. We have previously evaluated the contribution of *HTRA1* genetic variations in Chinese exudative AMD patients. In this study, we evaluated the contribution of *ARMS2* in our cohort. **Methods:** We performed whole gene sequencing of *ARMS2* in 156 exudative AMD patients and 248 unrelated normal control subjects aged over 60. **Results:** Thirty sequence variations were found, among which only 3 lead to amino acid change, p.R3H, p.R38X and p.A69S. Strong association was found between p.A69S and AMD (p=9.37x10^{-20}, OR=4.51). p.R38X was found to be predominant in control subjects, which confers a marginally significant protection to AMD under autosomal dominant model (p=0.037, OR=0.61). The reported 54 bp insertion and adjacent 443 bp deletion (indel) in the 3'UTR was also associated with AMD (p=5.78x10^{-18}; OR=4.22), and is in linkage disequilibrium with p.A69S and the *HTRA1* promoter SNP rs11200638. **Conclusions:** Our results indicated association of *ARMS2* with AMD. While further analysis on the functional consequences of the variants, both coding and non-coding, is warranted to substantiate our results.

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No evidence of association between complement factor I genetic variants and age-related macular degeneration. *V. Cipriani[1,2], B.K. Matharu[3], J.C. Khan[3], H. Shahid[3], C. Hayward[4], A.F. Wright[4], A.M. Armbrecht[5], B. Dhillon[5], S.P. Harding[6], P.N. Bishop[7], C. Bunce[2], D.G. Clayton[3], A.T. Moore[1,2], J.R.W. Yates[1,2,3], Genetic Factors in AMD Study Group.* 1) Institute of Ophthalmology, University College London, UK; 2) Moorfields Eye Hospital, London, UK; 3) Department of Medical Genetics, Cambridge Institute for Medical Research, University of Cambridge, UK; 4) Medical Research Council Human Genetics Unit, Institute of Genetics and Molecular Medicine, Edinburgh, UK; 5) Princess Alexandra Eye Pavilion, Edinburgh, UK; 6) Ophthalmology Research Unit, School of Clinical Sciences, University of Liverpool, UK; 7) Manchester Royal Eye Hospital and University of Manchester, UK.

Introduction Age-related macular degeneration (AMD) is the leading cause of visual impairment and blindness in the developed world. To date, four genetic loci have been confirmed to be associated with susceptibility to AMD, namely CFH, ARMS2/HTRA1, CFB/C2 and C3. Recently, Fagerness et al. reported association with a single nucleotide polymorphism (SNP), rs10033900, near the complement factor I (CFI) gene [1]. We had previously typed 533 AMD cases and 268 controls for four SNPs in and around CFI (including rs10033900) as well as variants at the established AMD loci. These data showed the expected association with CFH, ARMS2, CFB and C3 but no evidence of association with the CFI variants. Following the report by Fagerness et al. [1], we have typed rs10033900 in additional cases and controls to investigate this further. **Methods** A total of 859 cases (55% women, mean age=79 yrs) and 423 examined controls (60% women, mean age=75 yrs) were typed in our English sample. The cases comprised 29 with age-related maculopathy (ARM) and 830 with end-stage AMD of whom 688 had choroidal neovascularization (CNV) and 142 had geographic atrophy (GA). rs10033900 was also typed in an independent Scottish sample of 505 cases (63% women, mean age=78 yrs; 261 ARM, 189 CNV, 55 GA) and 351 examined controls (57% women, mean age=78 yrs). **Results** No evidence of association was found in the English sample (OR=0.94, 95% CI=0.79-1.11, P=0.46) or in the Scottish sample (OR=1.01, 95% CI=0.83-1.23, P=0.94). There was no evidence of association in subgroup analyses confined to cases with end-stage disease only, CNV only, GA only, or ARM only. **Discussion** We have been unable to replicate the association between CFI variants and AMD. Recent studies provide some support for the association with rs10033900 [2] and with variants in and around CFI [3]. Also, genome-wide scans have reported signals of interest in the neighbouring genes CCDC109B [4] and PLA2G12A [5]. Given the variability observed in these results, additional studies would be desirable to clarify the nature of the association between AMD and variants in the CFI region. **References** [1] Fagerness JA et al., *Eur J Hum Genet*, 2009, 17:100-104; [2] Kondo N et al., *Eur J Hum Genet*, 2010, 18:634-635; [3] Ennis S et al., *Eur J Hum Genet*, 2010, 18:15-16; [4] Neale BM et al., *PNAS*, 2010, 107: 7395-7400; [5] Chen W et al., *PNAS*, 2010, 107: 7401-7406.

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***SRBD1* gene polymorphism is associated with normal tension and high tension glaucoma.** *F. Mabuchi[1], Y. Sakurada[1], K. Kashiwagi[1], Z. Yamagata[2], H. Iijima[1], S. Tsukahara[1].* 1) Dept Ophthalmology, Univ Yamanashi, Chuo, Yamanashi, Japan; 2) Dept Health Sciences, Univ Yamanashi, Chuo, Yamanashi, Japan.

Purpose: Primary open angle glaucoma (POAG) is the most common form of glaucoma, and it is clinically classified into high tension glaucoma (HTG), in which an elevated intraocular pressure is a major feature, and normal tension glaucoma (NTG), in which the intraocular pressures are consistently within the statistically normal population range. We reported that the *SRBD1* gene polymorphism was associated with early onset NTG. As a next step, this study was performed to assess whether the *SRBD1* gene polymorphism was associated with HTG. Methods: Four hundred and twenty five Japanese patients with POAG, including NTG (n = 213) and HTG (n = 212) and 191 control subjects without glaucoma were analyzed for the *SRBD1* gene polymorphism (rs3213787). The mean age at the time of blood sampling was 64.0 ± 13.7 years (mean ± SD) in patients with NTG, 62.9 ± 14.8 years in patients with POAG and 65.7 ± 11.4 years in the control subjects. The genotype and allele frequencies were compared between the patients with NTG or HTG and the control subjects. Results: There was a significant difference (P = 0.0071) in the genotype frequencies between the NTG patients (GG: 0.5%, AG: 4.7%, AA: 94.8%) and control subjects, (GG: 0.5%, AG: 13.6%, AA: 85.9%) and the frequency of the A allele was significantly higher (p = 0.0033) in patients with NTG in comparison to the control subjects (97.2% vs. 92.7%). Moreover, a significant difference (P = 0.0018) was also found in the genotype frequencies between the HTG patients (GG: 0.5%, AG: 3.8%, AA: 95.7%) and control subjects, and the frequency of the A allele was significantly higher (p = 0.0013) in patients with HTG in comparison to the control subjects (97.6% vs. 92.7%). Adjusted for age, gender, and intraocular pressure, an almost 2.5 times increased risk of POAG (P = 0.043, odds ratio 2.48, 95% confidence interval 1.03 to 5.99) was found with the A allele. Conclusion: The *SRBD1* gene polymorphism is considered to be a genetic risk factor not only for NTG, but also for HTG.

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Rare variants in *BMP2*, *BMP4*, and *TGFB1* associated with otosclerosis in a German population. *M. Ealy[1], J. Cruz Corchado[1], I. Schrauwen[2], N.C. Meyer[1], M. Baur[3], M. Pfister[3], G. Van Camp[2], R.J.H. Smith[1].* 1) Molecular Otolaryngology and Renal Research Laboratory, Department of Otolaryngology, University of Iowa, Iowa City, Iowa; 2) Department of Medical Genetics, University of Antwerp, Antwerp, Belgium; 3) Department of Otorhinolaryngology, University of Tübingen, Tübingen, Germany.

Otosclerosis is a common form of adult-onset hearing loss. It is caused by abnormal bone remodeling of the otic capsule and can manifest as a conductive hearing loss when the otosclerotic lesion impairs movement of the stapes. While both genetic and environmental factors have been implicated in otosclerotic disease, the pathogenesis remains unclear. To identify genetic contributions to otosclerosis, a number of candidate gene case-control association studies have been successfully performed. Members of the TGFβ superfamily are excellent candidates due to their established role in bone remodeling and expression in the otic capsule. We sought to replicate an association between polymorphisms in genes of the TGFβ superfamily and otosclerosis first made in a Belgian population by studying a smaller German population of cases and controls. While associations with *BMP2*, *BMP4*, and *TGFB1* did not replicate in the German population, rare variants were identified that may contribute to the risk of developing otosclerosis. Most of these rare variants were located in a prodomain common to the three proteins. This regulatory prodomain (referred to as the latency-associated peptide in TGF-β1) is responsible for maintaining all three proteins in an inactive state. However, during the resorptive phase of bone remodeling, enzymes activate BMP2, BMP4, or TGF-β1 dimers by releasing them from the prodomain. Based on this mechanism of action, the rare variants we identified may modulate the ability of the prodomain to regulate protein activity. To test this hypothesis we established an *in vitro* reporter system that is responsive to TGF-β1, BMP2 or BMP4 signaling. Determining how these variants affect function of these TGFβ superfamily members will better our understanding of their role in otosclerosis.

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In Silico Genotype Imputation on Large Pedigrees. *C.Y.K. Cheung[1], E.A. Thompson[2], E.M. Wijsman[1,3].* 1) Dept. Biostatistics, University of Washington, Seattle, WA; 2) Dept. Statistics; 3) Div. of Medical Genetics.

Availability of dense SNPs or sequence data is commonplace but expensive. Many pedigree studies contain existing samples with sparse genotypes. In small pedigrees, it is already feasible to impute dense genotypes from the existing sample and a few densely-genotyped individuals. Here we show that “in silico” dense genotyping on large pedigrees is also now computationally practical. Using MORGAN, MCMC-based sampling conditional on genotype data provides realizations of inheritance vectors (IVs) on large pedigrees. We introduce GIGI (Genotype Imputation Given Inheritance), which imputes dense genotypes using sampled IVs and the few observed dense genotypes. GIGI uses probabilistic inference and threshold-based allele calling. Conditional on an IV, genotype probabilities are computed jointly over pedigree members, given population allele frequencies. Overall probability estimates are obtained by averaging over sampled IVs. A threshold is then used to call either a genotype or an allele. Coupled with the IBDgraph program, which identifies equivalent sampled IVs, this provides rapid and efficient imputation.

We used GIGI on a 95-member real pedigree with 5 generations. On a test chromosome, 21 STR genotypes were observed in 64 subjects of the lowest 4 generations, and 323 dense SNPs over a ~50cM region were observed on 60 subjects in the lowest 3 generations. SNPs from 13 individuals were used for imputation, with the SNPs in the remaining subjects used for validation. We explored both the threshold for genotype calling, and the effect of population allele frequency on the fraction of alleles called and the percentage of correct calls. With stringent genotype-calling threshold probabilities of .8 for both alleles and .9 for one of two alleles, 68% of alleles were called in the 47-subject validation sample, of which 97.6% were correct. Reduction of the threshold to .6/.8 for both/one allele(s) increased the call rate to 85%, of which 93% were correct. Allele call rates were inversely related to population allele frequency. These results demonstrate that accurate imputation in large pedigrees is practical, providing a cost-effective sequential approach for both design of further studies and to obtain dense marker genotypes from new platforms such as next-generation sequencing.

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Discovery of novel variants in the *SLC30A8* gene in the multiethnic cohort of the Diabetes Prevention Program (DPP) and their impact on diabetes incidence. *L.K. Billings[1,2,3], K.A. Jablonski[4], R.R. Fanelli[1], J.B. McAteer[1,5], A. Taylor[1,5], R. Ackerman[1], C. Guiducci[5], D. Dabelea[6], L.M. Delahanty[2,3], S.E. Kahn[7], P.W. Franks[8], R. Hanson[9], N. Maruthur[10], A. Shuldiner[11], E.J. Meyer-Davis[12], W.C. Knowler[9], J.C. Florez[1,5,2,3], Diabetes Prevention Program Research Group*. 1) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA; 2) Department of Medicine, Harvard Medical School, Boston, MA; 3) Diabetes Research Center (Diabetes Unit), Department of Medicine, Massachusetts General Hospital, Boston, MA; 4) The Biostatistics Center, George Washington University, Rockville, MD; 5) Program in Medical and Population Genetics, Broad Institute, Cambridge, MA; 6) Department of Epidemiology, Colorado School of Public Health, University of Colorado, Denver, CO; 7) Division of Metabolism, Endocrinology and Nutrition, VA Puget Sound Health Care System and University of Washington, Seattle, WA; 8) Genetic Epidemiology and Clinical Research Group, Department of Public Health and Clinical Medicine, Division of Medicine, Umea University Hospital, Umea, Sweden; 9) Diabetes Epidemiology and Clinical Research Section, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Phoenix, AZ; 10) Department of Medicine, Division of General Internal Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; 11) Department of Medicine, Division of Endocrinology, Diabetes and Nutrition, University of Maryland School of Medicine, Baltimore, MD; 12) University of North Carolina, Gillings School of Global Public Health, Department of Nutrition, Chapel Hill, NC.

The common missense single nucleotide polymorphism (SNP) rs13266634 in the *SLC30A8* gene is associated with type 2 diabetes (T2D) with an odds ratio ~1.15 in people of European descent. This SNP encodes a Trp®Arg change at position 325 in the islet-specific Zinc (Zn) transporter ZnT8, whose transport of Zn molecules into insulin granules is essential for insulin storage and processing. In vitro and null mouse models have demonstrated that disruption of *Slc30a8* alters insulin crystallization and secretion, and the T2D risk allele at rs13266634 is associated with impaired β-cell function and high proinsulin levels (adjusted for fasting insulin) in humans. These studies provide supportive evidence that *SLC30A8* contains the causal variant responsible for the association signal; however, in the DPP, the risk variant at rs13266634 was not associated with T2D or glycemic traits. Therefore, to discover additional functional variants in *SLC30A8*, we sequenced 8 exons, 50 base-pairs (bps) around each intron/exon junction, and 1 kb up- and down-stream of *SLC30A8* in 380 DPP participants (76 people in each five different ethnic groups, most of whom developed T2D) on an ABI sequencer. Ten PCR amplicons targeted 5,721 bps with an average of 2X coverage at a rate of 97.8%, for a total of 2.17 Mbp. We discovered 50 novel SNPs and genotyped 73 novel and known SNPs in 3,566 DPP participants on a Sequenom iPlex platform for validation. A total of 60 SNPs, of which 44 are novel, passed stringent quality control criteria. Of the novel SNPs, 11 had a minor allele frequency (MAF) between 1-5% in at least one of the ethnic groups, while the remaining had a MAF of less than 1%. Four SNPs were unique in Caucasians, 9 in African-Americans, 2 in Hispanics, 8 in Asians, and 2 in American Indians. Two novel missense variants, Ala87Thr and Asp295Glu, showed no evidence from bioinformatic analysis that these would be damaging. Among the SNPs with MAF > 1% (24 SNPs), we found no association with T2D incidence adjusted for intervention and no significant interaction between SNP and treatment arm. In stratified analyses by ethnic group, Caucasian participants, carriers of the minor allele at rs2464591, rs2466296 and rs2466297 were nominally less likely to develop diabetes than carriers of the major allele. In conclusion, we identified 44 novel low frequency (MAF < 5%) variants in *SLC30A8*. None of the SNPs with a frequency greater than 1% were significantly associated with T2D incidence.

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Implication of Next-Generation Sequencing on Association Studies. *H. Siu[1], Y. Zhu[3], M. Bainbridge[2], R. Gibbs[2], F. Yu[2], L. Jin[1], M. Xiong[1,3]*. 1) Dept Genetics, Fudan Univ, Shaghai, China; 2) 3Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas USA; 3) Human Genetics Center, University of Texas School of Public Health, Houston, Texas, U.S.A.

Genome-wide association studies (GWAS) have become the primary approach for identifying genes with common variants influencing complex diseases. Despite considerable progress, the common variations identified by GWAS account for only a small fraction of disease heritability, and are unlikely to explain the majority of phenotypic variations of common diseases. A potential source of the missing heritability is the contribution of rare variants. Next-generation sequencing technologies will detect millions of novel rare variants and open a new way for association studies. In this report, we focus on pilot 3 data of 1000 genome project to investigate the pattern of genetic variations and its implication for association studies. We found that the linkage disequilibrium (LD) between rare and rare alleles, and rare and common alleles are much weaker than the LD between common and common alleles. We estimated the coverage of pilot 3 data by the current commercial arrays. We found that the biggest coverage which Illumina 1M can achieve in the populations ranges from 23% to 38% at $r2 \geq 0.8$. We also estimated coverage of by pilot 1 data. Their coverage varies from 50% to 66% at $r2 \geq 0.8$,depending on populations. To our surprise, further increasing the number of SNPs in arrays may not increase too much coverage. We also investigated power of complete sequencing, and various tag SNP strategies under four disease models by case-control study design. We found that that power of complete sequencing can almost reach 1 with 5000 cases and 5000 controls for the additive model when the risk allele frequency is 0.03 at the significance level of 0.05. However, with the same model, parameters and sample sizes, pilot 1 data, Illumina 1M and Affymetrix 6.0 can only reach the power of 33.7%,15.5% and 4.5%, respectively. These results strongly demonstrated that any strategies based on tag SNPs may be work very well for association studies of common alleles, but will be ill suited for association studies of rare alleles.

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Efficiently combining single-variant test statistics rather than rare variants is the key to powerful association tests. *D.D. Kinnamon, E.R. Martin.* Dr. John T. Macdonald Foundation Department of Human Genetics, University of Miami Miller School of Medicine, Miami, FL.

Methods involving collapsing and summing over rare variants have been proposed to increase power to detect associations in case-control studies. However, these methods have not been compared to efficient combination of single-variant test statistics under realistic scenarios with linkage disequilibrium (LD), potential common risk variants, and missing genotype data. We considered a permutation test based on the maximum Cochran-Armitage test statistic over all variant positions in a locus (CA max) as well as two recently proposed collapsing and summing methods. Analytic power comparisons with varying LD were performed using a simple model of a locus comprising one rare risk and one rare neutral variant with the same minor allele frequency (MAF). Monte Carlo simulations were used to extend the basic conclusions of this simple model to a 100 kb locus with both rare and common risk variants and missing genotype data. Ten populations of 10,000 haplotypes with LD were generated under a standard neutral coalescent model with recombination, and 500 1:1 case-control samples with total sizes 500, 1,000, or 2,000 were drawn with replacement from each population according to a disease model. Each scenario either had complete or 5% randomly missing genotype data and α=0.05 or 0.01. In analytic power calculations with risk MAF=0.01 and minor allele odds ratio (OR)=2, the CA max test dominated collapsing or summing for negative and low-to-moderately positive LD. In simulations, all techniques demonstrated good control of Type I error under a null disease model. However, the multivariate test including both collapsed rare variants and common variants could not be performed with 5% missing genotypes because Hotelling's T^2 requires complete genotype data for common variants in an individual. For disease models with 50 rare risk variants (MAF<0.01, OR=2), the CA max test had power greater than collapsing or summing in two-thirds of scenarios. The CA max test also had power greater than collapsing or summing in >80% of scenarios for a disease model with 50 total risk variants randomly chosen from rare (MAF<0.01, OR=2) and common (0.01£MAF<0.05, OR=1.5; 0.05£MAF<0.1, OR=1.2) variants in the population. Efficient combination of single-variant test statistics with the CA max test offers greater power for detecting associations than collapsing or summing over rare variants under many scenarios likely to occur in actual sequence data.

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Comparison of methods and sampling designs to test for association between rare variants and quantitative traits. *S. Bacanu[1], M.R. Nelson[1], J.C. Whittaker[2].* 1) GlaxoSmithKline, Res Triangle Park, NC; 2) GlaxoSmithKline, Harlow, UK.

While genome wide association studies of complex traits have been very successful in detecting associations between common genetic variants and disease phenotypes, for most traits such variants explain only a small proportion of the estimated genetic variance. Several studies have demonstrated that some of the currently unexplained variance is due to rare variants. Until recently it was cost-prohibitive to investigate such variants except on a small and targeted scale, but current sequencing technologies are expanding this scale to large fractions of the genome on thousands of individuals. As is often the case, the development of methods for data analysis lags behind the technology for data generation. A commonly used analysis method is a trend statistic which contrasts the aggregate number of rare, putatively functional variants in a gene between subjects in the extreme tails of the trait distribution, assuming that the preponderance of variants have a similar effect on the trait of interest (i.e. effect homogeneity). The assumption of effect homogeneity is likely to be false; moreover, the aggregate trend method does not use the available rank information, nor does it incorporate in-silico predictions of the functional effects of genetic. To account for the possible heterogeneity in effect we propose new methods to test for heterogeneity, trend and heterogeneity and evaluate designs which test the tail and the middle of the distribution when just a fraction of the phenotyped sample is intended to be sequenced. We also propose new trend methods which use the information in ranks and functional prediction from methods such as PolyPhen. All methods and designs are tested via extensive simulation for different gene coding lengths, causal models and levels of heterogeneity for the effects rare variants. Based on this work we have identified four key findings: (1) the best analysis method depends on varying circumstances but a trend test combined with a trend and heterogeneity test appears to be a reasonable combination, (2) simplistic use of functional prediction of rare variants does not necessarily result in a gain of power and it may even result in a loss of power under some circumstances (3) depending on the causal model, incorporation of some subjects within the middle of the distribution can substantially improve power compared to a tail-only design in the presence of effect heterogeneity.

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Optimal Study Design for Targeted Re-Sequencing of Pooled DNA for Disease Association Studies. *A. Day-Williams, K. McLay, E. Howard, A.J. Coffey, A. Palotie, E. Zeggini.* Human Genetics, Wellcome Trust Sanger Institute, Hinxton, United Kingdom.

Genome-wide association studies have yielded an unprecedented number of associations between common genetic variants and complex traits. But the loci identified only explain a small proportion of the heritability of the analyzed traits. This has generated a huge interest in re-sequencing large numbers of individuals to find low frequency and rare variants that help account for the missing heritability. Although next-generation sequencing technologies have drastically reduced the costs of sequencing, it is still prohibitively expensive to sequence the whole genomes of thousands of individuals. Therefore targeted resequencing in pooled DNA is currently the preferred study design. But, there are many questions that need investigation in order to optimally design a targeted re-sequencing experiment in pooled DNA including choice of sequence enrichment technology and number of individuals to pool. We evaluate long-range PCR, array based pull down, and in-solution pull down enrichment methods for 6 genomic regions composing 1.6Mb. The enriched regions are sequenced in DNA pools of 1, 2, 10, 20, and 50 individuals with an Illumina GAII. The 50 individuals assayed are composed of 31 HapMap individuals and 19 control individuals from the WTCCC. Additionally, 22 of the individuals were also sequenced in Pilot 1 of the 1000 Genomes project. We have compared the SNPs called and their frequency estimates from our experiments to the SNPs and their known frequency in our pools from HapMap3 r27 and the final SNP call set from the 1000 Genomes Pilot 1, as well as to dbSNP129. In the pool of 10 HapMap individuals the PCR method found 6358 SNPs (60.5% in dbSNP), the array-based pull down method found 5874 SNPs (63.9% in dbSNP), and the in-solution method found 6783 SNPs (62.1% in dbSNP). Of the 6358 SNPs called using PCR enrichment the array-based pull down called 4272 of them, and the in-solution pull down called 4720. Of the SNPs called using array-based and in-solution pull down 5248 SNPs were called by both enrichment methods. Comparing the called SNPs to both the HapMap and 1000 Genomes data, the in-solution pull down method finds the highest proportion of the known variants with array-based pull down in second and PCR in third. We will present the results from the pool of 20 and 50 individuals, as well as a thorough investigation of the accuracy of the allele frequency estimates from all pools.

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Metabolic effects of common and rare variants in the Glucose Kinase Regulatory Protein. *D. Ng[1], M.G. Rees[2], S.L. Ruppert[1], J.C. Mullikin[3], M.C. Skarulis[4], L.G. Biesecker[1], NISC Comparative Sequencing Program.* 1) Genetic Disease Research Branch, NHGRI, NIH, Bethesda, MD; 2) National Center for Human Genome Research, NHGRI, NIH, Bethesda, MD; 3) Genome Technology Branch, NHGRI, NIH, Bethesda, MD; 4) Clinical Endocrine Section, NIDDK, NIH, Bethesda, MD.

ClinSeq™ is a large-scale medical sequencing (LSMS) project at the National Institutes of Health (NIH). The goal of ClinSeq™ is to study the technical, medical, and genetic counseling issues associated with LSMS and its application to personalized medicine. The research aims are twofold: 1) identify clinically relevant gene variants that impact on health and return these results to study participants to guide medical management; 2) generate hypothesis-driven clinical and molecular research to discover new genetic variants that contribute to disease susceptibility. Glucose Kinase Regulatory Protein (GCKR) was selected as a candidate gene for study in ClinSeq™ because a common coding SNP rs1260326 (p.Pro446Leu) was found to be associated with hypertriglyceridemia in a replication study of quantitative trait loci in type II diabetes mellitus. Rodent studies showed that GCKR regulates glucose kinase (GCK) activity in the liver. GCKR sequesters and stabilizes GCK in the hepatic nucleus in the fasting state. GCKR inhibition of GCK is enhanced by fructose 6-phosphate and reduced by fructose 1-phosphate. We hypothesized that GCKR variants with reduction or loss of GCK inhibition would result in abnormal cellular localization of GCK, altered glucose metabolism, and development of hepatic steatosis. To test this hypothesis, we analyzed over 700 ClinSeq™ participants with Sanger Sequencing to identify GCKR variants. Seventeen rare (n=42 individuals) and one common ((rs1260326), n=355 heterozygotes, n=141 homozygotes) GCKR coding variants were identified. Cellular effects of these genetic variants were studied by co-transfecting CFP-tagged mutant GCKR and YFP-tagged wild-type GCK constructs into Hela cells. Microscopy revealed a spectrum of GCK cellular localization ranging from entirely cytoplasmic (p.Val103Met) indicating a total loss of GCKR inhibition, to preservation of GCK nuclear to cytoplasmic shuttling (p.Arg540Gln). Select ClinSeq™ participants with GCKR variants were invited to the NIH for metabolic phenotyping. Oral glucose tolerance tests with and without fructose showed a spectrum of glucose metabolism ranging from accelerated to delayed glucose clearance. Preliminary data from hepatic MRI scans showed an increased triglyceride content in 3 out of 4 phenotyped individuals. Studies are ongoing to compare individuals who carry the same variant with each other and with wild-type controls to determine if there is a genotype-phenotype correlation.

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The ARRA Autism Sequencing Collaboration - Phase 1: Deep Whole Exome Sequencing in 1000 Autism Cases and 1000 Matched Controls. *C. Stevens*[*1], A. Sabo*[*2], B. Neale*[*1], S. Ripke[1], D. Muzny[2], I. Newsham[2], M. Rivas[1], M. Velankar[1], Y.Q. Wu[2], M. Wang[2], J. Reid[2], U. Nagaswamy[2], C. Kovar[2], J. Santibanez[2], H. Dinh[2], S. Gabriel[1], ARRA. Autism Investigators:[1,2,3,4,5,6], M. Daly[1], R. Gibbs[2], J. Buxbaum[3], B. Devlin[4], G. Schellenberg[5], J. Sutcliffe[6], * These authors contributed equally.* 1) The Broad Institute, Cambridge, MA; 2) Baylor College of Medicine, Houston, TX; 3) Mount Sinai School of Medicine, New York, NY; 4) University of Pittsburgh, Pittsburgh, PA; 5) University of Pennsylvania, Philadelphia, PA; 6) Vanderbilt University, Nashville, TN.

Autism (MIM209850) and additional autism spectrum disorders (ASDs) are pervasive developmental disorders defined by social disability and communication impairment as well as repetitive behaviors and/or restricted interests. The onset is generally before the age of 3 years, and the disorder has a prevalence of 0.6% in the population, affecting ~4 times as many more boys as girls. The heritability of autism is estimated at ~90%, making it one of the most heritable complex disorders. Despite the high heritability of autism, linkage and genome-wide association studies have not provided substantial insight into the root causes of idiopathic autism. Thus we have embarked on an ambitious collaboration between experienced large scale sequencing centers at the Baylor College of Medicine (BMC) and the Broad Institute (BI) and experienced autism genetics research groups to take advantage of dramatic advances in sequencing technology to study sequence variation across the entire genome. Phase 1 of this study, described here, aims to assess the entire spectrum of allelic variation using whole exome sequencing in 1000 cases and 1000 matched control samples. Our target parameters for the project are a median sample performance with greater than 80% of target bases covered at 20x per sample. The whole exome target consists of more than 32 Mb of exonic sequence, however, the flanking sequence near each exon also receives extensive coverage for analysis. Here, we present the experimental and analytic strategy of this study, quality control and detailed coverage analysis, as well as initial summaries of the rare and common coding variation found in ASDs. The complete dataset will provide a thorough assessment of variation found in all genes, allowing for a great range of analyses and should, after extension and confirmation of promising results in phase 2, yield discovery of genes and pathways currently not implicated in ASDs. As such it will provide a significant public resource for autism genetics, diagnostics and potential treatment.

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Common and rare variants in the PAX7 and VAX1 genes contribute to Orofacial cleft etiology. *A. Butali[1], M.A Mansilla[1], S. Suzuki[1], T.H Beaty[2], M.L Marazita[3], J.C Murray[1].* 1) Department of Pediatrics, University of Iowa 500 Newton Road, Iowa. IA 52242; 2) Johns Hopkins University, School of Public Health, Baltimore, Maryland, USA; 3) Center for Craniofacial and Dental Genetics, Department of Oral Biology, School of Dental Medicine, University of Pittsburgh, Pittsburgh. PA 15260.

Background: Orofacial clefts (OFC) are the most frequent congenital malformations of the head and neck, with birth prevalence approximately 1 in 700. They are commonly divided into cleft lip with or without cleft palate (CL(P)) and cleft palate (CP) groups based on anatomical, genetic and embryological findings. Advances in the knowledge of OFC etiology have shown strong evidence of association with markers in candidate genes including Interferon regulatory factor six (IRF6), Forkhead boxE1 (FOXE1) and Muscle segment homeobox (MSX1). Recently, genome wide association studies (GWAS) of CL(P) have identified genetic associations for non-syndromic CL(P) on chromosome 8q24.21, and in or near the IRF6, ABCA4, and MAFB genes. Signals approaching GWAS significance were also observed in or near the PAX7, VAX1 and NTN1 genes. The present study investigated the presence of common and rare variants in PAX7 and VAX1 genes as a follow up to the GWAS signals (Beaty et al., 2010). Methods: Direct sequencing was used to search for sequence variations in coding regions and conserved non-coding regions in and around the PAX7 and VAX1 genes in 360 individuals (90 Caucasian CL(P) cases and 90 controls, 90 Asian CL(P) cases and 90 controls). The observed variants were compared in cases and controls using the Fishers chi square test. Results: We found a total number of 16 new variants (three missense, two synonymous and eleven non coding variants). Of these, two missense mutations in the PAX7 gene (G411R in Caucasians and G412S in Asians),were observed in 1% 0f the cases and none in the controls. G411R was predicted as possibly damaging of the protein structure by PolyPhen and G412S as probably damaging. Also in the PAX7 gene, a missense mutation P397L predicted as benign by PolyPhen was observed only in 1% of Caucasian controls and none in cases. Common variants in the PAX7 gene showed significant association with CL(P)(rs4920523, p= 0.006 and rs 1416464, p= 0.001 using the Fisher's chi square test) , and new variant in the VAX1 gene were also identified which may contribute to the etiology of CL(P)(Chr10:118,880,130, p= <0.0001 using the Fisher's chi square test). Conclusions: Sequence analysis of chromosomal regions showing GWAS signals can identify both rare and common variants influencing a disease of interest. Grant: The project was supported by the NIH P50 grant DE-016215.

1190/F

Multiple gene and pathway analysis for rare variants in genome-wide associations. *H. Xu, V. George.* Dept Biostatistics, Med Col Georgia, Augusta, GA.

Much efforts have been devoted to genome-wide association studies (GWAS) of complex diseases. However, the genetic basis of most common complex diseases remain unknown. Multiple lines of evidence show that multiple rare alleles at several genes or genes in a pathway may contribute to common complex diseases. Current approach for GWAS has low power in detecting these rare alleles. We propose to an approach to detect multiple rare alleles by combining the alleles in multiple genes or a pathway. The combining strategy effectively increases the frequencies of the "pooled" alleles and hence improves the statistical power. Simulation studies show that it has higher power than the current approach of GWAS for detecting rare allele from multiples genes. This approach is useful in further analysis of the genome-wide data for association of rare alleles from genes in related pathways.

1191/F

Comprehensive Approach to Analyzing Rare Genetic Variants. *T. Hoffmann[1], N. Marini[2], J. Witte[1].* 1) Department of Epidemiology and Biostatistics and Institute of Human Genetics, University of California San Francisco, San Francisco, CA; 2) Department of Molecular and Cellular Biology, California Institute for Quantitative Biosciences, University of California Berkeley, Berkeley, CA.

Recent findings suggest that rare variants play an important role in both monogenic and common diseases. Due to their rarity, however, it remains unclear how to appropriately analyze the association between such variants and disease. A common approach entails combining rare variants together based on a priori information and analyzing them as a single group. Here one must make some strong assumptions about what to combine. Instead, we propose two approaches to empirically determine the most efficient grouping of rare variants. The first considers multiple different possible groupings using existing information. The second is an agnostic "step-up" approach that determines an optimal grouping of rare variants analytically and does not rely on prior information. To evaluate these approaches, we undertook a simulation study using sequence data from genes in the one-carbon folate metabolic pathway. Our results show that using prior information to group rare variants is advantageous only when information is quite accurate, but the agnostic step-up approach works well across a broad range of plausible scenarios. This agnostic approach allows one to efficiently analyze the association between rare variants and disease while avoiding strong assumptions required by other approaches for grouping such variants.

1192/F

Network-based burden test for the analysis of complex phenotypes by exome sequencing. *G.V. Kryukov, D.P. Nusinow, A. Kiezun, S.R. Sunyaev.* Division of Genetics, Brigham & Women's Hospital, Harvard Medical School, Boston, MA.

Identification of the genetic causes of disease through complete resequencing of individual genomes has become a reality. Until now this approach has been successfully applied to detect genes underlying phenotypes with simple Mendelian mode of inheritance. We have previously estimated that identification of genes involved in complex polygenic traits may require large sample sizes. However, combining multiple genes in a single association test can dramatically increase statistical power of the approach. We developed a network-based statistical method that searches for clusters of functionally related genes with a significant difference in rare variant counts between cases and controls. Given that the relevant pathways might not be known a priori, our method is not using any predefined gene sets, but rather searches for sub-networks in the STRING database. The STRING database incorporates heterogeneous information on gene-gene associations. As the internal control we perform an identical type of analysis also for synonymous and intronic variants, that are predominantly neutral and should not show differential burden of rare variants between cases and controls. We applied our method for the analysis of complete exome resequencing data for populations with cardiovascular and psychiatric phenotypes.

1193/F

Amplicon Ligation and Target Resequencing of Pooled DNA Samples for Effective Detection of Rare Variants. *T.S. Niranjan[1], A. Adamczyk[1], H.C. Bravo[4,5], S. Wheelan[3,4], R. Irizarri[4,5], T. Wang[1,2].* 1) The McKusick-Nathans Institute of Genetic Medicine, The Johns Hopkins University School of Medicine, Baltimore, MD; 2) The Department of Pediatrics, The Johns Hopkins University School of Medicine, Baltimore, MD; 3) The Department of Oncology Biostatistics and Bioinformatics, The Johns Hopkins University School of Medicine, Baltimore, MD; 4) The Center for Computational Genomics, The Johns Hopkins University School of Public Health, Baltimore, MD; 5) The Department of Biostatistics, The Johns Hopkins University School of Public Health, Baltimore, MD.

Rare deleterious variants of large effect in multiple genes may confer a substantial genetic risk to common diseases and complex traits. Next-generation sequencing of pooled DNA samples is an efficient way to identify rare variants in large sample sets within targeted genomic regions of interest at a relatively lower cost. We report an optimized sample pooling strategy and base-calling algorithms for reliable detection of rare sequence variants in pooled DNA samples. To sequence 120 exon-containing regions in 480 samples, we have tested different sample pooling strategies and conducted limited PCR amplification for template enrichment of individual exons. Twelve pools of PCR products, each consisting of 120 amplicons normalized at equal molar concentrations were ligated individually using a Quick Blunting Quick Ligation Kit (NEB) and fragmented by nebulization. Twelve indexed libraries were generated using multiplex primer sets and a Genomic DNA Library Preparation Kit for Solexa sequencing (Illumina), pooled at an equal molar ratio, and sequenced using one lane of a flow cell on a Genome Analyzer II to achieve an average depth of coverage at greater than 40-50 for individual bases. To reduce false positive calls contributed by errors accumulated from later sequencing cycles, we utilized *Srfim*, a model-based quality assessment and base-calling algorithm that captures base-cycle and other sequencing effects to create sequence read-specific estimates on the probability for each nucleotide position along individual reads (http://www.rafalab.org). Utilizing *Srfim* in a pilot study, we increased the total mapped reads by 5% reflecting more accurate base-calling and improved quality metrics, and reduced false positive calls by 30%. We confirmed all but one of all coding variants (n=34) identified by Illumina sequencing of 24 exons of a candidate gene using the Sanger method. Using optimized sample-pooling, library-indexing, and base-calling algorithms, we anticipate a reliable detection of rare variants with an allele frequency at 0.1% or higher in large sample sets.

1194/F

Rare variants in two functionally-related genes (*TREX1* and *PRF1*) and susceptibility to autoimmune diseases. *N. Barizzone[1,2], S. Monti[1], S. Mellone[1], G. Cappellano[1,2], E. Orilieri[1,2], A. Chiocchetti[1,2], M. Marchini[3], R. Scorza[3], M.G. Danieli[4], Y. Carlomagno[1], M.G. Sabbadini[5], G.D. Sebastiani[6], S. Migliaresi[7], M. Galeazzi[8], U. Dianzani[1,2], P. Momigliano-Richiardi[1,2,9], S. D'Alfonso[1,2].* 1) Department of Medical Sciences, University of Eastern Piedmont, Novara, NO, Italy; 2) IRCAD (Interdisciplinary Research Center of Autoimmune Diseases), Novara, NO, Italy; 3) Dipartimento Medicina Interna, IRCCS Ospedale Maggiore di Milano, Milano, MI, Italy; 4) Istituto di Clinica Medica Generale, Ematologia ed Immunologia Clinica, Ancona, AN, Italy; 5) Universita'Vita-Salute San Raffaele, Milano, MI, Italy; 6) Unita'Operativa Complessa di Reumatologia, Azienda Ospedaliera San Camillo Forlanini, Roma, Italy; 7) Unita'Operativa di Reumatologia, II Universita'di Napoli, Napoli, NA, Italy; 8) Struttura Semplice di Diagnosi e Cura delle Malattie Autoimmuni Sistemiche, Divisione di Reumatologia, Universita' Siena, Siena, SI, Italy; 9) Member of American Society of Immunogenetics (ASHG).

We performed a mutational analysis by direct sequencing on the whole coding region of two candidate genes (*PRF1* and *TREX1*), belonging to a common pathway, on three related autoimmune diseases: SLE (Systemic Lupus Erythematosus), SSc (Scleroderma) and SS (Sjögren's Syndrome). Secretory vesicles of CTLs and NK cells contain perforin (PRF1) and granzymes. PRF1 polymerizes on target-cells membrane, forming pores which allow the entry of granzymes, which in turn start the apoptotic response. In particular granzyme A triggers the translocation to the nucleus of the protein complex containing TREX1. Here DNA is digested into small fragments, which become substrates of the exonuclease TREX1 (DNase III). Mutations of *PRF1* gene have been associated with Familial Haemophagocitic Lymphohistiocytosis (FHL2), a rare autosomic recessive disease. Heterozygous *PRF1* variants have been associated with autoimmune diseases. We searched for *PRF1* variants in 2 new diseases: 154 SLE, 131 SSc and 559 controls. We identified 22 variants; 11 were non-synonymous and 4 of them have been observed in patients only. In silico analysis predicts for one of them (H514R) a possible damaging effect. The cumulative frequency of all non-synonymous variants is significantly higher in patients than in controls for both diseases (SLE: p=0.043 OR=1.63; SSc: p=0.0082 OR=1.89). The 2 most frequent non-synonymous variants (A91V and N252S) were further analysed in a larger sample set: (597 SLE, 204 SSc, 1856 controls) and they resulted significantly associated both with SLE (N252S p=0.039 OR= 2.35) and with SSc (A91V p=0.0054 OR=1.70). Heterozygous mutations in *TREX1* had been observed in 10/477 patients with SLE and in 1/169 subjects with SS. Those variations had not been found in 1712 controls (Lee-Kirsh). Furthermore *TREX1* mutations can cause three rare mendelian diseases with autoimmune features: (AGS, FLC, RVCL). We searched for *TREX1* mutations in 210 SLE, 58 SS and 150 cases of SSc. We observed 7 variants; two of them are non synonymous (E198K and M232V) and they are predicted as possibly damaging for protein function. They have been observed in one case of SS and in one case of SSc respectively and in none of the 200 controls typed in our lab or of the 1712 controls sequenced by Lee-Kirsh. E198K has recently been described in one AGS patient, while M232V is novel. In conclusion our work seems to confirm a role for rare variants in susceptibility to common autoimmune diseases.

1195/F

Approaches to Incorporating Uncertainty in Rare Variant Association Studies. *E. Zeggini, J.L. Asimit.* Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, United Kingdom.

In rare variant association studies there is a loss of power due to genotype misspecification. Quality scores are available for genotype and sequence-derived data, but they are not usually put to use in rare variant analyses. Imputation of rare variants is now possible due to the 1000 Genomes reference set, which contains variants with MAF as low as .01. We propose methods for rare variant analyses that incorporate quality scores derived from sequencing and probability distributions resulting from imputation. We consider extensions of two locus-based approaches: (i) collapsing the genotypes across rare variants and testing for an association with accumulations of rare variants; (ii) an allele-matching test based on combining single variant test statistics. The allele-matching test is nonparametric and does not require knowledge of the risk alleles. The SNP-level quality scores are incorporated in extensions of both methods, while the latter method includes genotype-level quality scores, as well. Use of imputation probabilities in the allele-matching test is also explored. In a comparison of the original and quality-weighted collapsing methods, the extension has a consistent, though small, gain in power. The table below provides simulation results for 10000 replications of 5000 individuals generated such that the maximum MAF of a causal SNP is .01, the maximum total MAF of the causal SNPs is .05, and the proportion of phenotypic variation due to rare variants (λ) varies.

Meth-odlambda	0.2%	0.4%	0.6%	0.8%	1.0%
Original	.14	.23	.32	.40	.48
Quality-weighted	.15	.25	.36	.43	.52

In conclusion, methods accounting for inaccuracies arising from sequencing and imputation processes increase power to detect association. We are extending our methods further to achieve greater power.

1196/F

Coding alterations in two adjacent genes at the PSORS2 locus co-segregate with psoriasis in a large human pedigree. *C.T. Jordan, E.D.O. Roberson, L. Cao, C. Helms, S. Duan, A.M Bowcock.* Department of Genetics, Washington University, Saint Louis, MO.

Psoriasis (PS) is a chronic, inflammatory skin disease that affects 2-3% of the European population. Psoriatic arthritis (PsA) is an inflammatory arthritis that occurs in up to 30% of affected individuals. We previously identified a susceptibility locus for PS and PsA at chromosome 17q25 (PSORS2) in a large family segregating a highly penetrant mutation (LOD = 5.84). This region spans ~ 3.5Mb and contains 77 genes. We implemented two sequence capture strategies to search for the causative mutation. First, we used whole-exome capture to obtain coding sequence from 4 affected individuals. Second, we performed genomic capture of the entire 17q25 region using DNA from pools of 8 unaffected and 14 affected individuals. All captured DNAs were sequenced using Illumina Solexa paired-end DNA sequencing technology. Whole-exome capture resulted in 30-fold enrichment of exons in the linkage region, and genomic capture enriched the region by greater than 200-fold. Both approaches identified two unique mutations altering the coding sequences of adjacent genes. Follow-up Sanger DNA sequencing confirmed that these mutations segregate with disease in the large pedigree. One mutation abolishes a splice site in a gene encoding a protein involved in regulation of apoptosis and the NF-κB pathway. The other mutation changes a phylogenetically conserved tyrosine to cysteine in a gene expressed in chondrocytes and high endothelial venule epithelial cells (HEVEC). As PS and PsA are complex diseases, either one or both mutations may contribute to disease pathogenesis. We are resequencing both genes in a larger cohort of unrelated patients and controls and will present an analysis of the frequency of these alterations, their potential functions and their significance in the general disease populations of PS and PsA.

1197/F

Targeted-genomic capture and high-throughput sequencing for genetic testing and new gene discovery in atypical hemolytic uremic syndrome. *T. Maga[1, 2], A. Deluca[3, 4], K. Taylor[3, 4], S. Scherer[5], T. Scheetz[3,4], R.J.H. Smith[1,2,6].* 1) Department of Otolaryngology-Head and Neck Surgery, Carver College of Medicine, University of Iowa, Iowa City , IA; 2) Interdepartmental PhD program in Genetics, University of Iowa, Iowa City, IA; 3) Electrical and Computer Engineering, University of Iowa, Iowa City, IA; 4) Center for Bioinformatics and Computational Biology, University of Iowa, Iowa City, IA; 5) Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX; 6) Department of Internal Medicine, Carver College of Medicine, University of Iowa, Iowa City, IA.

Atypical hemolytic uremic syndrome (aHUS) is characterized by acute renal failure, thrombocytopenia and microangiopathic hemolytic anemia. aHUS is far less common and more severe than typical HUS, which is caused by E. coli infection and manifests as diarrheal illness. The pathogenesis of aHUS is linked to dysregulation of the alternative pathway of the complement cascade. Mutations in the complement regulators factor H (*CFH*), membrane cofactor protein (*MCP*), factor B (*CFB*), factor I (*CFI*), complement component 3 (*C3*) and thrombomodulin (*THBD*) have been implicated in the disease. These loss- or gain-of-function mutations lead to uncontrolled complement activity and immune-mediated host cell damage. Twelve percent of patients who have a mutation in one of these genes have a mutation in a second gene, however in 50 percent of aHUS patients, no mutations are found, suggesting that other genetic causes remain to be discovered. Establishing a genetic etiology is important as it helps to direct treatment during the acute phase of disease and when transplantation is considered. To develop an improved protocol for genetic testing and to discover mutations in novel genes that are causally related to aHUS, we have optimized a platform called CASCADE (Capture and Sequencing of Complement-Associated Disease Exons), which is based on targeted-genome capture and next-generation sequencing of all complement genes. We report here a proof-of-principle study demonstrating the power of CASCADE to detect known and novel mutations involved in the pathogenesis of aHUS in four patients. (Funded in part by the Foundation for Children with Atypical HUS, RJHS).

1198/F

Exon sequencing of α-Actinin-4 for variants associated with non-diabetic end stage renal disease in African Americans. *P.J. Hicks[1], M.A. Bostrom[1,5,6], L. Lu[2], M.C.Y. Ng[4,5,6], C.D. Langefeld[2], B.I. Freedman[3], D.W. Bowden[1,5,6].* 1) Biochemistry, Wake Forest University, Winston-Salem, NC; 2) Biostatistical Sciences, Wake Forest University, Winston-Salem, NC; 3) Internal Medicine/Nephrology, Wake Forest University, Winston-Salem, NC; 4) Pediatrics, Wake Forest University, Winston-Salem, NC; 5) Center for Human Genomics, Wake Forest University, Winston-Salem, NC; 6) Center for Diabetes Research, Wake Forest University, Winston-Salem, NC.

Non-diabetic (non-DM) end stage renal disease (ESRD) disproportionately affects African Americans. Few studies have been done to identify genes responsible for this increased susceptibility. *ACTN4* (α-actinin-4) is an actin binding protein involved in maintaining the cell cytoskeleton. This gene is expressed in podocytes and rare mutations have been identified in familial focal segmental glomerulosclerosis. We sequenced 19 exons and 2800 bases of the promoter of *ACTN4* in 96 non-DM ESRD cases and 96 non-nephropathy controls. Sixty-seven SNPs were identified, including 33 intronic, 21 promoter, 12 exonic (1 non-synonymous), and 1 SNP in the 3' untranslated region. Forty-nine SNPs were novel. Sixty-one SNPs were genotyped in 278 African American non-DM ESRD cases and 327 non-nephropathy controls. A single SNP, rs10404257, located in the promoter region was associated with non-DM ESRD (p = 0.004, odds ratio (OR) = 0.60, confidence interval (CI) = 0.43 - 0.85; dominant model). Forty SNPs had minor allele frequencies less than 5%. These SNPs were collapsed into a single marker, designated by the presence (n=251) or absence (n= 404) of any rare allele. There was no association with having a rare allele at any of these SNPs with non-diabetic ESRD. SNPs were also tested for interaction with *MYH9*, a gene that has been shown to have a significant impact on risk for non-DM ESRD. No interaction with MYH9 was detected, either with single SNPs or with collapsed rare alleles. Twenty-one of the most significantly associated SNPs, including rs10404257, were genotyped in an independent set of 525 non-DM ESRD cases and 267 controls. Rs10404257 was not associated (p = 0.768, OR = 1.05, CI = 0.75 - 1.48; dominant model) however, when the samples were combined rs10404257 was still modestly associated (p = 0.033, OR = 0.78, CI = 0.62 - 0.98; dominant model). We completed a comprehensive analysis of the coding regions *ACTN4* gene and detected limited evidence of association with non-diabetic ESRD in African Americans. This negative finding suggests that the function of *ACTN4* is critical and common to moderately uncommon variants that alter this function may have a catastrophic impact on cellular processes.

1199/F

Resequencing of 10 susceptibility loci in 56 individuals using Next Generation Sequencing based on Long Range PCR enrichment. *S. May[1], M. Schilhabel[1], I. Thomsen[1], M. Forster[1], P. Rosenstiel[1], S. Schreiber[1,2], A. Franke[1].* 1) Institute for Clinical Molecular Biology, CAU Kiel, Kiel, Schleswig- Holstein, Germany; 2) 1st Medical Department, University Clinic Schleswig-Holstein (Campus Kiel), Germany.

One of the key applications of Next Generation Sequencing is to study the genetic variation between healthy individuals and patients using whole - genome or targeted resequencing. The perhaps best-established approach for high throughput population-based sequencing of targeted intervals in the human genome is to amplify the regions of interest using long-range PCR followed by Next Generation sequencing. In our current project we resequenced with the SOLiD platform 10 validated disease susceptibility loci in 56 individuals (30 cases, 20 controls and 6 HapMap control samples). In order to enrich the samples for genetic diversity at the locus of interest, haplotype analyses were carried out using dense genotyping data from available genome-wide association scans. Cases and healthy controls carrying the associated risk haplotypes were preferentially selected and individuals with the remaining haplotypes were used for filling the pools. This means that patient sets that are resequenced vary between targets so we obtained full sequence variation - i.e. in coding and non-coding genomic sequence - and individual haplotypes for these 10 disease-associated genes. For these ten loci, more than 5500 long-range PCR amplicons have been successfully generated. This large number requires a high degree of automation and logistics. On the conference, we will show first results of our sequencing data and we will give a demonstration of the employed workflow.

1200/F

Exploring alternative splicing profiling in autism, a proof of concept study. *Z. Talebizadeh[1], R. Aldenderfer[1], B. Han[2], X. Chen[2].* 1) Pediatrics, Children's Mercy Hosp and University of Missouri-Kansas City School of Medicine, Kansas City, MO; 2) 2Electrical Engineering and Computer Science Department, University of Kansas, Lawrence, KS.

Autism is a complex, heterogeneous neurobehavioral disorder with many causes and varying degrees of severity. To date, the main technique used to screen autism candidate genes has been direct sequencing of exons using genomic DNA. It is surprising that for strong candidate genes such as NLGN3 and NLGN4, few functional mutations have been identified in association with autism. This suggests that implications may be at a higher level than the genomic sequence, including alternative splicing. Previously, we reported novel alternative splicing variants in the NLGN3 and NLGN4 genes using RT-PCR and DNA sequencing. The dysregulation of alternative splicing has been associated with some cancers and neurological diseases such as Parkinson's and Alzheimer's. Despite the growing evidence for the regulatory influence of alternative splicing on gene expression, particularly in brain function, this gene regulatory process has not been previously evaluated in autism spectrum disorders. In order to assess the potential role of alternative splicing, we performed a pilot study to examine global exon expression between autistic subjects (n=5) compared with unrelated controls (n=5) using Affymetrix GeneChip Human Exon 1.0 ST arrays on lymphoblastoid cell line (LCL) derived RNA. Data analyses was limited to the 'core' probe set comprised of 17,800 transcripts. In doing so, 10,072 genes (57%) met the applied filtration criteria and were identified as being "present" above background levels. Within this reliably expressed gene set, ANOVA and multi-test correction were used to identify differential exon expression in autism samples. Applying Bonferroni multiple test correction to the 10,072 genes identified 57 genes that appear to have differential expression between autism and control samples. This list includes genes associated with neurological diseases such as Alzheimer's (SLC30A7). Several genes identified have been linked to nervous system development or autism including CYFIP1, ROBO1, and CDKL5 (ROBO1 and CDKL5 in females only). Our results indicate that changes in mRNA splicing patterns should be considered in the etiology of autism. This pilot study demonstrates that even though LCLs are not neuronal cells, they are a viable cellular model for human neurological diseases and can facilitate understanding the role alternative splicing plays in complex neurodevelopmental disorders such as autism.

1201/F

Multivariate analysis of clinical data for age related hearing impairment diagnosis. *C.H. White, L. Fisher, R. Friedman, J. Ohmen.* Cell Biology and Genetics, House Ear Institute, Los Angeles, CA.

Presbycusis, or Age Related Hearing Impairment (ARHI) results from the complex interaction of genetic susceptibility to aging in the inner ear and unknown environmental components. The identification of the genes underlying this disease requires an accurate phenotypic assessment. The primary tool for doctors to assess presbycusis is the audiogram for pure tone thresholds. Classically, a diagnosis of ARHI is suggested by an audiogram that reflects the loss of ability to hear high frequency tones. Recently, in an effort to convert the qualitative diagnosis of ARHI, into a quantitative measure of disease, geneticists have attempted to apply mathematical tools to audiometric data to derive quantitative phenotypes. Due to the large number of data points present in individual audiograms which results in audiograms with a variety of shapes, one approach to data reduction involves the application of Principle Component Analysis (PCA). We have applied this and other multivariate statistics to our current hearing data, as well as collected additional samples, to derive better a quantitative definition of ARHI.

1202/F

Population-based survival analysis of children born with orofacial clefts in Texas. *T.G. Barbaro Dieber[1], S.S. Hashmi[1], J.L. Salemi[2], M. Canfield[3], W. Nembhard[2], J.T. Hecht[1].* 1) University of Texas Medical School at Houston, Houston, TX; 2) College of Public Health, University of South Florida, Tampa, FL; 3) Birth Defects Epidemiology and Surveillance Branch, Texas Department of State Health Services, Austin, TX.

Orofacial clefts are the most common craniofacial birth defects in humans and are associated with high morbidity. An increased rate of infant mortality has been reported among children with all types of congenital anomalies. Increased mortality in children with isolated orofacial clefts has been inconsistently reported. Gender, ethnicity and socioeconomic status are known risk factors for infant mortality but have not been systematically evaluated among children with orofacial clefts. Therefore, this study assessed infant mortality in children with all types of orofacial clefts born in Texas between 1996-2003 accounting for these risk factors. All cases were identified through the Texas Birth Defects Registry (TBDR) and were cross-referenced with Texas Vital Statistics through 2005 to ascertain survival and underlying cause of death (COD). Standardized mortality ratios (SMR) were calculated for all cases and separately for isolated and for syndromic/multiple congenital anomalies (MCA) cleft cases. Cox regression analysis was performed to compute hazard ratios (HR) for both isolated and MCA cases of orofacial clefts. A total of 3,713 cases of orofacial clefts were identified, of which 2,543 (68.5%) were isolated cases and 1,170 (31.5%) had MCAs. There were a total of 483 deaths with 343 (71%) in patients with MCA. Overall 10 year survival for all, isolated and MCA cases was 87%, 94% and 71%, with the majority of deaths occurring in the first year of life. The SMRs for all, isolated and MCA cases of clefts were 18.24, 7.62 and 41.34, respectively. There was a two-fold increased risk of death in African-Americans compared to nonHispanic whites (HR=2.096, p=0.007). Higher but nonsignificant risk of death was found for females and Hispanics for both isolated and MCA cases. Low birth weight and gestational age were risk factors for higher mortality among all cases. "Congenital malformations, deformations, and chromosomal abnormalities" were the most common COD (79.0% (n=347) for all cases and 72.7% (n=93) for isolated). In conclusion, this population-based study of orofacial clefts shows that there is a seven-fold increase in death in children with isolated CLP. This information should be used in the treatment and management of these children.

1203/F

The polygenic nature of glaucoma: Dissecting sub-phenotypes to facilitate the characterization of modifier genes. *P. Belleau[1], K. Lebel[1], S. Dubois[1], R. Arseneault[1], J.L. Anctil[2], E. Shink[1], G. Cote[2], M. Amyot[3], V. Raymond[1], The Quebec Glaucoma Network.* 1) Biology of Sensory Systems, Ocular Genetics & Genomics, Laval University Hospital (CHUL) Res Ctr, Québec City, PQ, Canada; 2) Ophthalmology, Laval Univ, Québec City, PQ, Canada; 3) Ophthalmology, Univ of Montréal, Montréal, PQ, Canada.

Open-angle glaucoma (OAG), a major cause of blindness worldwide, is characterized by optic nerve degeneration and visual field impairments. Potential mechanisms leading to OAG involve elevation of intraocular pressures, optic nerve hypersensitivity to stressful events and inflammation. Although OAG is primarily considered a complex genetic disease, families were essential in characterizing 4 disease-genes. We studied the CA family, a huge French-Canadian pedigree showing very wide variability of its autosomal dominant glaucoma phenotype. The myocilin (MYOC) K423E mutation caused OAG in the family. Modifier genes can account for wide variability of ages at onset (AAO). To facilitate the identification of these modifier genes for AAO, we dissected the disorder according to four additional subphenotypes. The records of 155 $MYOC^{K423E}$ heterozygotes were reviewed to extract the values of their respective quantitative variables: 1. maximal intraocular pressures (IOP) in mm Hg, 2. maximal IOP after beginning of treatment, 3. optic nerve degeneration as cup-to-disk (C/D) ratio and, 4. progression of optic nerve degeneration (ΔC/D) as the variation of the C/D ratio divided per the time period between 2 C/D ratios. Ages at onset ranged from 7 to 63 years old with 16 % of the men showing onset < 20 years old as compared to 11 % of the women. IOP sub-phenotypes were classified in 3 categories with 94 heterozygotes classified in the high severity group with maximal IOPs $\geq$ 27, 12 carriers in the moderate group with IOPs between 22 and 27 while 49 heterozygotes were in the low severity group with IOP below 22 mm Hg. Of the 104 heterozygotes who were treated, 84 were categorized in the high severity group with maximal IOP $\geq$ 22 mm Hg following the beginning of the treatment whereas 71 were in the low severity category below this value. When C/D ratios were measured in 142 heterozygotes, 56 were classified as severe with a ratio $\geq$ 0.8, 12 moderate with C/D between 0.7 and 0.8, and 74 in the low category with C/D < 0.7. When ΔC/D was tested in the 142 carriers, 3 groups could be defined with 88 carriers in the low severity group below 0.02, i.e. a variation < 0.2 C/D in 10 years, 13 as moderate: from 0.02 to 0.03 and, 41 as high with a ΔC/D $\geq$ 0.03. In conclusion, we established a novel classification system for severity of glaucoma that should be useful to characterize modifier genes for the disorder. This system can be used to analyze dependency between subphenotypes.

1204/F

Limits in processing speed as a possible endophenotype in dyslexia. *B. Peter[1, 2], M. Matsushita[2], W. Raskind[2, 3].* 1) Speech & Hearing Sci, Univ Washington, Seattle, WA; 2) Medicine; 3) Psychiatry and Behavioral Sciences, University of WA.

This study investigated the hypothesis that limits in processing speed constitute an endophenotype in dyslexia, which, if confirmed, would provide the motivation to identify its genetic origin. Dyslexia is a disability interfering with the acquisition of written language at the word level, characterized by deficits in accurate and/or fluent word recognition, decoding, and spelling. There is strong evidence for a genetic basis. Dyslexia is associated with left-hemisphere white matter abnormalities. Of note, in typical adults, white matter volume and motor speeds decrease with age. Children with dyslexia show slowed speed in various tasks, compared to typical peers, but it is unknown whether slowed processing is a latent dimension in dyslexia. Exploratory factor analysis (FA) was conducted in a sample of 289 multigenerational families, each ascertained through a child with dyslexia. FA was conducted separately in four cohorts (all children, n = 390; children with low reading scores, n = 105; children with typical reading scores, n = 198; and adults with typical reading scores, n = 444) and confirmed in male and female subcohorts. In a first FA model, nine input variables represented verbal and nonverbal processes and alphabet writing; four of these were timed. In a second FA model, two timed motor sequencing tasks involving oral and finger movements were added. Processing speed formed the first factor in all cohorts and models. Leading variables in the first FA model were three timed measures with verbal output, rapid automatic naming with and without category switches and inhibition switch times from a naming task with alternating conditions, followed by a timed measure with motor output, alphabet writing speed. In the extended model, timed oral and hand motor sequencing loaded on the speed factor along with alphabet writing speed, ranking below the speed measures with verbal output. Children with poor reading scores showed lower speed factor scores than typical peers. The speed factor was negatively correlated with age in the adults. Timed measures with verbal output were more closely associated with processing speed than those with motor output. Results are consistent with a unified theory of processing speed as a quadratic function of age in typical development and with slowed processing in poor readers. These findings motivate an investigation of slowed processing speed as an endophenotype in dyslexia.

1205/F

Further investigation of the SNP rs17563 in the *BMP4* gene which was previously associated with in cleft lip and/or palate. *T.K. Araujo, M. Simioni, C.V. Maurer-Morelli, R. Secolin, I. Lopes-Cendes, V.L. Gil-da-Silva-Lopes.* Department of Medical Genetics, Faculty of Medical Sciences, University of Campinas - UNICAMP, Campinas, SP, BRAZIL.

The pathogenesis of cleft lip and (or) palate (CL/P) involves genetic and environmental factors. The *BMP4* gene has distinct roles in embryonic development, including facial development. Overexpression of *BMP4* has been found in the maxillary prominence. The presence of a CL/P in *BMP4* knockout or null mutant mice indicate the importance of *BMP4* for embryonic facial fusion. The nonsynonymous polymorphism rs17563 (538T®C) of *BMP4* gene changes a valine to an alanine residue at codon 152. This polymorphism was significantly associated to the risk of nonsyndromic CL/P in Chinese population and was also described in patients with microforms of CL/P in a study involving different populations. The aim of our study was to investigate further the evidence for association between the rs17563 SNP and CL/P in a different population than previously reported. To date, we have included in our study 15 CL/P (8 non-syndromic and 7 syndromic) and 100 normal control individuals (50 M: 50F). Patients and controls were ascertained in our University Hospital which is located in the southeastern part of Brazil in Sao Paulo state. Selection criteria for the control group were: negative family history for CL/P in three successive generations, no evidence of any other serious illness and no oriental ancestry. Genotyping was performed using PCR-digestion. The frequencies of the TT, TC, and CC genotypes 538T®C were 40%, 46,7%, and 13,3% in the CL/P group, and 20, 8%, 53,1%, and 26,1% in the controls, respectively. The genotype distribution of the polymorphism is in Hardy-Weinberg equilibrium in both groups. In addition, our preliminary results showed no significant difference in the genotypes and allele frequencies of the *BMP4* 538T®C polymorphism between CL/P and control groups (p=0,119, OR=0,523, 95%CI). Financial Support: FAPESP, CNPQ.

1206/F

Pleiotropic genetic effects contribute to the correlation between bone mineral density and quantitative ultrasound measurements. *S.C. Nguyen[1], N.D. Nguyen[1], J.R. Center[1,3], J.A. Eisman[1,2,3], T.V. Nguyen[1,2].* 1) Osteoporosis and Bone Biology, Garvan Institute of Medical Research, Darlinghurst, NSW, Australia; 2) School of Public Health and Community Medicine, University of New South Wales, Kensington, NSW, Australia; 3) St Vincent's Hospital and St Vincent's Clinical School, Sydney, NSW, Australia.

The heritability of bone mineral density (BMD) has long been established using both twin and familial models. Similarly, various quantitative ultrasound (QUS) parameters of bone have been shown to be under genetic influence. BMD is correlated with QUS, but it is not known whether this is due to genetic or environmental influences. The present study was designed to examine whether BMD and QUS are affected by the same genetic factors. The study involved 622 individuals from 33 multigenerational families who were part of the Dubbo Osteoporosis Genetics Study, all aged 18+ at the time of participation. BMD at the femoral neck (FNBMD), lumbar spine (LSBMD) and total body (TBBMD) was measured by DXA (GE-Lunar Corp, Madison, WI, USA). Speed of sound (SOS) at the distal radius (DRSOS), midshaft of tibia (MTSOS) and proximal phalanges (PPSOS) were measured by Sunlight Omnisense (Sunlight Medical). The indices of heritability (h^2) of BMD and QUS measurements were estimated using the variance components model as implemented in the SOLAR program, taking into account the effects of age, sex, and anthropometric variables. Moreover, the genetic and environmental correlations were estimated by bivariate genetic analysis. The genetic correlation represents a measure of pleiotropic effects of genes influencing both traits concomitantly. Between 42% and 59% of the variation in BMD at different skeletal sites was attributable to genetic factors (h^2 for FNBMD 0.42 (SE: 0.09), LSBMD: 0.59 (0.07), and TBBMD: 0.54 (0.14)). The effect of genetic factors on QUS was slightly lower than for BMD (DRSOS: 0.21 (0.09), MTSOS: 0.39 (0.12), and PPSOS: 0.23 (0.12)). More importantly, there was a strong genetic correlation between the three BMD measurements (FNBMD vs. LSBMD: 0.55 (0.10), FNBMD vs. TBBMD: 0.77 (0.06), and LSBMD vs. TBBMD: 0.81 (0.09)). Moreover, there was a genetic correlation between DRSOS and FNBMD (0.52 (0.22)) and LSBMD (0.40 (0.17)), but the correlation was not statistically significant for other QUS measurements (0.08 to 0.40; $P > 0.05$). Furthermore, a modest genetic correlation was observed between total fat mass and LSBMD: 0.38 (0.17). These results suggest that pleiotropy contributes to the additive genetic variation in BMD measurements, and to a lesser extent, between BMD and QUS measurements. The presence of genetic correlation provides the rationale for multivariate analyses to identify novel genetic loci with pleiotropic effects on these bone traits.

1207/F

Blood group antigens related to transfusions in Italian population. *A. Saluto, O. Camerini, R. Prestinice, F. Rossini, F. Petrazzi, D. Lajolo di Cossano, P. Ottone.* Laboratory of Immunohematology and Transfusion Medicine, Ospedale San Luigi Gonzaga, Orbassano (TO), Italy.

Blood group antigens, present on the cell membrane of Red Blood Cells (RBCs), may be proteins, glycoproteins or glycolipids. Most of them are accommodated in 30 blood group systems; the gene encoding each blood group system has been cloned and sequenced and the molecular bases associated with the vast majority of blood group antigens have been determined (Veldhuisen, 2009). RBCs carrying a particular antigen, if introduced into the circulation of an individual who lacks that antigen (through transfusion or pregnancy) can elicit an immune response. Pretransfusion compatibility tests include ABO grouping and Rh typing of the recipient and donor blood and the detection of clinically relevant RBCs antibodies in the recipient that could potentially cause a severe hemolytic transfusion reactions; the donor blood is not routinely tested for other antigens. To evaluate the frequency of alloimmunization to RBCs antigens in the north-west Italian population we performed a seven years retrospective study (2003-2009) of patients referred to our hospital. 232 new antibody specificities were found in 194 alloimmunized patients; multiple new antibody specificities (2-6) were seen in 38 patients (19.6%). The identified alloantibodies, as a percentage of all alloantibodies observed, were E (19.8%), K (18%), D (9.5%), Jk^a (6.5%), C^w (6%), Lu^a (5.6%), C (5.6%), S (3.9%), e (3.9%), c (3.9%), M (3.5%), Fy^a (3.4%), Kp^a (2.6%), Jk^b (2.2%), Le^a (2.1%), Le^b (1.7%), Fy^b (0.86%), s (0.43%) and P_1 (0.43%). Our results are similar to those previously reported for south-west Dutch population, except for C^w, Lu^a, Kp^a, Le^a and Le^b alloantibodies that were not found (Schonewille, 2006). These findings suggest that relative frequency of C^w, Lu^a, Kp^a, Le^a and Le^b antigens could be different between Italian and Dutch populations. We observed a high frequency of Jk^a alloantibody (6.5%) as in Dutch study (7.9%); this result suggest that, as in Dutch population, there is a significant increase in relative immunogenicity of Jk^a antigen compared to the original result of Giblett, probably reflecting the use of more sensitive antibody screening over the years (Gibblet, 1961). Although RBCs antigen frequencies among whites are known, our work suggest that the relative frequency for the most clinically relevant antigens should be assessed in each European country.

1208/F

Genome-wide linkage scan for QTL influencing physical activity levels of children from the Jirel ethnic group in Eastern Nepal. *K.D. Williams[1], J. Blangero[2], R. Nahhas[3], T.D. Dyer[2], J. Subedi[4], B. Jha[5], J.L. VandeBerg[2], S. Williams-Blangero[2], B. Towne[3].* 1) Temple University, Philadelphia, PA; 2) Southwest Foundation for Biomedical Research, San Antonio, TX; 3) Wright State University School of Medicine, Dayton, OH; 4) Miami University, Oxford, OH; 5) Tribuvan University Institute of Medicine, Kathmandu, Nepal.

Familial resemblance for physical activity has been established in several studies, but relatively few genome-wide scans have been conducted on quantitative measures of physical activity, and most of those have been carried out in adults from Western populations. Here we present the results of initial heritability and genome-wide linkage analyses of physical activity levels of healthy children from the Jirel ethnic group of Eastern Nepal who participate in the Jiri Growth Study. A total sample of 383 boys and girls aged 3 to 18 years, most belonging to one very large extended pedigree, each wore an Actical tri-axial accelerometer for one week that collected a data point every 15 seconds during that time period. Data were aggregated into 1 minute epochs and examined over 12-hour daylight periods (6 am to 6 pm). Activity count data in each of four categories (sedentary, light, moderate, and vigorous) were analyzed using a variance components-based method for pedigree data. All measures of physical activity were found to be significantly heritable: (sedentary $h^2 = 0.68 \pm 0.15$, $p < 0.0001$; light $h^2 = 0.85 \pm 0.13$, $p < 0.0001$; moderate $h^2 = 0.53 \pm 0.15$, $p < 0.001$; vigorous $h^2 = 0.33 \pm 0.17$, $p = 0.02$). Genome-wide linkage analyses were conducted in a subset of 220 children typed for ~400 STR markers at an average density of 10 cM. Sedentary activity count was significantly linked to chromosome 1q31-32 (LOD = 3.80). Modestly suggestive linkages at other chromosomal locations were observed for other activity level counts. Despite issues in some study environments regarding the sensitivity of accelerometers to capture variation in physical activity, our study demonstrates that the Actical accelerometer is a useful tool to measure and document variation in intensity of physical activity, especially in a free-living, largely agrarian population such as the Jirels. In sum, these preliminary results demonstrate a significant heritability of physical activity levels in Jirel children, and the feasibility of localizing and eventually identifying genes that influence everyday physical activity. This work was supported NIH grants F32HD053206, R01HD40377, R01AI37091, R01AI44406, and R37MH59490.

1209/F

Application of a genetic risk model of celiac disease to a prospective cohort from the United States. *J. Romanos[1], A. Szperl[1], C.C. van Diemen[1], G. Trynka[1], H.M. Boezen[2], A. Perlman[3], J.M. Norris[4], K. Barriga[3], G. Eisenbarth[3], M.J. Rewers[3], C. Wijmenga[1], E. Liu[3].* 1) Genetics Dept, UMCG, Groningen, Netherlands; 2) Epidemiology Dept, UMCG, Groningen, Netherlands; 3) Barbara Davis Center for Childhood Diabetes, University of Colorado Health Sciences Center, Denver, USA; 4) Epidemiology Dept, Colorado School of Public Health, Aurora, USA.

Celiac disease (CD) is a multi-organ chronic inflammatory disease affecting up to 1% of the general population. Besides the HLA-DQ, several non-HLA genes have been associated with CD in case-control studies, but not yet validated in population-based cohorts. Recently, we have proposed a genetic risk model which classifies individuals into groups of low-, intermediate-, and high-risk for CD, based on their HLA and non-HLA genotypes. Here, we applied this risk model to a large unique population-based cohort of children followed annually from birth to up to 16 yrs of age for development of transglutaminase autoantibodies (TGA) and CD. The phenotype of interest - CD-autoimmunity - was defined as persistent TGA positivity on 2 or more consecutive visits. We have genotyped 6 HLA and 10 non-HLA single nucleotide polymorphisms (SNPs) in 1124 non-Hispanic white independent children, selected from 31,690 newborns from Denver, Colorado, screened at birth for HLA-DR-DQ genotypes. CD-susceptibility HLA genotypes categorized these children into 3 risk groups: low (no-DQ2 or DQ8), intermediate (DQ8 and/or one dose of DQ2) and high (double dose of DQ2). Next, children with intermediate HLA-defined risk who had ≥13 non-HLA risk alleles were reclassified into the high risk group. Finally, CD-autoimmunity status was unmasked. Using non-HLA risk alleles, we were able to reclassify 3.5% CDA cases from the intermediate HLA-defined risk in the high risk group, increasing the number of cases in the high risk group by 39.5%. In Cox regression analysis, individuals with high-HLA risk and £12 non-HLA risk alleles (hazard ratio HR=2.70) and individuals with high-HLA risk and ≥13 non-HLA risk alleles (HR= 6.28) were at a significantly higher risk of CD-autoimmunity than those with intermediate-HLA risk and £12 non-HLA risk alleles. Moreover, persons with a high number of non-HLA loci appeared to develop CD-autoimmunity earlier in life. The risk model based on HLA- and non-HLA risk alleles holds promise of improved risk prediction for TG-based phenotype preceding clinical manifestation of CD.

1210/F

Specific epithelium sodium channel genotypes are strongly associated with fast decline in lung function among children with cystic fibrosis (CF). *M.J. Emond[1], T. Louie[1], J. Emerson[2], S. Ruuska[2], S. McNamara[2], K.T. Buckingham[2], K. Joubran[2], B.W. Ramsey[2], M. Rosenfeld[2], R.L. Gibson[2], M.J. Bamshad[2], EPIC Observational Study Group.* 1) Biostatistics, University of Washington, Seattle, WA; 2) Dept of Pediatrics.

Background: Patients with CF exhibit high variability in disease severity, even within families, suggesting genetic factors in addition to CFTR mutations contribute to decline in lung function. The epithelial sodium channel (ENaC) is a membrane-bound heterodimer that interacts with CFTR to regulate airway surface liquid volume and composition. Patients with double mutations in ENaC or single mutations in both ENaC and CFTR have been shown to have CF-like disease. Objectives: We sought to determine whether variations in ENaC among children with CF modify severity of CF disease. Methods: Thirty-five ENaC SNPs were genotyped in 617 CF children born between 1992 and 2002 who had lung function measurements from the nation-wide EPIC study. Genotypes were evaluated for association with CF-specific FEV1 percentiles via longitudinal analysis, with modeling for population structure, potential confounders and effect-modifiers such as CFTR mutation class and sex. Results: Homozygous minor genotypes at two unlinked SCNN1B SNPs were strongly associated with faster decline in lung function, regardless of adjustments and sensitivity analyses. Differences in annual change in CF-specific FEV1 percentiles (95% confidence intervals) were -0.019 (-0.013, -0.025) and -0.021 (-0.013, -0.026) compared to other genotypes ($p< 1e-4$ for both after Bonferroni correction); children with these genotypes were more likely to have a negative slope over the study period (OR = 3.0, $p < 0.01$). Sweat test values were significantly higher in subjects with these genotypes compared to those without (105.1 vs. 100.1, $p<0.05$). Conclusions: These results provide strong evidence that variation in SCNN1B is associated with CF lung disease severity, complementing the family-based study of Stanke, et al, 2006. Identifying genetic modifiers can guide early intervention strategies for patients in high risk groups. Supported by the Cystic Fibrosis Foundation.

1211/W

Complex chromosomal re-arrangement and imbalances in a family with retinoblastoma and multiple phenotypes. *S.P. Shankar[1,2,3], G.B. Hubbard[2,3], J.R. Wells[2,3], K. Coleman[1,3], E.C. Thorland[4], D. Rushlow[5], B.L. Gallie[5], P.M. Fernhoff[1,3], M.R. Rossi[1].* 1) Department of Human Genetics, Emory University, Atlanta, GA; 2) Department of Ophthalmology, Emory University, Atlanta GA; 3) Children's Healthcare of Atlanta Pediatric Hospital, Atlanta GA; 4) Department of Lab Med & Pathology, Mayo Clinic, Rochester, MN; 5) Retinoblastoma Solutions, Toronto Western Hospital, Toronto, Canada.

Retinoblastoma (RB) is the most common pediatric intraocular tumor and accounts for 3% of all childhood malignancies. Mutations in the gene *RB1* cause retinoblastoma in the majority of patients and, chromosomal deletions involving chr13q14 accounts for 5-7.5% of retinoblastoma. We report a family with retinoblastoma in proband and her mother having a different underlying etiology. The proband presented at birth with leukocoria and was found to have multifocal unilateral retinoblastoma associated with Hirschsprung disease, bilateral hearing loss, hypotonia, and abnormality of the brain showing unusual cortical folding pattern. Microarray analysis of the proband revealed interstitial deletion on chromosome 13(arr 13q14.11q31.1(40,615,956-84,609,688)X1 [hg18]), a region that includes the *RB1* gene and a number of other genes accounting for the multiple medical issues in her. The proband's mother had bilateral retinoblastoma diagnosed at age 6 months but has no other associated systemic abnormality. Mother's karyotype was reported as apparently balanced insertional translocation, ins(18;13)(18pter->q23::13q14.1::18q23->18qter;13pter->13q14.1::13q32.3->13qter). A genome wide microarray analysis in mother was normal. However, quantitative mutiplex-PCR and long-range PCR of the *RB1* gene showed 2 copies of all *RB1* exons with the exception of exon 12 suggesting disruption of one of the copies of *RB1* gene at exon 12 that resulted in her predisposition to the development of retinoblastoma. The proband has a 5 year old brother with microcephaly, developmental delay and ADHD but no retinoblastoma. His microarray analysis revealed duplication on chromosome 13 (arr 13q14.11q31.1(40,760,629-84,609,688)x3[hg18]), a region that includes *RB1* gene and corresponds to the region of deletion in the proband. Thus, this family demonstrates multiple phenotypes resulting from different molecular and cytogenetic abnormalities. It is not certain which copy of the *RB1* gene has non-amplification of exon 12 although, the most likely possibility is the *RB1* on the translocated copy. Any future children of mother have an overall 50% risk for developing retinoblastoma. The various possibilities of inheritance patterns have to be considered during risk counseling of such families and both molecular testing of *RB1* gene and karyotyping/microarray analysis should be offered to all at risk individuals in the family.

1212/W

Cytogenetics, Fluorescence in situ hybridization (FISH) and Oligo aCGH (OaCGH) techniques applied together in a case of acute myeloid leukemia help in further delineating a concomitant occurrence of the amplified MYC and MLL genes in a ring chromosome and the possible mechanism behind the ring formation. *R. Bajaj[1], A. Dulau[2], J. Gong[2], T. Holdbrook[2], M. Weiss[3], S. Peiper[4], Z. Wang[4].* 1) Molecular Cytogenetics, Thomas Jefferson University Hospital, Philadelphia, PA; 2) Division of Hematopathology, Thomas Jefferson University Hospital, Philadelphia, PA; 3) Department of Medical Oncology, Thomas Jefferson University Hospital, Philadelphia, PA; 4) Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University Hospital, Philadelphia, PA.

Acute myeloid leukemia (AML) is a heterogeneous disease with several morphologic and genetic subtypes that show recurring and uncharacterized chromosomal aberrations that are of etiologic, diagnostic, prognostic and therapeutic significance. AML may be induced by disruption of the normal structure and function of genes that control the balance of proliferation and differentiation in hematopoietic precursors. Two such genes are the proto-oncogenes MYC and MLL. Abnormalities in MYC expression, which is involved in cell cycle progression, apoptosis and cellular transformation, are often associated with leukemia. AML's with abnormalities of the MYC and MLL genes are associated with a poor prognosis and poor response to chemotherapy. MLL and MYC gene amplification have previously been described occurring together and independently in leukemia cell lines and in some AML patients. We describe the first patient, to our knowledge, with AML to demonstrate both MLL and MYC amplification on a ring chromosome and the possible mechanism for the formation of the ring chromosome identified by cytogenetics, fluorescence in situ hybridization (FISH) and OaCGH techniques. The chromosome analysis revealed a complex karyotype with two cell lines showing loss of both copies of chromosome 11, additional material on chromosome 17p leading to the loss of the TP53 gene, additional material on chromosome 18p, a ring chromosome and double minute chromosomes. FISH performed using MLL and MYC probes showed the presence of both amplified genes in a ring chromosome. In addition, a pattern of deletion of the 5' end and the amplification of the 3' end of the MYC gene in some interphase cells reveals a possible evolutionary mechanism of different cell lines. The OaCGH helped in further characterizing the possible mechanism that might have led to the formation of the ring chromosome.

1213/W

Germinative copy number changes in familial cancer predisposition. *C. Rosenberg[1], A. Gonçalves[2], E.M.M. Santos[2], L. Capelli[2], S. Costa[1], M.I.W. Achatz[2], R.R. Brentani[2], A.C.V. Krepischi[2].* 1) Dept Genetics and Evolutionary Biology, Univ Sao Paulo, Sao Paulo, SP, São Paulo, Brazil; 2) Antonio Prudente Foundation, A.C. Camargo Hospital, São Paulo, Brazil.

Detection of rare CNVs may reveal susceptibility genes in families with cancer predisposition. Such cases have already been reported for neuroblastomas, prostate, breast and pancreas tumors. On the other hand, individuals from such families may also display increased number of CNVs as the result of new mutations. Specifically, the number of CNVs in Li-Fraumeni patients has been reported to be 3 times higher than in normal controls. To ascertain the number and gene content of constitutive copy number changes in patients with cancer predisposition, we have screened germinative CNVs by array-CGH (4x180K Agilent) in 130 unrelated probands from families with clinical diagnosis to cancer predisposition: 60 from families with hereditary breast-ovarian cancer, 70 from Li-Fraumeni and Li-Fraumeni like families, and compared to 80 normal controls. In this study, the probands of breast-ovarian cancer families were prescreened for BRCA1 and BRCA2 gene mutations, and the individuals from Li-Fraumeni families for TP53 mutations; individuals with mutations for either gene were excluded. We subdivided the CNVs into common and rare, the latter being designated by occurring £ 3 times within the Database Genome Variants (DGV), which corresponds to ~15% of CNV detection events. The initial results point to a slight increase in the average number of all CNVs per individual in cancer patients (8.2 for breast-ovarian cancer, and 9.3 for Li-Fraumeni) as compared to the controls (6.8). However, the preliminary data for the category of so-called "rare alterations" indicates that the difference between groups is larger, and occurs approximately twice as frequently in cancer patients (average of 0.96±1.28 per individual) than in controls (average of 0.42±0.67 per individual). Some of the gene changes encompassed by these constitutive CNVs have been previously described in tumors. In conclusion, our results suggest a somewhat higher frequency of constitutive CNVs in cancer patients than controls, with a stronger effect noted for the "rare CNV" category. ***Support: FAPESP and CNPq***.

1214/W

Interstitial deletion of 8p11.2-p12: clinical report. *J.A. Fahrner, N.L.M Sobreira, C. Dinsmore, A. Hamosh.* McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins School of Medicine, Baltimore, MD.

Deletions of the proximal part of 8p (8p11.1 to 8p23.1) have a heterogeneous phenotype characterized by spherocytosis, cardiac anomaly, hypogonadism, ocular abnormality, facial dysmorphism, microcephaly, growth retardation, and psychomotor retardation. Spherocytosis is caused by the deletion of *ANK1* on 8p11.2 and a critical region associated with congenital heart defects has been assigned to 8p23.1. About 20 cases of proximal 8p deletion have been described in the literature and the majority of these cases were characterized by standard karyotyping methods and the sizes of individual deletions were very variable. The delineation of the precise cytogenetic defects in these patients is important to understand their clinical phenotype and implicate novel genes that underlie the pathogenesis. Here we describe a 4-year-old girl with microcephaly, growth retardation, psychomotor retardation, spherocytosis, difficulty feeding, obstructive and central apnea, seizure disorder, mild hearing loss in the high frequencies, ear pits, bilateral retinal detachments and blindness, opaque corneas with band keratopathy in both eyes, deep set eyes with upslanting palpebral fissures, and a sacral dimple. Echocardiogram and abdominal ultrasound were normal. A standard karyotype study and FISH analysis revealed a minimal 5.3 Mb deletion, from 37.3 Mb to 42.6 Mb (8p11.2-p12). This region includes 49 annotated RefSeq genes. Approximately 13 cases of proximal 8p deletion reported in the literature were found to have regions of the deletions that partially overlap with 8p11.2p12 and of these, seven are known to have ocular abnormalities and two do not have ocular abnormalities. The ocular abnormalities usually include strabismus and nystagmus. SNP genotyping of this patient followed by more precise definition of the breakpoints of the deletion will determine what genes are involved in the deletion and comparing this deletion to deletions that overlap may disclose a critical region associated with ocular abnormalities or disclose candidate genes for the features of the deletion.

1215/W

Immunodeficiency-Centromere Instability-Facial Anomalies (ICF) syndrome resulting from a novel homozygous *DNMT3B* mutation. *H. Kearney[1], J. Kearney[1], M. Shook[1], R. Casey[1], J. Tarleton[1], S. Roberts[1], D. Russell[2], E. Boyd[1].* 1) Fullerton Genetics Center, Mission Hospitals, Asheville, NC; 2) Allergy Partners of Western North Carolina, Asheville, NC.

Immunodeficiency-Centromere Instability-Facial Anomalies Syndrome (ICF Syndrome) is a rare autosomal recessive disorder described in fewer than 50 individuals in the medical literature. ICF syndrome is characterized by immunodeficiency (agammaglobulinema or hypogammaglobulinemia resulting in frequent childhood infections and mortality), variable facial dysmorphism, and characteristic non-clonal chromosome abnormalities involving the centromeres of chromosomes 1, 16, and 9. Growth deficiency, failure to thrive and mild mental retardation are also described in a number of patients. The majority of ICF syndrome cases are shown to be caused by mutations in the *DNMT3B* gene, encoding a de novo DNA methyltransferase.

Our patient is a 23 year old male with hypogammaglobulinemia recognized in the first year of life, followed by regular IVIG therapy. Height and weight were below the 3rd centile, and mild dysmorphic features, developmental delays and cognitive disabilities were also noted. Routine cytogenetic analysis of PHA-stimulated lymphocytes showed breaks, isochromosomes, and radial formations involving the centromere of chromosome 1 in approximately half of the cells examined. SNP-based microarray revealed no clinically significant copy number imbalances, but did show multiple large homozygous regions, encompassing approximately 10% of the genome. Review of his family history confirmed that he was the product of a consanguineous union. Given the cytogenetic findings, the *DNMT3B* gene at 20q11.21 was checked for homozygosity, and was found in a homozygous segment. Subsequent sequence analysis revealed a novel homozygous missense mutation (c.1760G>A) that is predicted to result in a deleterious amino acid substitution in the methytransferase domain of the DNMT3B protein (p.G587D). The clinical features of this individual, the hallmark cytogenetic findings, and the predicted pathogenic nature of the missense mutation establish with reasonable confidence that this is a novel case of *DNMT3B*-associated ICF syndrome.

1216/W

Clinical and molecular characterization of chromosome 7p22.1 microduplication detected by array CGH. *J.V. Chui[1], J.D. Weisfeld-Adams[1,2], J. Tepperberg[3], L. Mehta[1].* 1) Department Genetics and Genomic Sciences, Mount Sinai School of Medicine, 1 Gustave L. Levy Place Box 1497, New York, NY 10029; 2) Department of Pediatrics, Mount Sinai School of Medicine, 1 Gustave L. Levy Place Box 1512, New York, NY 10029; 3) LabCorp, Center for Molecular Biology and Pathology, 1912 Alexander Dr., Research Triangle Park, North Carolina 27713.

A 28-month-old Peruvian male presented with speech delay and abnormal facial features including prominent forehead, anteverted nares, and ocular hypertelorism. The patient had a unilateral preauricular pit and a normal hearing evaluation. Closure of the anterior fontanel and bone age were significantly delayed. Motor development and stature were normal. Following normal routine chromosome analysis and subtelomeric FISH, diagnostic considerations included Greig cephalopolysyndactyly, Robinow syndrome or a skeletal dysplasia. However, characteristic features of these conditions were absent. Array CGH revealed a novel 1.7 Mb interstitial 7p22.1 duplication. FISH studies on the proband and his father and mother, who were 47 and 35 years old, respectively, at the time of conception, confirmed that the duplication had occurred de novo. To our knowledge, this finding represents the smallest interstitial 7p duplication reported to date. The Database of Genomic Variants lists 27 genes, 13 of which are OMIM annotated genes, within this region of chromosome 7p. However, none of the known functions of these genes make them obvious candidates for our patient's phenotype. 7p duplications can occur as a result of mis-segregation of unbalanced translocations or as "pure" duplications without any other chromosomal involvement, making genotype-phenotype correlations difficult. Some common phenotypic features of 7p duplication include macrocephaly, hypertelorism, large anterior fontanel, and mental retardation. Based on the findings in our patient, and those in previously reported cases of 7p duplication, we propose a role for genes in the 7p22.1 region in skeletal maturation and intellectual development.

1217/W

A FAMILY BALANCE TRANSLOCATION 46,XX,ins(7;10) (q21;q32q35) RESULTING IN A PARTIAL CHROMOSOME 7 TRISOMY IN THE PROGENY. A CASE REPORT AT THE HOSPITAL PARA EL NINO POBLANO, MEXICO. *J. Aparicio[1,8], M.L. Hurtado-hernandez[2], M. Barrientos-Perez[3], N.C. Gil-Orduna[7,8], W. San Martin-Brieke[7], S.I. Assia-Robles[4], R. Zamudio-Meneses[5], B. Vazquez-Juarez[6], F. Almanza-Flores[4], K. Villarreal-Borrego[4].* 1) Dept Genetics; 2) Cytogentics; 3) Endocrinology; 4) Pediatrics; 5) Cardiology; 6) Neurology; 7) Estomatology, Hosp para el Nino Poblano, Puebla,; 8) Estomatology, Benemerita Universidad Autonma de Puebla, Mexico.

INTRODUCTION. A reciprocal chromosome translocation between 7q and l0q was found in a healthy 44-year-old woman, with no physical or mental alterations was observed. However her unique male progeny, the propositus of this study, had a chromosomal 10q+ translocation. The clinical features of the patient were physical malformations as micrognathia, abnormal ears, and overlapping fingers and toes, cifoescoliosis and mental retardation. MATERIALS AND METHODS. A 7 years old male patient was clinically examined at the departments of Genetics, Neurology and also underwent karyotyping. The latter's karyotype was 46, XY (10q+). Trypsin-Giemsa banding analysis of the consultand's peripheral blood lymphocytes showed a chromosomal translocation. The patient mother was also studied by cytogenetics to find any possible healthy carrier. One translocations and insertion involved a portion of the long arm of a number 7 and number 10 chromosomes in a reciprocal rearrangement was observed in the patient's mother. Brain ventriculomegaly and subcortical atrophy was observed with a cranial tomography. DiSCUSSION. A balanced rearrangement between 7q and 10q was observed in one of the parents in this study, while the progeny had a partial chromosome 7 trisomy. The clinical features in the patient were bone malformations and mental retardation, mainly. Studies on large kindred with three cytogenetic markers were described by Jacobs et al. (1970). The healthy mother, ascertained through a cytogenetic survey, was found to have a balanced translocation and insertion between a number 7 chromosome and a 10 chromosome. There was only one pregnancy, however no miscarriages were reported. The propositus of this study had the chance to inherited 25% (7;10) normal, 25% (7;10q+) like the patient, 25% del(7q-;10), 25% balance translocation like the mother (7q-;10q+). The patient translocation t(7;10q+) leaded to a partial 7 chromosome trisomy. An early cytogenetic diagnosis should be important for an earlier treatment and a better quality of life.

1218/W

Micronuclei in bone marrow cells of mice exposed to methylparathion, in vivo. *I. Aranha, T. Ornelas.* Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil.

The use of pesticides is still the main strategy to fight plagues in agriculture. Organophosphate esters represent a significant proportion of the world production of pesticides. Methylparathion is an organophosphate pesticide largely used. In order to exert its biological effect it must be biotransformed into its oxon-analogous, methylparaoxon, in the liver. Previous work in our laboratory, using the chromosomal aberrations assay in human lymphocytes exposed to different concentrations of these drugs(0.1, 0.25 and 0.5 ppm)in vitro have shown that while methylparathion had no clastogenic or aneugenic effect on the chromosomes, methylparaoxon in the same concentrations was responsible for the alterations in structure observed. The objective of the present work was to study the effect of methylparathion on chromosomes of mice bone marrow in vivo, using the micronucleus assay. Animals were separated into four groups. In the first group, 6 animals received methylparathion intraperitoneally during five consecutive days in a concentration equivalent to 25% of the LD_{50}. In the 6^{th} day, animals were sacrificed, their femurs removed, the bone marrow collected and smears were made for slides preparation. After 24h cells were stained with Giemsa Gurr (2%) and analyzed under optical microscope. As positive control, 6 animals received cyclophosphamide (50mg/mL) once. Six animals were injected intraperitoneally with the solvent(corn oil) and 6 animals not exposed to any drug served as negative control for the experiment. In the test group, 12000 cells were observed and 199 showed micronuclei. In the group exposed to the solvent, 12000 cells were analyzed, and none had micronucleus. In the positive control group, 12000 cells were observed and 102 had micronuclei and in the negative control group, of 12000 cells observed, none had micronucleus. The chi-square test for independence showed that our results were extremely significant ($P<0.0001$). They suggest that methylparathion is responsible for the micronuclei observed.

1219/W

Inversions in the genome can both lead to triplications and contribute to rearrangement complexity. *C. Carvalho[1], M. Ramocki[2, 3], D. Pehlivan[1], L.M. Franco[1], L. Seaver[4, 5], L. Friehling[6], S. Lee[4, 5], R. Smith[7], D. del Gaudio[1], M. Withers[1], P. Liu[1], P.J. Hastings[1], S.W. Cheung[1], J.W. Belmont[8], H.Y. Zoghbi[1, 2, 3], J.R. Lupski[1, 2, 3].* 1) Dept Molecular Human Genetics, Baylor Col Medicine, Houston, TX; 2) Pediatrics, Baylor College of Medicine, Houston, TX; 3) Texas Children's Hospital, Houston, TX; 4) Kapiolani Medical Specialists; 5) Departments of Pediatrics, John A. Burns School of Medicine, Honolulu, HI; 6) Children's Medical Associates, Alexandria, VA; 7) Department of Pediatrics, Maine Medical Center, Portland, ME; 8) Division of Cardiology, Department of Pediatrics, Baylor College of Medicine, Houston, TX.

Genomic duplication involving the *MECP2* gene causes a clinically recognizable syndrome that includes infantile hypotonia, global developmental delays, progressive spasticity, recurrent infections, absent speech, and autism or autistic features. We identified complex rearrangements involving *MECP2*, characterized by a triplicated segment embedded within the duplication, in seven subjects. Here we provide detailed molecular characterization of those rearrangements obtained by customized oligonucleotide array CGH (aCGH), southern blot, breakpoint sequence and SNP array (Illumina Human Omni1-Quad). The complex rearrangements vary in size from ~430 kb to ~653 kb, whereas the triplicated regions range in size from ~33 kb to ~537 kb. Remarkably, in all cases one of the duplicated breakpoints and one of the triplicated breakpoints map within a low copy repeat (LCR) or within a small repeat (854 bp) in inverted orientation, suggesting that one of the breakpoint junctions was produced by a Non-Allelic Homologous Recombination (NAHR) mechanism and generated an inversion of the triplicated segment. Sequencing of the second breakpoint showed that the inverted triplicated segment is once more connected to the duplicated segment in opposite orientation (based on the reference genome) suggesting the following genomic organization: DUP-PIRT-DUP. Microhomologies or lack of homology were observed at those breakpoints suggesting the involvement of a second mechanism for formation underlying those rearrangements. All complex rearrangements were inherited from mothers (in one case it was demonstrated to be inherited from the available grandmother) and who display a skewed X-inactivation pattern in DNA isolated from blood. In summary, our data clearly show that inversions can both mediate triplication formation and contribute to the complexity. We also provide further evidence that the presence of LCRs in the *MECP2* vicinity can be associated with genome instability and potentially induce DNA strand lesions, generating complex rearrangements involving *MECP2* and flanking genes.

1220/W

CLINICAL CHARACTERISTICS AND IDENTIFICATION OF CHROMOSOMAL ABNORMALITIES IN MEXICAN PATIENTS WITH PIGMENTARY MOSAICISM. *D. CERVANTES-BARRAGAN[1], R. CRUZ[1], C. SALAS[1], C. DURAN-MCKINSTER[2], V. DEL CASTILLO-RUIZ[1], P. PEREZ-VERA[1].* 1) DEPARTMENT OF HUMAN GENETICS NATIONAL INSTITUTE OF PEDIATRICS, MEXICO CITY, MEXICO; 2) DEPARTMENT OF DERMATOLOGY, NATIONAL INSTITUTE OF PEDIATRICS, MEXICO CITY, MEXICO.

Introduction: Mosaicism represents a phenomenon which encompasses two or more genetically different cell populations that proceed from the same zygote. Pigmentary mosaicism (PM) is a clinical condition that represents an example of phenotypic expression of genetic mosaicism. PM is a term that refers to hypopigmented and hyperpigmented skin lesions that follows the Blaschko's lines, including patients with Hypomelanosis of Ito and some others with linear and whorled nevoid hypermelanosis. Patients with PM can be associated with abnormal systemic features including central nervous, musculoskeletal and ocular systems (60-90%). Cytogenetic analysis of blood and fibroblasts reveals chromosomal mosaicism with structural or numerical abnormalities (30-60%). The purpose of this study was to determine the clinical systemic involvement and blood/fibroblast cytogenetic analysis of Mexican patients with PM. Methods: We tested 18 patients with PM in a period from 2002-2009, the patients were evaluated by Dermatology, Neurology, Orthopedics, Ophthalmology and Genetics Departments. Cytogenetic analysis of all patients were performed in T-lymphocytes from peripheral blood, and in fibroblasts from dark/light skin biopsies cultures. Fifty metaphases were analyzed by standard GTG bands in at least two cultures. Results: Clinical evaluation of the systemic involvement of the patients showed: mental retardation in 15/18 (83%) seizures 8/18 (15%), musculoskeletal involvement was present in 5/18 (27%), and ophthalmologic changes 4/18 patients (22%). Six out of the 18 PM patients (33%) presented chromosomal mosaicism: a)Three patients with rings: two cases with r(22)(p11.2-q13.3), one with r(7)(p22-q36); b) Two patients with deletions of the same chromosome, del(18)(q21.3) and del(18)(q21.1); and c) One with an apparently balanced translocation t(X;16)(p11.2; p13.3). Conclusions: Cytogenetic analyses in blood and fibroblast from dark and light skin is useful in the etiologic approach for patients with PM.

1221/W

Reciprocal insertion: a rare chromosomal rearrangement in a phenotypically normal mother explaining interstitial deletion and duplication in a patient with developmental delay and dysmorphic features. *S. Chenier, G. Mitchell, G. Mathonnet, E. Lemyre, F. Tihy.* Medical Genetics, CHU Sainte-Justine, Université de Montréal, Montreal, Canada.

Array comparative genomic hybridization (aCGH) is a clinically valuable molecular cytogenetic method for analyzing DNA copy-number variations. Not only aCGH can detect submicroscopic anomalies, but it can also help reveal, in conjunction with fluorescence in situ hybridization (FISH), genomic rearrangements previously thought to be exceedingly rare. We have recently detected by aCGH a 1p31.1p32.1 interstitial deletion and a 13q32.1q32.1 microduplication in a patient with developmental delay and facial dysmorphisms. Interestingly, parental studies using FISH analysis revealed that the mother of the child carried a balanced reciprocal insertion (1;13)(p31p32;q32.1q32.1). Reciprocal insertion is a rare chromosomal rearrangement that requires at least four interstitial breakage events and a reciprocal exchange of DNA fragments. Carriers of reciprocal insertions are at an increased risk of creating gametes with unbalanced chromosomal rearrangements leading to miscarriages or children with abnormalities. To our knowledge, less than five cases of constitutional reciprocal insertions have previously been reported. It can be expected that the increased resolution achieved by aCGH analysis in comparison to karyotype analysis as well as its common use in clinical practice will demonstrate that reciprocal insertion occurs more frequently than previously thought. This case illustrates the need to confirm the location of copy number deletions and duplications detected by aCGH with techniques such as FISH analysis in order to guide genetic counselling.

1222/W

Cytogenetic analysis in fetal blood. *J. Choi[1], M. Woo[1], N. Song[1], H. Ko[1,2], S. Oh[1,2], C. Park[1,2], J. Park[1,2], J. Jun[1,2], S. Kim[1,2], Y. Choi[1,2], S. Moon[1,2].* 1) Institute of Reproductive Medicine and Population, Medical Research Center, Seoul National University, Seoul, Korea; 2) Department of Obstetrics and Gynecology, College of Medicine, Seoul National University, Seoul, Korea.

This study reviewed cytogenetic results in 568 fetal blood samples by cordocentesis from second- and third-trimester pregnancies to identify associated problems with fetus and to confirm abnormal karyotype of cultured amniotic fluid cells. Chromosome abnormality was detected in 80 cases (14.1%) which contained 60 cases (75%) of numerical aberration and 20 cases (25%) of structural aberration. The most frequent abnormalities were sex chromosomal mosaicisms (23 cases, 28.8%) fallowed by autosomal trisomies (22 case, 27.5%) and sex chromosomal trisomies (7 cases, 8.8%). Twenty-two cases (27.5%) were consistent with autosomal trisomies, including eleven cases of trisomy 18, eight cases of trisomy 21 and three cases of trisomy 13. Among structural aberrations, translocations (6 cases, 7.5%), maker chromosomes (4 cases, 0.5%) and additions (4 cases, 0.5%) were prevalent. One of three cases with unbalanced reciprocal translocation and one of two cases with balanced translocation were inherited from male translocation carriers. In order to obtain definitive results, 44 cases with chromosomal aberration in amniotic fluid cells investigated in fetal blood and 25 cases (56.8%) showed the same abnormality in fetal blood. Three of twenty-one cases previously defined as true mosaicism in amniotic fluid cells revealed a normal karyotype in fetal blood. Interestingly, one of three cases with mosaic trisomy 20 confirmed in fetal blood, whereas the others showed a normal karyotype in fetal blood. Cytogenetic analysis in fetal blood by cordocentesis is necessary for prenatal genetic diagnosis in pregnancies that indicated high risk for fetal abnormalities or presented abnormal karyotypes of cultured amniotic fluid cells.

1223/W

Mosaic ring (9) syndrome, report a new case. *M.M. Duenas Roque, G. Campoverde Avila.* Hospital Guillermo Almenara Irigoyen , Lima, Lima, Peru. Resident.

The ring 9 syndrome is a rare genetic disorder observed in the children with variable clinical presentation and phenotype. The syndrome is caracterized by growth and developmental delay, mild hirsutism, dysmorphic face, microcephaly and hipoacusia. We present the case of a nine year old girl, who was born post-term by spontaneous vaginal delivery. Birth weight was 2560g,birth height was 43cm, presenting with microcephaly, prognatism, clynodactyly, short stature, language and learning disabilities, and mild to moderate mental retardation. Auditory evoked potencial was normal. The karyotype was a mosaic of three cell lines: 46, XX/46, XX, r(9)/46, XX, r(dup 9) . The parents karyotypes were normal.

1224/W

Mosaic partial trisomy 19p12-q13.11 in Asperger Syndrome due to a small supernumerary marker chromosome, suspected through array-based comparative genomic hybridization. *F.R. Faucz[1,2], J. Souza[2], A. Bonalumi[2], V.S. Sotomaior[2], E. Frantz[3], S. Antoniuk[4], J. Mokry[5], S. Raskin[2,6].* 1) Section of Endocrinology and Genetics, Program on Developmental Endocrinology & Genetics (PDEGEN), National Institute of Child Health & Human Development (NICHD), National Institutes of Health (NIH), Bethesda, MD, USA; 2) Laboratory of Molecular Genetics, Core for Advanced Molecular Investigation (NIMA), Center for Health and Biological Sciences (CCBS), Pontificia Universidade Católica do Paraná (PUCPR), Curitiba, PR, Brazil; 3) Department of Neurology, Blumenau, SC, Brazil; 4) Departamento de Pediatria UFPR, Curitiba, PR, Brazil; 5) Signature Genomics, Spokane, Washington, USA; 6) Laboratory Genetika, Curitiba, PR, Brazil.

Small supernumerary marker chromosomes (sSMC) are present in 0.075% of unselected prenatal and 0.044% of postnatal cases. In the mentally subnormal population the sSMC rate is 0.288%. The risk of phenotypic abnormalities in de novo prenatally detected cases of sSMC is estimated to be 26%. Here we report the case of a four-year-old boy with Asperger Syndrome. The patient is the first child of a healthy 39-year-old mother and 54-year-old father. Pregnancy was complicated in the last month by oligoamnio. The mother had no history of medication or drug use. There was no family history of any malformation or mental retardation. The weight, height and Apgar score at birth were normal. The neonatal period was complicated by lack of breast suction and mild hypotonia. His developmental milestones were delayed and now, at 4 years and 10 months of age, he is on preschool education and has short attention span and few social interaction with other children. He had long face with high nasal bridge and thin upper lip. No other dysmorphic features were noted. An auditory brain response showed unilateral mild neurosensorial hearing loss. Neurological examination revealed diffuse mild hypotonia with normal deep tendon reflexes and impairment in social interaction, obsessive/compulsive behavior, emotional immaturity, attention deficit, specific interests in certain activities, mood disorder, insecurity and gullibility. The intellect level, eyesight and hearing were normal, fitting with a diagnosis of Asperger Syndrome. Conventional cytogenetic study showed a small supernumerary marker chromosome (sSMC) on around 60% of the cells. To identify the origin of the sSMC a microarray-based comparative genomic hybridization (aCGH) was performed using a 135K-feature whole-genome microarray and a partial trissomy in a pericentromeric region of chromosome 19 was seen. The region spans 18.4Mb, contains at least 55 genes and is located between 19p12 and 19q13.11. To confirm this result and the mosaicism, a FISH was performed using BAC clones to visualize the abnormalities and 43.3% of mosaicism was detected. Based on the size and genic content is expected that the partial trisomy detected to be responsible for the characteristics observed in the patient. In that case it could be an indication of a novel locus associated with Asperger syndrome.

1225/W

Characterization of Chromosomal Aberrations in the NIGMS Human Genetic Cell Repository by the Affymetrix GeneChip SNP 6.0. *N. Gerry, L. Toji, C. Beiswanger, B. Frederick, J. Leonard, D. Berlin.* Coriell Institute for Medical Research, Camden, NJ.

The Affymetrix SNP Array 6.0 was used to define the chromosomal segments gained and lost in more than 700 samples from the NIGMS Human Genetic Cell Repository Chromosomal Aberrations collection. This collection contains a wide variety of copy number variants, including micro or sub-micro deletions and duplications, translocations, aneuploidies, and isodicentric chromosomes. All of the samples had been previously characterized by G-band, FISH and/or PCR. Data were analyzed for copy number changes using Affymetrix Genotyping Console Software. The resolution of the Affymetrix SNP Array 6.0 data allowed characterization of chromosomal copy number changes in the kilobase range. Within the samples studied, the smallest change detected was a 35 kb deletion of an exon in the SNRPN gene in a Prader-Willi sample. This result, along with many other submicroscopic changes detected on the array, agreed with prior molecular mapping experiments. The high resolution of the array also provided the ability to distinguish between chromosome breakpoints in multiple samples of the same disorder that appeared nearly identical by traditional G-band analysis, but in fact were all unique. In addition, the array provided more accurate breakpoints for submicroscopic changes that had previously been identified by FISH using only one, or at most a few, markers in the critical region. Finally, previously unrecognized complexities such as cryptic translocations and duplications adjacent to syndrome-specific deletions were identified by the array.

The copy number segment data and graphic output from Affymetrix Genotyping Console are being used to upgrade the descriptions of these samples on the Coriell Cell Repositories web-site (http://ccr.coriell.org/) to better inform users in their decisions for experimental design and choice of reference samples. Each of the samples is publicly available as a culture and many are available as DNA. In addition, the raw data for all of the samples has been deposited with the database of Genotypes and Phenotypes (dbGaP) and is available for download.

1226/W

A familial 12.7 Mb deletion in the proximal short arm of chromosome 3 with no apparent phenotypic abnormality. *A. Hajianpour, J.C. Wang, A. Khanna, J. Szymanska, R. Habibian.* Dept Cytogenetics, Genzyme, Monrovia, CA.

Amniocentesis was performed on a 43 year old woman G1, P0, SAB1 at a gestational age of 17 weeks due to AMA, abnormal maternal serum screen with increased risk of chromosome abnormality. There were no abnormal ultrasound findings. Cytogenetic analysis revealed an abnormal chromosome 3 with an interstitial deletion in the proximal short arm: 46,XX, del (3)(p11.2p13). The same abnormal chromosome 3 was present in the mother, who apparently does not have any phenotypic abnormality. Array CGH analysis on the mother and the fetus showed a 12.7 Mb deletion in 3p12.3-3p11.1. This deletion contains at least 12 OMIM genes and is expected to be clinically relevant due to size and gene content. However, its clinical significance is less clear in this case since the same chromosome abnormality is present in a phenotypically normal parent,. An apparently normal baby girl was born at 38 weeks. The baby is doing well at age of three months. A follow up examination at age of 8 months is scheduled and will be presented. This case illustrates the many challenges at prenatal diagnosis and the uncertainty in view of abnormal cytogenetic findings with normal follow up of the pregnancy.

1227/W

Genetic, environmental, age, and chromosome-specific influences on acquired aneuploidy frequencies in lymphocytes: A twin study. *C. Jackson-Cook[1,2], K. Jones[1], C. Rehder[1,3], Y-J. Chen[1], T. York[2].* 1) Dept Pathology, VA Commonwealth Univ, Richmond, VA; 2) Dept Human & Molecular Genetics, VA Commonwealth Univ, Richmond, VA; 3) Dept Pathology & Duke University Health Systems Clinical Laboratories, Duke Univ, Durham, NC.

Although acquired chromosomal abnormalities have been associated with several age-related health conditions, little is known about the genetic or environmental factors influencing their frequency in healthy individuals. Therefore, we initiated a twin study to determine the: (1) frequency of acquired aneuploidy in cultured lymphocytes; and (2) variation attributable to genetic versus environmental factors. To date, 191 twins (63 males; 128 females with 88 complete twin pairs) ages 7 to 85 have been studied. Acquired aneuploidy was evaluated using: (1) a micronuclei (MN) assay (n=145) coupled with a SKY analysis (n=79); and (2) an interphase FISH assay (n=191) using probes for each of the 24 chromosomes. For all chromosomes, loss was seen more frequently than gain ($p<0.05$). In both assays significant increases in abnormalities involving the X (female) or Y (male) chromosome were observed, with this effect being positively correlated with age ($p<0.0001$, $p=0.04$). In addition to age, the completion of puberty in either gender ($p=0.036$) and menopause in females ($p=0.024$) was associated with a significant increase in MN frequencies. Interestingly, a significantly increased frequency of aneuploidy "rescue" was observed for the X (females) and Y (males) chromosomes in the MN from younger compared to older twins ($p=0.03$), suggesting that different mechanisms result in the aneuploidy present at different ages. A non-random pattern of autosomal abnormalities was detected ($p<0.0001$ in MN and FISH), with chromosomes 4, 8, & 9 and 1, 18, & 16 having the highest frequencies in the MN and FISH assays, respectively. Thus, 4 of the 6 chromosomes having the highest frequencies are heterochromatin-rich. The lowest frequencies were observed for chromosomes 22 (MN) and 20 (FISH). Genetic model fitting indicated that influences from both additive genetic (46.8%) and unique environmental (53.2%) sources best explained the observed variance in MN data. Specific environmental factors that significantly influenced MN frequencies included dietary [decreases correlated with consuming fruit ($p=0.016$), green, leafy vegetables ($p< 0.001$), and/or folate-enriched bread ($p=0.035$)], and lifestyle [increases in females using tobacco ($p<0.001$)] choices. In conclusion, the frequency of acquired aneuploidy in humans appears to be a complex trait that is influenced by both environmental and additive genetic components, with chromosome-specific and age-related differences being present.

1228/W

High Resolution Analysis of Human Chromosome 13 Partial Aneuploidies. *V. Jobanputra, A. Burke, A. Muhlhauser, O. Nahum, K. Anyane-Yeboa, W. Chung, B. Levy, D. Warburton.* Columbia University Medical Center, New York, NY.

Partial deletions and duplication of chromosome 13 (Chr13) have been described with widely varying phenotypes. Some cases have major malformations such as oligodactyly, holoprosencephaly, microopthalmia and heart defects: others have only minor developmental delay or dysmorphic features. Although several attempts have been made to establish genotype-phenotype correlations based on banding patterns in patients with Chr13 abnormalities, it is still unclear as to which regions or gene(s) on Chr13 contribute to specific phenotypic features in these patients. The ability to make these correlations is compromised by the limited resolution of standard karyotyping. Oligonucleotide-based genomic microarrays provide an opportunity to re-evaluate these data at an ultra-high resolution. To date we have recruited 16 cases of partial deletions and 4 cases of partial duplications of Chr13. We are collecting detailed clinical information from these patients. We have performed SNP microarray analysis (Affymetrix 6.0) for precise characterization of the chromosome abnormality in 17 cases identified by conventional cytogenetic, array CGH, or targeted clinical microarrays. In addition, cell lines from 3 patients carrying partial trisomy or tetrasomy of distal Chr13 were also analyzed. In preliminary analyses, the deletions (n=16) ranged from 3.28 Mb to 45.23 Mb and the duplications (n=7) ranged from 564 kb to 64.78Mb. To construct a genotype-phenotype map, a smallest region of overlap is identified and the copy number changes from these segmental aneuploidies are compared with the phenotypic features recorded for the patients. As an example of the type of information that can be obtained, we identified 7 cases with duplications that included the ZIC2 gene in 13q32 that leads to holoprosencephaly when deleted or mutated. None had holoprosencephaly, contrary to some experimental data suggesting ZIC2 overexpression would be deleterious. Collection of similar data in other regions will improve our ability to predict phenotype prenatally or in infancy and to establish the genes associated with specific phenotypic abnormalities in these patients.

1229/W

Chromosomal aberrations delineated by aCGH and RT-PCR in the families with Mental Retardation. *J. Kasnauskiene[1], Z. Ciuladaite[1], A. Alexandrou[2], G. Koumbaris[2], P.C. Patsalis[2], V. Kucinskas[1].* 1) Department of Human and Medical Genetics, Vilnius University, Vilnius, Lithuania; 2) Department of Cytogenetics and Genomics, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus.

The purpose of our study was to identify pathogenic chromosomal aberrations for patients with mental retardation. We present the results of analysis in 26 patients with mental retardation, developmental delay and/or congenital malformations with normal karyotype. Oligo aCGH 105K (Agilent Technologies) for identification of copy number changes and submicroscopic deletions/duplications and RT-PCR for confirmation and heritability of rearrangements were used. De novo CNVs were identified in 8 % (del(5)(q14.3q14.1) and dup(16)(p13.3). Del(16)(p11.2) for one patient maternal origin was identified. 16p11.2 microduplication syndrome associated with autism and in published cases arose as de novo rearrangements. We found inherited CNVs (deletions and duplications) not previously described in the normal location in approximately 20% of the cases: dup(1)(p21), dup(3)(p22.2), dup(3)(p26.3), del(4)(q28.3), dup(7)(p21.1), dup(12)(q14.2), dup(12)(q24), and dup(17)(q22q23). Sizes of the variation are from 0.1 to 1.3 Mb. The CNVs can be involved in mental retardation and developmental delay due to altering the expression of the genes in genomic regions. The research is founded by the FP7/2007-2013 under grant agreement No. 223692, CHERISH project.

1230/W

Molecular cytogenetics using array-CGH in three deletion groups of chromosome 6q. *E.M Kwon[1], E.J Seo[1,2,4], S.Y Shin[1,2], K.J Kim[4], M. Hong[4], H.W Yoo[1,3,4], J.Y Lee[1].* 1) Medical Genetics Clinics & Laboratory, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea; 2) Department of Laboratory Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea; 3) Department of Pediatrics, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea; 4) Genomic Research Center for Birth defects and Genetic disorders, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea.

Interstitial deletions of chromosome 6q are a relatively rare finding. Deletions have ranged from the loss of a single band to larger deletions spanning multiple bands. The clinical phenotype varies, but some features commonly seen include cardiac anomalies, hypotonia, facial dysmorphism and mental retardation. To further delineate the syndrome, we report a detailed clinical and molecular characterization of three patients with three different deletions, namely, proximal (q12-q14.3), middle (q16.3-q22.31), and terminal (q27) deletion of chromosome 6q. Conventional chromosome analysis revealed an interstitial deletion in the long arm of chromosome 6. The deletion was confirmed and visualized at the molecular level by array comparative genomic hybridization (array CGH). Case 1 was 1-year-old child with a de novo 19.5 Mb deletion in the 6q12-q14.3 ascertained by array CGH. The clinical features of this patient include developmental delay, dolicocephaly, facial asymmetry, dysmorphism such as laterally upslant eye with narrow palpebral fissure, low-set ear, receded chin, flat nasal bridge and bulbous nose, high arched palate, micropenis with both undescended testis, umbilical hernia, slender and pencil-like hands, and hypotonia. Case 2 was a 4-month girl with microcephaly, dysmorphism, and congenital heart defect of VSD. Array CGH showed a 16.2Mb deletion in the 6q16.3-q22.31, demonstrating higher resolution than the conventional cytogenetic findings, del(6)(q16.2q22.2). Case 3 with vetriculomegaly and cerebellar hypoplasia had a prenatally detected to have a de novo 3.8Mb terminal deletion in 6q27. Phenotypes and array CGH results shown in these 3 patients were compatible with clinical features and chromosomal regions described in literatures. Array CGH can provide precise location information linked to physical and genetic maps of the human genome.

1231/W

Copy number gain at Xp22.31: mechanisms and clinical phenotypic consequences. *P. Liu[1], A. Erez[1], S.C. Sreenath Nagamani[1], W. Bi[1], C.M.B. Carvalho[1], A.D. Simmons[1], J. Wiszniewska[1], P. Fang[1], P.A. Eng[1], M.L. Cooper[1], V.R. Sutton[1], E.R. Roeder[4], J.B. Bodensteiner[5], M.R. Delgado[6], S.K. Prakash[1], J.W. Belmont[1], J.S. Berg[7], M. Shinawi[8], A. Patel[1], S.W. Cheung[1], J.R. Lupski[1,2,3].* 1) Molec & Human Gen, Baylor College Med, Houston, TX; 2) Dept of Pediatrics, Baylor College of Med, Houston, TX; 3) Texas Children's Hospital, Houston, TX; 4) Dept of Pediatrics, Univ of Texas Health Science Center at San Antonio, San Antonio, TX; 5) Division of Child Neurology, St. Joseph's Hospital and Medical Center, Phoenix, AZ; 6) Dept of Neurology, Univ of Texas Southwestern Medical School, Dallas, TX; 7) Dept of Genetics, Univ of North Carolina, Chapel Hill, NC; 8) Division of Genetics and Genomic Medicine, Dept of Pediatrics, Washington Univ School of Med, St Louis, MO.

Genomic instability is a feature of the human Xp22.31 region wherein deletions are associated with X-linked ichthyosis, mental retardation, and attention deficit hyperactivity disorder. A recombination hotspot motif is enriched within the low copy repeats that mediate recurrent deletion at this locus. To date, few efforts have focused on copy number gain at Xp22.31. Clinical testing has revealed a high incidence of duplication of Xp22.31 in subjects ascertained and referred with neurobehavioral phenotypes. We designed a high-density custom oligonucleotide array comparative genomic hybridization (aCGH) assay to interrogate Xp22.31 and systematically studied sixty-one unrelated subjects with rearrangements revealing gain in copy number. We detected not only the anticipated recurrent and simple nonrecurrent duplications, but also unexpected recurrent triplications and other complex rearrangements. Breakpoint analyses enabled us to surmise the mechanisms for many of these rearrangements; the mechanisms include "hotspot motif" associated NAHR in the majority of the patients, NHEJ (at least 2/61 subjects), and replication based mechanisms. Clinical studies in our subjects with Xp22.31 recurrent duplications and recurrent triplications uncovered potential clinical neurobehavioral consequences of these structural variations. Our findings reveal the spectrum of different mechanisms for genomic duplication rearrangements at a given locus, and provide insights into aspects of strand exchange events between paralogous sequences in humans.

1232/W

Two Cases with Rare Chromosomal Abnormality of Chromosome 12p Presenting Pallister-Killian Syndrome Phenotype. *G. Luleci[1], E. Mihci[2], Z. Cetin[1], S. Yakut[1], I. Keser[1], S. Berker-Karauzum[1].* 1) Medical Biology, Akdeniz University,Faculty of Medicine, Antalya, Turkey; 2) Pediatric Genetics,Akdeniz University,Faculty of Medicine, Antalya, Turkey.

Pallister-Killian Syndome (PKS) is a rare genetic disorder usually characterized by mosaic tetrasomy of isochromosome 12p detected in cultured fibroblast cells. We report here two rare cases with unusual cytogenetic findings including; nonmosaic, pure and complete trisomy of 12p and intrachromosomal triplication of short arm of chromosome 12 which are rare chromosomal abnormalities observed in patients with Pallister-Killian Syndrome phenotype. Case 1; is a unique patient with nonmosaic trisomy 12p which resulted from centric fission of maternal chromosome 12. 12p fission product have observed both in peripheral blood lymphocytes and skin fibroblast cells. Conventional cytogenetic and fluorescence in situ hybridization (FISH) techniques were used to detect proband's karyotype which is 47,XX,+fis(12)(p10)mat, and mother's karyotype is 47,XX,-12,+fis(12)(p10),+fis(12)(q10). Case 2: is a rare case diagnosed as Pallister-Killian Syndrome with a karyotype designated as 46,XX,inv trp(12)(p11.2p13) [34]/ 46,XX[16] de novo in fibroblast cells but not peripheral blood lymphocytes. According to the literature, case 1 is the first case with centric fission of chromosome 12 resulting in formation of telocentric fission product. Also review of the published reports shows that our case 2 is third case with PKS and intrachromosomal triplication of whole short arm of chromosome 12. Our findings show that non-mosaic trisomy 12p and mosaic intrachromosomal triplication of 12p are very rare but possible mechanisms for formation of trisomy 12p syndrome clinical phenotype.

1233/W

Breakpoint determination of X;autosome balanced chromosomal translocations in four patients with premature ovarian failure. *A. Nishimura[1], T. Wada[2], G. Bano[3], T. Kosho[4], N. Ando[5], H. Hamanoue[1,6], H. Sakakibara[6], Y. Tsurusaki[1], H. Doi[1], N. Miyake[1], H. Saitsu[1], Y. Fukushima[4], F. Hirahara[6], N. Matsumoto[1].* 1) Dept Human Gen, Yokohama City Univ Med, Yokohama, Japan; 2) Division of Neurology, Clinical Research Institute, Kanagawa Children's Medical Center, Yokohama, Japan; 3) Department of Cellular and Molecular Medicine, St George's University of London, London, UK; 4) Department of Medical Genetics, Shinshu University School of Medicine, Matsumoto, Japan; 5) Division of Obstetrics and Gynecology, Yokohama Municipal Citizens Hospital, Yokohama, Japan; 6) Department of Obstetrics and Gynecology and Reproductive Science, Yokohama City University Graduate School of Medicine, Yokohama, Japan.

Premature ovarian failure (POF) is a disorder characterized by amenorrhea and elevated serum gonadotropins before 40 years of age. The risk of this disorder or natural menopause before 40 years is approximately 1%. Heterogeneous etiology should be involved in POF, such as environmental, autoimmune, and genetic factors. X chromosomal abnormalities [partial monosomies and X;autosome-balanced translocations] are often observed in POF patients. These rearrangements cluster at Xq13-q26 called as the critical region (for POF). The critical region is separated into two, critical region 1 at Xq13-q21 and critical region 2 at Xq23-q26. It may be possible that ovarian development and/or function are hampered by haploinsufficiency of genes in these regions. However, genetic factors of POF are more complex as X;autosome-translocations often disrupt no genes and other factors such as position effects of autosomal genes are proposed. We had an opportunity to analyze four cases of POF each having t(X;autosome). Chromosome analysis revealed 46,X,t(X;4)(q21.3;p15.2) in case 1, 46,X,t(X;2)(q22;p13) in case 2, 46,X,t(X;4)(q22.1;q12) in case 3, and 46,X,t(X;14)(q24;q32.1) in case 4. We hypothesized that precise determination by cloning and sequencing of translocation breakpoints in these patients should bring us direct evidence of POF-related genes. We could successfully clone the junction fragments from all the patients. Data of breakpoint sequences in relation to genes will be presented and discussed.

1234/W

A new case of dup/del8p associated with autism and epilepsy detected by Genome array-CGH. *A. Nucaro[1], T. Pisano[2], I. Chillotti[2], S. Zorco[3], N. Santini[3], C. Montaldo[3], C. Cianchetti[2], D. Pruna[2].* 1) CNR, Inst Neurogen Neurofarmacolog, Monserrato, Italy; 2) Clinica di Neuropsichiatria Infantile, Azienda Ospedaliero- Universitaria, Cagliari, Italy; 3) OBL, Dipartimento Scienze Chirurgiche, Università di Cagliari, Italy.

Autism is a neurodevelopmental disorder with early childhood onset and a prevalence of as much as 5/10,000. Symptoms that may contribute throughout life include qualitative impairments in reciprocal communication and social interaction, as well as repetitive and stereotyped behavior. We report on a case of 8-year-old male with partial trisomy 8p (22;23.1)/partial monosomy 8p(23.2;pter) associated with autism, mild dysmorphic features, epilepsy and moderate learning disability. The cryptic deletion has been detected by Genome array- CGH. Although mental retardation is a common finding in patients with mosaic trisomy 8 or partial trisomy of various regions of chromosome 8, only few cases associated with autism and epilepsy have been reported so far (Glancy et al. 2009). In our case a cryptic deletion is also present. Clinical manifestations were mild compared to other patients with duplication of the same region of chromosome 8. Recently, Chien et al (2010) identified a terminal deletion of about 2.4 Mb at 8p 23.2;pter in a boy with autism and epilepsy. It is known that there is a close relationship between autism and epilepsy as about 30% of autistic individuals also have a history of seizures. Although there has been no strong evidence for linkage on chromosome 8p in any of the genome-wide linkage studies so far, the possibility that this segment includes genes involved in the etiology of both autism and/or epilepsy should be further explored.

1235/W

Prenatal Diagnosis of a Recombinant Chromosome 5 Resulting from a Complex Maternal Chromosome Rearrangement. *V. Potluri[1], J. Smith[1], J. Hoskovec[2], T. Solomon[1], P. Papenhausen[3].* 1) Dynagene/Labcorp, Houston, TX; 2) University of Texas Houston Medical School, Houston, TX; 3) Laboratory Corporation of America, Research Triangle Park, NC.

A 23 year old G2P1 woman was referred for prenatal testing at 30 weeks gestation due to abnormal targeted ultrasound findings including unilateral cleft lip, colpocephaly, possible ventricular septal defect and intrauterine growth restriction. An MRI confirmed the presence of the cleft lip without a cleft palate; no other brain malformations in addition to colpocephaly were detected. She has a previous child from a different partner with developmental delay. Chromosome analysis of amniotic fluid showed additional chromatin of unknown origin on the short arm of chromosome 5. A complex rearrangement involving chromosomes 5 and 9 was observed on the maternal analysis, but the maternal derivative 5 was not the same as the fetal derivative 5. After chromosome painting, the derivative chromosomes were described as follows: der(5)(9pter->9p13::5p15.1->5q13::9p13->9p11::5p15.1->5pter),der(9)(5qter->5q13:: 9p11->9qter). The baby received the normal maternal 9 and a recombinant abnormal 5. A whole arm exchange between the complex maternal der(5) and her normal 5 must have occurred during meiosis to produce the baby's rec(5) which can be described as follows: rec(5)(9pter->9p13::5p15.1->5qter). Therefore, the fetus is trisomic for 9p13 to 9pter and monosomic for 5p15.1 to 5pter. A whole genome microarray analysis was performed on the maternal sample using the Affymetrix 6.0 SNP platform. A 980 kb interstitial deletion was detected in 5p15.1, one of the breakpoints assigned by G banding to her derivative 5. The linear positions of the deletion are 16,338,682 and 17,318,602. Four known OMIM annotated genes are in the deleted region (FAM134B; MYO10; ZNF622; BASP1) with minimal known copy number variant overlap. Additional studies are in progress on her developmentally delayed son and on her mother. The maternal rearrangement appears to represent a translocation with an additional pericentric inversion of the derivative 5 which is likely to predispose to unbalanced recombinants, but which in itself, may not be balanced.

1236/W

Jumping translocation: A conundrum. *K. Reddy.* Cytogenetics department, Kaiser Permanente of Southern California, 4580 Electronics Place, Los Angeles, CA 90039.

Jumping translocations (JTs) are rare constitutional or acquired rearrangements involving a donor and several receiver chromosomes. They may be inherited or de novo. JTs can be found as a cultural artifact, in normal individuals or in pathological conditions. The clinical consequences range from spontaneous abortion, loss of fetus, chromosome syndrome, congenital abnormalities, infertility to malignancy. The breakpoints of JTs, are localized predominantly in repeat regions such as pericentromeric, centromeric, subtelomeric, telomeric and occasionally interstitial regions that may be a low copy repeat(LCR) or variant telomere sequence. Differences between the constitutional and acquired JTs donor breakpoints suggest an independent mechanism in their formation. We describe: a new jumping translocation found during CVS prenatal diagnosis of a twin pregnancy using molecular cytogenetic techniques; discuss two new JTs in hematological malignancy involving rare chromosome donor sites (3q21 and 11q13) and consolidate our knowledge on JTs. This study highlights the diagnostic challenges of JT, recognizes differences in donor sites in constitutional versus hematological malignancies and within different types of hematological disorders.

1237/W

Sporadic Aneuploidy in PHA-Stimulated Lymphocytes of Trisomies 21, 18 and 13. *O. Reish[1,2], M. Regev[1], S. Girafi[1], M. Mashevich[1].* 1) Gen Inst, Assaf Harofeh Med Ctr, Zerifin, Israel; 2) Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel.

Background: In accordance with previous study demonstrating monosomy for one sex X-chromosome is associated with sporadic loss and/or gain of other chromosomes, we studied here whether this instability is a consistent finding in constitutional autosomal trisomies. **Methods:** We used PHA-stimulated lymphocytes derived from 14 patients (10 patients with trisomy 21, 2 with trisomy 18 and 2 with trisomy 13). Fourteen healthy controls were compared. Fluorescence in-situ hybridization (FISH), applied at interphase, was used to evaluate the level of aneuploidy for three, randomly selected chromosomes (autosomes 8, 15 and 16) in each sample. **Results:** For each tested chromosome, our results showed a significantly higher level of aneuploid cells in the samples from the patients than in those from controls (P<0.05 -0.001) with no difference between the patients (P>0.2). The mean level of aneuploid cells (percentage) for all three tested autosomes (8, 15 and 16) was almost twice as high in the patients samples as in the control samples (2.51 vs 1.17, 5.05 vs 2.71, 2.59 vs 1.67 respectively). Aneuploidy level was mainly due to monosomy which was significantly higher in the samples from the patients than in those from controls for each one of the tested chromosomes (P<0.05- 0.001), with no difference between the patients (P>0.53). The mean level of monosomic cells (percentage) for all three tested chromosomes was almost twice as high in the patients samples as in the control samples (2.2 vs 1.03, 3.77 vs 2.14, 2.04 vs 1.46 respectively). **Discussion:** Our study shows that variable constitutional autosomal trisomies are associated with increased frequency of non-chromosome specific aneuploidy. It is possible that primary aneuploid cells destabilize their own genome resulting in variable aneuploidy of other chromosomes. It is also possible that a common factor(s) is involved with both constitutional and sporadic aneuploidy. As cancer is associated with aneuploidy and incidence of cancer is higher in Trisomic patients comparable to controls, our study may uncover a possible mechanism linking this newly detected instability with cancer and may assist in future therapy planning.

1238/W

Monozygotic twins discordant for submicroscopic chromosomal anomalies detected by array CGH. *M. Rio[1], MC. de Blois[1], C. Turleau[1], S. Gobin[1], G. Royer[1], C. Ozilou[1], L. Colleaux[1], A. bernheim[2], M. Vekemans[1], V. Malan[1].* 1) Département de Génétique, Hôpital Necker-Enfants Malades, Paris, France; 2) Laboratoire de genomique cellulaire des cancers (FRE 2939 CNRS) & Département de Pathologie Moleculaire Institut de Cancérologie Gustave Roussy, Paris-Villejuif Cedex, France.

Although discordant phenotypes in monozygotic twins used to be considered as an exception, an increasing number of reports indicate that this phenomenon is not so rare. Most of reported patients have numerical chromosomal anomalies, with only few cases having structural chromosomal anomalies. Here, we report on the clinical and cytogenetic details of 4-year-old female monozygotic twins with discordant phenotypes. Twin 1 exhibited global developmental delay with walk at 35 months, absence of speech, and hyperactivity. Twin 2 had an autistic spectrum disorder without motor delay. Extensive investigations including blood karyotype, metabolic screening and brain MRI were normal in both twins. Molecular karyotyping in twin 1 identified a 2p25.3 deletion, further confirmed by FISH analysis on leukocytes in all cells. Interestingly, array-CGH was normal in twin 2 but FISH analysis performed on leukocytes using the same probes showed mosaicism with 33 % of deleted cells, 33 % of duplicated cells, and 33% of normal cells. Genotyping confirmed the monozygosity of the twins and ruled out uniparental disomy for chromosome 2. We propose that the discordant chromosome imbalance may be due to a mitotic non-allelic recombination occuring during blastomeric divisions of a normal zygote. Such event will result in 3 distinct cell populations whose proportion in each embryo formed after separation from the inner cell mass, may differ, leading to discordant chromosomal anomalies between twins. To our knowledge, this is the first report of monozygotic twins with discordant phenotypes ascribed to a distinct submicroscopic rearrangement detected by array-CGH.

1239/W

Deletions flanked by breakpoints 3 and 4 on 15q13 may contribute to abnormal phenotypes. *J.A. Rosenfeld[1], L.E. Stephens[2], J. Coppinger[1], B.C. Ballif[1], J.J. Hoo[3], B.N. French[3], V.C. Banks[4], W.E. Smith[4], D. Manchester[5], A.C-H. Tsai[5], K. Merrion[5], R. Mendoza-Londono[6], L. Dupuis[6], R. Schultz[1], B.S. Torchia[1], T. Sahoo[1], B.A. Bejjani[1], D.D. Weaver[2], L.G. Shaffer[1].* 1) Signature Genomic Laboratories, Spokane, WA; 2) Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN; 3) Department of Pediatrics, University of Toledo Medical College & NW Ohio Regional Genetics Center, Toledo, OH; 4) Division of Genetics, Maine Medical Center, Portland, ME; 5) Division of Clinical Genetics and Metabolism, Department of Pediatrics, The Children's Hospital, University of Colorado Denver School of Medicine, Aurora, CO; 6) Division of Clinical and Metabolic Genetics, The Hospital for Sick Children and University of Toronto, Toronto, Canada.

Non-allelic homologous recombination (NAHR) between segmental duplications in the proximal chromosome 15q breakpoint (BP) regions can lead to microdeletions and microduplications. Several individuals with deletions flanked by BP3 and BP4 on 15q13, immediately distal to and not including the Prader-Willi/Angelman syndrome (PW/AS) critical region and proximal to the BP4-BP5 15q13.3 microdeletion syndrome region, have been reported, but because the deletion has also been found in normal relatives, the significance of these alterations is unclear. We have identified six individuals with deletions limited to the BP3-BP4 interval and an additional four individuals with deletions of the BP3-BP5 interval from 34,046 samples submitted for clinical testing by microarray-based comparative genomic hybridization (aCGH). Of four individuals with BP3-BP4 deletions for whom parental testing was performed, two were apparently de novo, and two were maternally inherited. A comparison of the clinical features, available for five individuals in our study (four with deletions within BP3-BP4 and one with a BP3-BP5 deletion), to those in the literature shows common features of short stature and/or failure to thrive, microcephaly, hypotonia, and premature breast development in some individuals. Although the BP3-BP4 deletion does not yet demonstrate statistically significant enrichment in abnormal populations compared to control populations, the presence of common clinical features among probands and the presence of genes with roles in development and nervous system function in the deletion region suggest this deletion may play a role in abnormal phenotypes in some individuals.

1240/W

The Genetic Variations that Cause Down Syndrome and Oral Manifestations. *E. Severin[1], A. Stan[2], L. Savu[2], D. Stanciu[1].* 1) Dept Human Genetics, Carol Davila Univ Med Pharm, Bucharest, Romania; 2) Genetic Lab, Bucharest Romania.

Various genetic mechanisms such as, full trisomy 21, mosaic trisomy 21 or translocation trisomy 21, may be contributors to the variability of the Down syndrome (DS) phenotype. The severity of clinical features varies from an individual with DS to another. How trisomy 21 leads to the phenotype of DS is not fully understood. The aims of the study: to evaluate the karyotype pattern in the patients with DS and to find out if there are correlations between the severity of the oro-dental phenotype and chromosomal abnormality mechanism of trisomy 21. A total of 26 individuals with DS ranging from 2 to 30 years of age were chromosomally investigated in order to confirm the clinical diagnosis of DS. All the cases were diagnosed cytogenetically after birth. A karyotype of the parents was required in the case of a child with robertsonian translocation trisomy 21. To characterize the oro-dental phenotypic spectrum of the patients were performed oral, clinical and radiological evaluations. Based on cytogenetically analysis results three groups were identified: patients with full trisomy 21, mosaicism for trisomy 21 and translocation trisomy 21. Most oro-dental features including oral, dental, and periodontal and occlusion anomalies were variable in both frequency and expression. The most common dental anomaly was hypodontia followed by microdontia. Concomitant hypodontia and supernumerary teeth occurred in one patient. Eruption of dentition was delayed in most cases. Taurodontism occurred in few patients. Variation of oro-dental phenotype is not associated with specifically chromosomal mechanisms of trisomy 21.

1241/W

Usefulness of molecular techniques in characterization of sSMC^T. *F. Sheth[1], J. Pani[1], M. Desai[1], J. Andrieux[2], T. Liehr[3], A. Weise[3], S. Mehta[1], H. Patil[1], H. Sheth[4], J. Sheth[1].* 1) Department of Cytogenetics, FRIGE's Institute of Human Genetics,Satellite, Ahmedabad, Gujarat, India; 2) Laboratory of Medical Genetics, Jeanne de Flandre Hospital CHRU de Lille, Lille Cedex France; 3) Jena University Hospital, Institute of Human Genetics and Anthropology, Kollegiengasse 10, D-07743 Jena, Germany; 4) School of Biomedical Sciences, Newcastle University, Framlington Place, Newcastle Upon Tyne, NE2 4HH, UK.

Small supernumerary marker chromosomes [sSMC] are usually observed in addition to numerically normal "essential karyotype". However, they are rarely seen in numerical abnormal karyotypes similar to Turner syndrome [sSMC^T]. Around 565 cases have been identified till date with the frequency of 1 in 100,000 in the general population. In the present study, sSMC^T is documented in three cases where case-1 is a 13-years-old female having clinical presentation of Turner syndrome and cytogenetic finding showed 46,X,+mar. Array-CGH, cenM-FISH, subcenM-FISH mix analysis characterized the sSMC as a del(14)(q11.1) which consisted only of heterochromatic region according to array-CGH and FISH. Case-2 is an 8 month-old child born to consanguineous parents having clinical features of intersex. Karyotypic pattern revealed mosaic cell line with 46,X,mar/45,X of equal number. subcenM-FISH mix for Y-chromosome confirmed marker to be idic(Y)(q11.2) having both euchromatic and hetrochromatic region. Case-3 is a 24 year-old phenotypically male, which was referred due to clinical features of ambiguous genitalia. Chromosomal analysis initially showed 45,X cell line with positive SRY-PCR. Further analysis after the application of subcenM-FISH mix for Y-chromosome analysis and DNA studies for a total of 18 different Y chromosomal loci showed that the rearranged Y was accompanied by SRY region and partial deletion of pseudoautosomal region (PAR1) involving SHOX as a del(Y)(:p11.2->q11.1:) which was missed by routine banding technique. This shows that the sSMC, when associated with a numerical abnormal karyotype, mainly originates from Y chromosome and they are inverted duplicated/isodicentric. sSMC^{T}s of a non gonosomal origin might be easily missed if they are not further characterized by molecular approaches. Overall, this shows the usefulness of oligonucleotide array-CGH is the best initial technique for the characterization of sSMC that can precisely measure the size of the aberration (covering euchromatic region) in a single experiment followed by DNA analysis which together with FISH gives additional information for the structure and the level of mosaicism. This part of the study was supported by DBT-India.

1242/W

Complex chromosomal rearrangements in males: complexity of rearrangements affects spermatogenesis. *S. Shim[1,2], J. Kim[2], S. Song[4], M. Chin[1], K. Kang[1], T. Yoon[2], D. Cha[1,3].* 1) Genetic Lab, CHA General Hosp, Seoul, Korea; 2) Fertility Center of CHA Gangnam Medical Center; 3) Department of Obstetrics and Gynecology, Kangnam CHA Hospital, CHA University; 4) Department of Urology, CHA University.

Objective: Complex chromosome rearrangements (CCRs) are very rare structural aberrations involving at least three chromosomes or three or more chromosomal breakpoints. Carriers of CCRs have a risk for having a conception with various anomalies and reproductive failure. The most of males with CCRs showed infertility problems resulting from hypospermatogenesis or spermatogenic failure. **Methods**: Ten males with CCRs, who were phenotypically normal, were referred to the Genetic laboratory of CHA Gangnam Medical Center from 2002 to 2009. Seven patients were azoo- or oligozoospermia and the other three were habitual abortions(HA). Sexual development was normal in all patients. The patients were carried out chromosome analysis and histological examination as well as basic laboratory tests such as hormone and sperm assays. Chromosome abnormalities were characterized by FISH and M-FISH as well as routine G-banding. For seven patients with spermatogenetic defects, testicular biopsies were performed and azoospermic factors (AZFs) region defects were also examined. **Results**: Three cases showed OAT and four cases were diagnosed as azoospermia. The level of testosterone was slightly lower in one case. None of the patients with impaired spermatogenesis had a deletion of AZF regions. One azoospermic case was defined as sertoli cell only, and the other three azoospermic cases showed maturation arrest at the early spermatid stage. CCRs identified in ten patients were classified as follow: two cases were group I, three cases were group II and five cases were group III. All three cases with HA and two of three cases with OAT had group III CCRs. On the other hand, CCRs identified in one OAT case and all four azoospermic cases belonged to group I and II. Several breakpoints, located at chromosome 4q, 5q, 7q, 9p, 14q, were frequently reported in HA and breakpoints related to spermatogenetic failure were apt to locate in chromosome 3, 4q, 11q, 12q, 13q. However, the breakpoints randomly distributed, in general. **Discussion**: We understood that not locations of breakpoints but also complexity of chromosomal rearrangement in patients with CCRs could have a role in male infertility and might affect the spermatogenetic process, although more fine characterizations of the chromosomal breakpoints using an advanced technique such as array-based comparative genomic hybridization are necessary.

1243/W

Double and Triple Aneuploidy in Spontaneous miscarriages: A single institution experience. *S. Subramaniyam, LM. Alonso, VR. Pulijaal, S. Mathew.* Pathology and Laboratory Medicine, Cytogenetic laboratory, Weill Cornell Medical College/New York Presbyterian Hospital, New York, NY:10065.

Chromosomal analysis on products of conception (POC) is useful to determine the cause for pregnancy loss and aid in recurrence risk estimates for future pregnancies. More than 50% of first trimester miscarriages are due to chromosomal abnormalities. Double and triple aneuploidy (gain or loss of two or three chromosomes) is rare and is reported in 0.21~ 2.8% of the aborted fetuses with a chromosomal analysis. We present data on double/triple aneuploidy encountered at our institution from January 2006 to December 2009. We retrospectively reviewed 1599 cases of POC. Successful karyotypes were obtained in 94% (1502/1599) of the cases. Of these, 63.2% (949/1502) had an abnormal karyotype and the rest 36.8% (553/1502) showed a normal karyotype. The different abnormalities included loss/monosomy (10%), single trisomy (65%), double aneuploidy (7.4%), triple aneuploidy (0.6%), triploidy (10.8%), tetraploidy (2.3%), and structural abnormalities (3.9%). Among the total 1502 (abnormal and normal) karyotyped cases, 70 cases (4.6%) with double aneuploidy and six cases (0.4%) with triple aneuploidy were observed in this study. Median gestational age for double and triple aneuploidy was 8 weeks (range, 5 ~ 13 weeks), and the median maternal age was 41 years (range, 31 ~ 44 years). Of these 76 cases, 13 women had an abnormal karyotype in the previous pregnancy of which two cases had double aneuploidy, with no recurrence of the same chromosomes. The chromosomes most frequently involved in double/triple aneuploidy were chromosomes 21 (35.5%), 16 (26%), 22 (18.5%), X (17%), 18 (13%), 13 (10.5%) and 15 (10.5%). The most frequent chromosome combinations observed were: X/21 (8 cases, 10.5%), 21/22 (4 cases, 5%), 16/21 (3 cases, 4%), and 7/21 (3 cases, 4%). In conclusion, we have observed a high percentage of double (4.6%) and triple (0.4%) aneuploidy in our series when compared to the previous studies. This abnormal rate is attributed due to the high success rate in obtaining metaphases by both direct and long term cultures at our institution.

1244/W

Complex chromosome 4 rearrangement characterized by array CGH. *J. Szymanska, A. Hajianpour, R. habibian, J.C. Wang.* Cytogenetics, Genzyme Genetics, Monrovia, CA.

A 34 year old woman was referred for prenatal diagnosis due to the abnormal ultrasound findings including IUGR, cardiac anomaly, and clubbed feet. Cytogenetic analysis of G-banded metaphases showed additional genetic material of unknown origin on the short arm of chromosome 4, interpreted as add(4)(p15.3). Parental karyotypes were normal. Array comparative genomic hybridization (aCGH) revealed DNA copy number changes, including: a 2.95 Mb terminal deletion of chromosome 4 at 4p16.3, and a 20.7 Mb gain of chromosome 4 long arm, including 4q32.3-q35.2. FISH analysis using BAC clones from the regions 4p16.3 (RP11-1150B4) and 4q35.2 (RP11-706F1), plus the chromosome 4 centromere probe (D4Z1) identified a derivative chromosome 4 with the 4q35.2 clone inserted into the distal short arm at 4p16.3 with simultaneous loss of 4p16.3 sequences. The rearrangement was described as ish der(4)del(4)(p16.3)ins(4)(p16.3q32.2q35.2)(RP11-1150B4-,RP11-706F1+) The fetus is therefore trisomic for the segment 4q32.3-q35.2 and monosomic for 2.95 Mb DNA sequences within 4p13.6. The deleted region at 4p16.3 contains at least 30 genes, including the Wolf-Hirschhorn syndrome critical region (WHSC1/WHSC2). The 4q32.3-q35.2 duplication region contains at least 36 currently known OMIM genes. This case illustrates the importance of comprehensive testing in prenatal diagnosis, combining conventional cytogenetics, aCGH, and FISH analyses. It also demonstrates how all three techniques are necessary for an accurate interpretation.

1245/W

A familial complex chromosome rearrangement (CCR), der(8)t(8;21)(q24.3;q22.1)idic(21)(q22.1), resulting in a 18.6 Mb duplication of proximal long arm of chromosome 21 with minimal dysmorphic features. *G.V.N. Velagaleti[1], E.R Roeder[2], V.S. Tonk[3], D.M. Farley[4], R.W Huff[4].* 1) Dept Pathology, Univ Texas Hlth Sci Ctr, San Antonio, TX; 2) Dept Pediatrics, Univ Texas Hlth Sci Ctr, San Antonio, TX; 3) Dept Pediatrics, Texas Tech Univ, Lubbock, TX; 4) Dept. Obstetrics and Gynecology, Univ Texas Hlth Sci Ctr, San Antonio, TX.

The widespread use of comparative genomic microarrays (CGH) has resulted in identification of many genomic imbalances including large genomic duplications. The phenotypic variability associated with these duplications precludes identification of carriers of these duplications based on phenotypic evaluation. Here we report on a family where a CCR is identified in a mother and her children with variable phenotype. A 24-year- old, Hispanic woman was referred for prenatal counseling due to a maternal translocation. She has had 5 first trimester miscarriages and one preterm male that died at 3 months. He had congenital heart disease and clubbed feet. She underwent amniocentesis at 16 weeks gestation. Her height is 60 inches (152 cm) and weight 184 lb (83.5 kg). She had mild dysmorphic features including bilateral ptosis, mild cubitus valgus and short 4th metatarsals bilaterally and 4th toes overlap 5th toes, mild 2-3 toe syndactyly and mildly short great toes with a gap between 1st and 2nd toes. She does not have Down syndrome facies. She is mildly retarded and has a seizure disorder. An echocardiogram obtained recently because of her son's history was normal. Her fetus showed no anomalies on ultrasound at 28 weeks gestation with normal growth. Chromosome analysis from peripheral blood lymphocytes showed a CCR with karyotype, 46,XX,der(8)t(8;21)(q24.3;q22.1)idic(21)(q22.1), idic(21)(q22.1). Array CGH studies showed an 18.6 Mb duplication involving the 21q11.2-q22.11 region with no imbalance on the chromosome 8. Subtelomeric FISH studies showed both the 8q and 21q subtelomeres present on the der(8). Amniotic fluid chromosome analysis showed two cell lines on her fetus with the karyotype 46,XY,der(8)t(8;21)(q24.3;q22.1)idic(21)(q22.1), idic(21)(q22.1)[16]/ 46,XY,idic(21)(q22.1)[3]. Chromosome analysis on her son that expired at 3 months showed partial monosomy with 45,XY,der(8)t(8;21)(q24.3;q22.1). Chromosome studies from the grandparents showed that the mother of our proband is the carrier of 5 different cell lines and transmitted the CCR to her daughter who in turn transmitted it to her children. This is the first case to our knowledge of a CCR with large duplication segregating in several members of the family with minimal phenotypic manifestations and normal fertility.

1246/W

8p23.1 duplication and 15q25-26 duplication due to an (11;15)(p13;q25q26) insertion characterized by high resolution array in a patient with aniridia. *T.P. Vieira[1], M. Simioni[1], I.C. Sgardioli[1], C.V. Maurer-Morelli[1], I. Lopes-Cendes[1], A.C. Fett-Conte[2], V.L. Gil-da-Silva-Lopes[1].* 1) Department of Medical Genetics, Faculty of Medical Sciences,-University of Campinas, Campinas, SP, Brazil; 2) Molecular Biology Department, Medicine School in São José do Rio Preto, São José do Rio Preto , SP, BRAZIL.

High resolution array technology has provided a better understanding and insight into the delineation of chromosomal abnormalities. Here we report on a two years old male patient referred to us for investigation of velocardiofacial syndrome. He presented cardiac anomaly (ventriculoseptal defect), submucous cleft palate, dysmorphic facies, developmental delay, renal anomaly (hydronephrosis) and aniridia. Multiplex ligation-dependent probe amplification analysis with the SALSA P250 kit, was normal for the 22q11 region, but it showed a duplication in 8p23.1. Subsequent studies with a high resolution array (Genome-Wide Human SNP Array 6.0, Affymetrix®), confirmed an 8p duplication of 3.8 Mb at p23.1 region (8.129.435-11.934.586 [hg18]), and revealed an additional interstitial duplication of 17.09 Mb at 15q25-26 region (81.869.248-98.962.477 [hg18]). In order, to confirm the large duplicated segment detected, we performed chromosome (chr) analysis that revealed an insertion of chr 15 on chr 11 [46,XY,der(11)-ins(11;15)(p13;q25q26)]. Both cytogenetic and array analyses of parents were normal. Since our patient has aniridia, a more detailed analysis of the array results was performed for chr 11p13, and a small deletion of approximately 49 kb was also detected. This deletion is 12 kb downstream of the PAX6 gene and includes the ELP4 gene. The 8p and 15q duplications such as these identified in our patient are well characterized in the literature, and interestingly our patient presents typical clinical features of both: i) cardiac anomaly as described in patients presenting 8p duplication, and ii) renal anomaly as described in individuals with 15q25-26 duplication. Additionally, the (11;15) insertion probably altered the normal expression of the PAX6 gene by a position effect or deletion of a regulatory element, which explains the aniridia phenotype that is not described in association with 8p23.1 nor 15q25-26 duplications. This case report shows that array analyses together with classical cytogenetic techniques are making an extraordinary impact in our way of seen chromosome abnormalities and their phenotypic correlates. Financial support: Fapesp and CNPq.

1247/W

Cytogenetic abnormalities in amniotic fluid cells. *M. Woo[1], J. Choi[1], N. Song[1], H. Ko[1,2], S. Oh[1,2], C. Park[2], K. Park[2,3], J. Park[2], J. Jun[2], B. Yoon[2], Y. Choi[1,2], S. Moon[1,2].* 1) Institute of Reproductive Medicine and Population, Medical Research Center, Seoul National University, Seoul, Korea; 2) Department of Obstetrics and Gynecology, College of Medicine, Seoul National University; 3) Seoul National University Bundang Hospital, Bundang, Korea.

This study reviewed cytogenetic results in amniotic fluid cells and analyzed chromosomal abnormalities according to patient's indications. The 2438 cases obtained by amniocentesis were performed using traditional cytogenetic methods at the Institute of Reproductive Medicine and Population, Seoul National University between January 2005 and December 2009. The cytogenetic results of 2438 cases on cultured amniotic fluid samples were observed normal chromosomes (2283 cases, 93.6%), chromosome heteromorphisms (77 cases, 3.2%), and chromosomal abnormalities (78 cases, 3.2%). A total of 78 cases of abnormal fetal karyotypes were identified including 54 cases (2.2%) of numerical aberrations, 14 cases (0.6%) of structural aberrations, and 10 cases (0.4%) of mosaicism. Numerical abnormalities consisted of autosomal aneuploidies with trisomy 9 (1 case), 13 (3 cases), 18 (12 cases), and 21 (29 cases), sex chromosome abnormalities (7 cases), and polyploidy (1 case). Structural abnormalities showed balanced reciprocal translocations (10 cases), deletion (1 case), addition (1 case), and marker chromosome (2 cases). The 10 fetuses with balanced reciprocal translocations were inherited from three maternal carriers, five paternal carriers and two de novo, respectively. The rate of indication with chromosome aberrations was most frequently detected in advanced maternal age (35.9%), followed by abnormal ultrasonographic findings and abnormal maternal serum marker positive. Of fetuses with an abnormal karyotype, the abnormal ultrasonographic findings in the cases observed trisomy 21 could be considered a predictive marker. These data provides a comprehensive review of the prenatal diagnosis and helpful information in genetic counseling for patients with the high-risk pregnancy.

1248/W

Clinical and Molecular-Cytogenetic Characterization of Partial Monosomy 13 (13q33-13qter) in Adults with Ring Chromosome 13. *R. Badilla-Porras[1], D.J. Stavropoulos[2], L. Dupuis[1], R. Mendoza-Londono[1].* 1) Division of Clinical and Metabolic Genetics, The Hospital for Sick Children and University of Toronto,Toronto, Canada; 2) Department of Paediatric Laboratory Medicine, The Hospital for Sick Children and University of Toronto, Toronto, Canada.

Partial monosomy of chromosome 13q is a rare condition. Ring chromosomes result from the breakage of a portion of both arms of a chromosome with fusion of the points of fracture. Loss of the distal fragments result in a partial monosomy and are commonly associated with congenital malformations and mental retardation. We present the clinical characterization of two adult patients with ring chromosome 13 with overlapping deletions of 13q33-13q34. We define the cytogenetic abnormality by array comparative genome hybridization (Array CGH), and compare their features with other cases of partial monosomy 13q. Patient 1 is an 18 year-old male born after pregnancy complicated by IUGR. His birth parameters at 36 weeks of gestation were below the third centile. He developed self-resolving early chylothorax and PDA. Urogenital malformations included hydronephrosis and posterior ureteral valves. Additional findings included microcephaly ataxia, short stature, strabismus and constipation. He has severe developmental delay and lack of verbal language. He completed life-skills training. Karyotype analysis revealed a ring chromosome 13. Array CGH identified a deletion of chromosome region 13q33.1 to 13qter with an estimated size of 13.528 Mb. Patient 2 is a 33 year-old female born at 38 weeks of gestation with birth parameters below the third centile. She developed early feeding difficulties. She developed diabetes mellitus type 2 at age 20 and hyperlipidemia at age 28. She has mild to moderate developmental delay with speech delay but was able to complete school to grade 10 and is now able to hold a job and live with supervised independence. Additional findings include short stature and myopia. Karyotype analysis revealed a ring chromosome 13. Array CGH identified a deletion of chromosome region 13q34 to 13qter with an estimated size of 1.957 Mb. The cases presented herein highlight the clinical variability in the phenotype of patients with partial monosomy 13q due to ring chromosome and allow a better genotype-phenotype correlation. Common features in patients with monosomy of distal 13q include short stature, developmental delay and dysmorphic features. Our findings also demonstrate that patients with this cytogenetic abnormality survive into adulthood without major complications, which is distinctive from patients with more proximal breakpoints involving band 13q32.

1249/W

Deletion 12Q: A first patient with 12q14 microdeletion syndrome Delineated by Whole Genomic Array Comparative Genome Hybridization. *C. Chong Kun.* Pediatrics, Pusan National Univ Chld Hosp, Geungsang Nam Do, Korea.

The 12q14 microdeletion syndrome is a rare chromosomal abnormality. Characteristic features of the 12q14 microdeletion syndrome include low birth weight, failure to thrive, short stature, learning disabilities and Buschke-Ollendorff lesions in bone and skin.We report a 12(q14.3q14.3) deletion identified by array-CGH analyses in a boy resenting with growth retardation, developmental delay, and dysmorphic features. We utilized whole genomic array comparative genome hybridization (CGH)with 4,000 selected bacterial artificial chromosomes (BACs) to define the chromosomal breakpoints and to delineate the extent of the microdeletion in more detail. To our knowledge, this is the novel microdeletion, rarely reported to-date, defined by whole genomic array CGH.

1250/W

Further delineation of the 17p13.3 microdeletion involving *YWHAE* but distal to *PAFAH1B1*: four additional patients. *A. Delahaye[1], M. Schiff[2,3], J. Andrieux[4], D. Sanlaville[5,6], S. Passemard[2,3,7], A. Labalme[5], L. Perrin[7], S. Bouquillon[4], M. Elmaleh-Berges[3,8], A. Aboura[7], S. Drunat[7], S. Manouvrier-Hanu[9], B. Benzacken[1,3,7], P. Edery[5,6], A. Verloes[3,7], C. Vincent-Delorme[9].* 1) Histology-Embryology & Cytogenetics Department, AP-HP - Jean Verdier Hospital, Bondy, France; Paris 13 University, UFR SMBH, Bobigny, France; 2) Department of Pediatric Neurology , APHP - Robert DEBRE University Hospital, Paris, France; 3) Inserm, U676, Paris, France; 4) Laboratoire de Génétique Médicale, Centre Hospitalier Régional Universitaire, 59000 Lille, France; 5) Service de Génétique, Hospices Civils de Lyon, Hôpital de l'Hotel Dieu, 69288 Lyon, France; 6) EA 4171, Université Claude Bernard, 69622 Lyon, France; 7) Department of Genetics, APHP - Robert DEBRE University Hospital, Paris, France; 8) Pediatric Imaging Department, AP-HP - Robert DEBRE University Hospital, Paris, France; 9) Service de Génétique Clinique, Hôpital Jeanne de Flandre, Centre Hospitalier Universitaire de Lille, 59000 Lille, France.

BACKGROUND: The 17p13.3 deletion syndrome (or Miller Dieker syndrome, MDS, MIM 247200) is characterized by lissencephaly, mental retardation and facial dysmorphism. The phenotype is attributed to haploinsufficiency of two genes present in the minimal critical region of MDS: *PAFAH1B1* and *YWHAE*. Whereas isolated *PAFAH1B1* deletion causes lissencephaly, *YWHAE* is a candidate for the dysmorphic phenotype associated with MDS.

OBJECTIVE: We describe clinical, neuroradiological and molecular data in four patients with a 17p13.3 deletion distal to *PAFAH1B1* involving *YWHAE*.

RESULTS: All patients presented with mild or moderate developmental delay and pre and/or post-natal growth retardation. Patients A, B and C had leucoencephalopathy with macrocephaly (patients A and C), Chiari type 1 malformation (patient A) and paraventricular cysts (patient C). Patient B had patent ductus arteriosus and pulmonary arterial hypertension. Patient C had unilateral club foot. Patient D had enlarged Virchow Robin spaces, microcornea and chorioretinal and lens coloboma. Array-CGH revealed *de novo* terminal 17p13.3 deletions for patient A and B, and showed interstitial 17p13.3 deletions of 1.4 Mb for patient C and of 0.5 Mb for patient D.

CONCLUSION: Our patients confirm that 17p deletion distal to *PAFAH1B1* have a distinctive phenotype : mild mental retardation, moderate to severe growth restriction, white matter abnormalities and developmental defects including Chiari type 1 malformation and coloboma. Our patients contribute to the delineation and clinical characterization of 17p13.3 deletion distal to *PAFAH1B1* and highlight the role of the region containing *YWHAE* in brain and eye development and in somatic growth.

1251/W

WAGR Syndrome associated with unbalanced segregants of a balanced insertion. *I. Gadi[1], j. Tepperberg[1], V. Jaswaney[1], S. Schwartz[1], R. Burnside[1], B. Williford[1], H. Risheg[1], P. Papenhausen[1], D. Day-Salvatore[2], M. Horner[2].* 1) Dept Cytogenetics, Lab Corp America, Res Triangle Park, NC; 2) St. Peters Medical Center New Brunswick, N.J.

In this study we report cytogenetics , FISH and SNP array analyses in a family in which some individuals presented with Wilms tumor, abnormal genitalia and mental retardation while others presented with Beckwith Wiedemann syndrome. Our initial cytogenetic analysis of a normal adult, who has two sons, a sister and a niece with WAGR, revealed an apparent deletion of the proximal short arm of chromosome 11. A previous cytogenetic analysis had described the deletion break points p13->p15.1 region of chromosome 11. However no loss of chromosome 11 DNA was apparent from the whole genome SNP array analysis (Affymetrix 6.0 version) in this apparently normal individual. Subsequent FISH using a whole chromosome 11 paint probe (Abbott Molecular Inc.) showed partial hybridization in the proximal short arm of chromosome 6. A cytogenetic analysis of both children showed a deletion in the proximal short arm of chromosome 11. Both children presented with hypospdias and aniridia consistent with a deletion of 11p. The whole genome SNP array analysis on his children confirmed the cytogenetic studies and revealed a 12.381 Mb deletion of the chromosome 11 short arm p14.3->p13 segment (linear position 22,392,777-34,774,080). This study is important as it illustrates the importance of combining SNP analysis along with FISH and cytogenetic studies to determine chromosome structure and recurrence risk. The array has delineated the precise identity of the material deleted (leading to WAGR) or duplicated (leading to Beckwith-Weidemann Syndrome), while FISH and cytogenetic studies were instrumental in illustrating a high risk family.

1252/W

Complex rearrangements in monosomy 1p36. *M. Gajecka[1], J. Karolak[1], J. Shen[2], C. Glotzbach[3], L.G. Shaffer[3].* 1) Institute of Human Genetics, Polish Academy of Sciences, Poznan , Poland; 2) Children's Hospital Central California, Madera, CA, USA; 3) Signature Genomic Laboratories, LLC, Spokane, WA, USA.

Deletions of 1p36 occur in approximately 1 in 5,000 newborns. To date, we have ascertained 150 cases with monosomy 1p36, representing four possible classes of rearrangements: pure terminal deletions, interstitial deletions, unbalanced translocations, and complex rearrangements. Here we present five complex rearrangements involving duplications in monosomy 1p36. For each individual, deletion and duplication sizes, and parental origin of the rearrangements were determined using array CGH and genotyping. To further understand the mechanisms for concurrent deletions and duplications, we narrowed the breakpoints and investigated the junctions using molecular cytogenetics and molecular biology methods. Our results show high complexity at the breakpoint junctions and indicate involvement of multiple mechanisms in the DNA breakage and repair process during rearrangement formation.

1253/W

Clinical features in two patients with de novo overlapping interstitial deletions of chromosome 1q21.2. *J. Goldstein[1], A. McConkie-Rosell[1], E. Cordoba[2], P. Jayakar[3], S. Schwartz[4], I. Gadi[4], Y.H. Jiang[1].* 1) Pediatrics, Duke University Medical Center, Durham, NC; 2) St. Mary's Hospital, West Palm Beach, FL; 3) Miami Children's Hospital, Miami, FL; 4) Laboratory Corporation of America, Research Triangle Park, NC.

We report the clinical features of two children with de novo overlapping interstitial deletions of chromosome 1q21.2, one of which extends into chromosome 1q21.3. Patients with deletion of this chromosomal region have not been previously reported; the deleted region in both patients is distinct from the 1q21.1 microdeletion that has been associated with autism spectrum disorder (ASD) and schizophrenia. Patient 1 is a 12 year-old Caucasian old male with classic ASD, seizure disorder, cognitive impairment, amblyopia, astigmatism, and mild dysmorphic features including small chin and flat nasal bridge. His karyotype was normal (46, XY) but chromosome SNP array analysis revealed a 210 kb deletion of chromosome 1q21.2 (position 149,100,105 - 149,309,823). Fluorescence in situ hybridization (FISH) analysis, using a BAC probe from the deleted area, was normal for the patient's parents and sister, all of who are healthy and developmentally normal, suggesting that the deletion is de novo. Patient 2 is a 3 year-old Filipino male born at 34 weeks gestation and conceived by in vitro fertilization. He is the product of a twin pregnancy that was complicated by twin-twin transfusion. He has global developmental delay, hypotonia, history of febrile seizure, microcephaly, failure to thrive, cleft palate, and mild-to-moderate bilateral hearing loss. He has had a normal EEG and brain MRI. Chromosome SNP array revealed a 1.68 Mb deletion of chromosome 1q21.2-q21.3 (position 148,224,484 - 149,900,535). This deletion encompasses the deletion identified in Patient 1. FISH analysis, using a BAC probe from the deleted area, was normal for both parents, suggesting that the deletion is de novo. Results of FISH analysis for the twin of Patient 2 are pending. Further genetic and biochemical analysis of these two patients, as well as documentation of clinical features in other patients with deletions of chromosome 1q21.2, may lead to hypotheses regarding the pathogenesis of their clinical features, and whether altered expression of a particular gene(s) in this region may be associated with ASD.

1254/W

Isolation and characterization of cis-regulatory elements at the deletion junction for 9p- syndrome. *X. Hauge, P. Hord, J. Ezcurra, K. Traver.* Dept Biol & Physics, Kennesaw State Univ, Kennesaw, GA.

Deletions of the terminal region of the short arm of chromosome 9 (9p-) are associated with trigonocephaly, dysmorphic facial features, and mental retardation. The deletion of cerberus 1 (CER1) gene located at 14.7 Mb from the 9p terminus has been postulated as the cause of trigonocephaly. However, two independent studies showed that patients whose phenotype is consistent with 9p- syndrome possess smaller than 12.4 Mb deletions. Therefore, these patients have two, intact copies of CER1 gene. It raises the possibility that cis-regulatory elements for CER1 gene could be located in the first 12.4 Mb of 9p. Previously, we identified ~270 highly conserved sequences (CNSs) between 10 and 12.4 Mb from the 9p terminus. We report here cloning of 5 CNSs located at the deletion junction region (12.4 Mb) and characterization of their potential regulatory functions by using a dual luciferase reporter assay. The genomic DNA sequences corresponding to these 5 CNSs were amplified and cloned into a pGL4 vector at the 5' of the luciferase gene, which is controlled by a minimal promoter. A co-transfection of the CNSs-luciferase gene construct and a renilla gene, an internal control gene, was performed. To evaluate the tissue specificity of CNSs enhancer/silencer activity, two human cell lines were transfected. Of the 4 CNSs-luciferase constructs tested, one dramatically increased the luciferase expression in 2 types of cells, neuroblastoma and embryonic kidney cells. The second clone significantly reduced the luciferase expression in both types of cells, while the third clone showed tissue specific regulatory functions, acting as an enhancer in one cell type but a silencer in the other cell type. The last clone suppressed expression of luciferase in neuroblastoma cells but did not alter the expression level in the embryonic kidney cells.

1255/W

Chromosome 10p terminal deletion in a fetus with novel congenital type2 cystic adenomatoid malformation and renal agenesis. *V. Hucthagowder, TC. Liu, F. White, S. Kulkarni.* Cytogenomics and Molecular Pathology, Pathology and Immunology, Washington University School of Medicine, St Louis, MO 63110.

The terminal deletion on the short arm of chromosome 10 is a rare chromosomal abnormality and in some case is associated with DiGeorge syndrome/ Velocardiofacial, syndrome (DGS/VCFS; OMIM#601362 and 192430) like phenotypes and the hypoparathyroidism, sensorineural deafness, and renal disease (HDR) syndrome (OMIM#146255). Congenital cystic adenomatoid malformation (CCAM) is a rare developmental symptom of the lung with unclear pathogenesis, unknown incidence and unpredictable prognosis. We report here an affected 27-week male fetus with multiple anomalies and a previously undocumented CCAM who carried a terminal deletion on the short arm of chromosome 10. Overall, the gross anatomy, general measurements and organizations were appropriate for the gestational age of the fetus. The face was mildly abnormal with slight receding of the chin and features often associated with oligohydramnios. The autopsy revealed the right and left lung had three and two lobes respectively and were hypoplastic with the missing pulmonary veins. Microscopic findings indicated congestion, hemorrhage, and early cannalicular alveolar pattern of the lung. In addition, type II- CCAM was identified in the right lower lobe. The kidney and bladder were not present which is concordant with the characteristic involvement of chromosome 10p deletion (HRD syndrome). High resolution chromosomal analysis was conducted using cultured skin fibroblast and the metaphase spreads showed a deletion in the terminal region on the short arm of the chromosome 10. Fluorescence in situ hybridization (FISH) analysis further confirmed the presence of the identified deletion in this proband. While compared to fifty prviously reported individuals with 10p deletion, our proband showed some overlapping clinical presentations but stood out unique with the involvement of type II-CCAM. In conclusion, 10p deletion syndrome is rare and the underlying mechanism is complex; however, the identification of type-II CCAM in our proband with 10p deletion suggests that any suspected CCAM patient early in the development are warranted for chromosome and molecular cytogenetic studies to rule out 10p deletion. On the contrary, some affected individuals may manifest atypical symptoms with absence of the classic features of 10p syndrome.

1256/W

A recurrent interstitial microdeletion at Xp22.13 transmitted in a family presenting Nance-Horan syndrome. *H. Liao[1,8], Y. Chen[2,3], J. Fang[4], D. Niu[5,6], S. Chen[7], C. Chen[8,9].* 1) Institute of Biotechnology, National Tsing Hua University, Hsinchu,, Taiwan; 2) Department of Life Sciences and Institute of Genome Sciences, National Yang-Ming University, Taipei, Taiwan; 3) Department of Teaching and Research, Taipei City Hospital, Taipei, Taiwan; 4) Institute of Molecular Biology and Human Genetics, Tzu-Chi University, Hualien, Taiwan; 5) Institute of Clinical Medicine, School of Medicine; National Yang-Ming University, Taipei, Taiwan; 6) Department of Pediatrics, Veterans General Hospital-Taipei, Taiwan; 7) Department of Ophthalmology, Taipei Veterans General Hospital-Taipei, Taiwan; 8) Division of Mental Health and Addiction Medicine, Institute of Population Health Sciences, National Health Research Institutes, Miaoli, Taiwan; 9) Department of Psychiatry, Chang Gung Memorial Hospital at Linkou and Chang Gung University School of Medicine, Taoyuan, Taiwan.

Nance-Horan syndrome (NHS) is a rare X-linked disorder characterized by congenital cataracts, dental anomalies, and mental insufficiency. The disease has been linked to a novel gene NHS located at Xp22.13. The majority of pathogenic mutations of the disease are nonsense mutations that lead to truncation of the NHS protein, while some small deletions and insertions at the coding sequences of the NHS gene also contribute to the frame shift of open reading frame and premature stop codon of the NHS gene. Recently, several patients with NHS or X-linked congenital cataracts were found to be associated with copy number variations (CNVs) of the NHS locus, suggesting genomic rearrangement also plays a role in the pathogenesis of NHS. Here we describe the genetic analysis of a novel interstitial microdeletion of ~0.92 Mb at Xp22.13 detected by array-based comparative genomic hybridization (array CGH) in two brothers presenting congenital cataract, dental anomalies, facial dysmorphisms, and mental insufficiency. This microdeletion was transmitted from their carrier mother who presented only mild ophthalmological symptoms. The deleted region encompasses the REPS2, NHS, SCML1, and RAI2 genes, and is similar to a microdeletion of ~ 0.9 Mb encompassing the NHS, SCML1 and RAI2 genes reported recently in a NHS patient. Thus, the interstitial microdeletion found in our subjects might be a recurrent microdeletion associated with NHS, and that the deletion of NHS, SCLM1 and RAI2 genes in this family contributes to the genotype-phenotype correlations of patients with NHS and X-linked congenital cataracts.

1257/W

Phenotypic consequences - deletion 8pter vs duplication 15qter. *J. Pani[1], F. Sheth[1], J. Andrieux[2], S. Mehta[1], M. Desai[1], H. Patil[1], J. Sheth[1].* 1) Department of Cytogenetics, FRIGE's Institute of Human Genetics, Ahmedabad, Gujarat, India; 2) Laboratory of Medical Genetics, Jeanne de Flandre Hospital CHRU de Lille, Lille Cedex, France.

Subtelomeric rearrangements have been identified as a major cause of mental retardation and/or malformation syndromes and are observed in ~2-5% of the cases. Deletions involving these regions can lead to severe phenotypic consequences. We here report a 4-year-old girl born to a non-consanguineous parent having multiple congenital anomalies such as microcephaly, convergent strabismus, micropthalmia, dolichocephaly, congenital heart defects in form of atrio-ventricular septal defects, seizures, chronic anemia and mental retardation. Cytogenetic study revealed 46,XX,add(8)(p23). On further analysis by array-CGH using 44K oligonucleotide probe and BAC FISH confirmed deletion on 8p23.3p23.1 of 7.3 Mb and duplication involving 15q23q26.3 of 30.2 Mb. Phenotypic presentation in our case may have resulted from a balancing effect due to trisomy 15q23qter and *IGF1R* overgrowth on one hand and monosomy 8p23.1pter and loss of one copy of microcephalin on the other hand resulting in a borderline microcephaly, which suggests that deletion of 8p distal segment has shown more powerful phenotypic expression than trisomy of 15q distal segment.

1258/W

Mitigative Role of Melatonin on Mercury induced Cytogenetic Alterations *In Vitro*. *M.V. Rao, T.A. Patel, A.R. Purohit.* Human Genetics Centre, Department of Zoology, University School of Sciences, Gujarat University, Ahmedabad-380009, India.

INTRODUCTION: Mercury present in the environment is a potent toxicant to human health. Environmental contamination is the main cause of primary exposures which results due to mining, smelting, extensive industrial and agricultural usage including inhalation and ingestion via the food chain. It is also very well known genotoxic heavy metal. Melatonin is a hormonal product of the pineal gland and is also produced by certain plants. It is an antioxidant as it has a capacity to act as an electron donor and reduces the oxidative stress. METHODOLOGY: An *In Vitro* study was made in peripheral blood lymphocyte cultures (PBLC) to evaluate the protective effect of melatonin on mercury induced genotoxic potential at various dose levels. The cultures were exposed to four different doses of mercury for 24 hours. Melatonin alone and in combination with mercury were added to the cultures. EMS was used as a positive control. The genotoxic indices evaluated were sister chromatid exchanges (SCEs), cell cycle proliferative index/replicative index (CCPI/RI), average generation time (AGT), population doubling time (PDT) and total chromosomal aberrations (CAs) using suitable statistical analysis. RESULTS: A dose dependent increase in the SCEs, total aberrations, AGT and PDT, with a concomitant reduction in CCPI values. Melatonin supplementation to treated cultures reduced the genotoxicity exerted by mercury. CONCLUSION: Melatonin had a protective effect due to its antioxidant properties as observed by percent amelioration in this study. The importance of these findings is discussed.

1259/W

Identification of a recurrent microdeletion of 3q13.2q13.31 associated with hypotonia and developmental delay. *J.B. Ravnan[1], J.A. Rosenfeld[1], N.J. Neill[1], M.G. Bialer[2], C. Moore[2], P. Wheeler[3], S.E. Wallace[4], M.C. Hannibal[4], M.F. Murray[5], M.A. Giovanni[5], R.A. Schultz[1], B.C. Ballif[1], L.G. Shaffer[1].* 1) Signature Genomic Laboratories, Spokane, WA; 2) Cohen Children's Medical Center of NY, Manhasset, NY; 3) Nemours Children's Clinic, Orlando, FL; 4) Seattle Children's Hospital, Seattle, WA; 5) Brigham and Women's Hospital, Boston, MA.

The use of microarray technology in clinical diagnosis has greatly increased the detection of copy number alterations and allowed correlation between the gene content of the alteration and the clinical phenotype of the affected individuals. Here we describe the clinical and molecular characterization of four individuals with a recurrent 3.4 Mb deletion of the proximal long arm of chromosome 3 at 3q13.2-3q13.31 detected by array-based comparative genomic hybridization (aCGH). These individuals ranged from 16 months to 38 years old at age of diagnosis. All four individuals have hypotonia and motor delays; three have mild to moderate cognitive delays. Common facial features include ptosis, downslanting palpebral fissures with epicanthal folds, a slightly bulbous nose, and a large head (greater than 75th centile). Additional anomalies include abnormalities of the brain (Chiari malformation, agenesis of the corpus callosum, cerebellar agenesis), and vision (myopia, strabismus, nystagmus). The deletions were apparently de novo in the three individuals for whom parental samples were available. At least 25 genes have been mapped to the deleted region, including four genes, *DRD3*, *ZBTB20*, *GAP43*, and *BOC*, that have been implicated in neuronal growth or brain development. The *DRD3* protein product (dopamine receptor D3) is localized to the limbic areas of the brain, and the *ZBTB20* protein (zinc finger- and BTB domain-containing protein 20) appears to be involved in the development of the hippocampus. The protein product of *GAP43* (growth-associated protein 43) regulates the growth of axons, modulates the formation of new connections and appears to be involved in neuronal growth across the midline. Of particular interest is *BOC* (brother of *CDON*), a member of the cell surface Ig/fibronectin superfamily. *BOC* appears to play a role in axon guidance across the midline through the sonic hedgehog signaling pathway as well as in muscle development by promoting the differentiation of myogenic cells. Analysis of the shared deletion breakpoints revealed no segmental duplications; however, long terminal repeats (LTRs) were identified at both breakpoints in direct orientation. These repeats contain a shared 4.3 kb region of approximately 95% homology which could mediate non-allelic homologous recombination (NAHR) at this location and create the observed recurrent breakpoints.

1260/W

High resolution prenatal array CGH improves detection rate of clinically significant copy number abnormalities in the clinical laboratory. *A. Breman[1], I. Van den Veyver[1,2], P. Eng[1], W. Bi[1], S. Darilek[1], A. Pursley[1], P. Ward[1], A. Patel[1], L. White[1], J. Lupski[1], A. Beaudet[1], S. Cheung[1].* 1) Dept of Molec & Human Genetics, Baylor College of Med, Houston, TX; 2) Dept of Ob-Gyn, Baylor College of Med, Houston, TX.

Objectives: Current Array Comparative Genomic Hybridization (aCGH) performed at Baylor College of Medicine utilizes the latest microarray technology to detect unbalanced chromosome abnormalities associated with over 210 clinical disorders. Inclusive of our reported experience with 300 prenatal cases (PMID 19012303), we now have aCGH results on 682 clinical prenatal samples. Notably, 250 were analyzed on our custom high-resolution clinical BCMV7 array having 105,000 oligonucleotides to interrogate regions of the human genome at an average resolution of 30 kb, with increased coverage at known disease loci. Coverage also includes all the known microdeletion/duplication syndromes, 41 unique subtelomeric regions and all 43 unique pericentromeric regions. Methods: Analysis by aCGH was performed on DNA extracted directly from amniotic fluid (AF) for 352 (52%) samples, directly from chorionic villus sampling (CVS) for 87 (13%) samples and from cultured cells (amniocytes/CVS) for 234 (34%) samples. For the remaining 9 samples, the sources of fetal DNA were tissues, fibroblasts, or fetal blood. Results: Copy number changes were detected in 131 (19.2%) of the total 682 cases. Of these, 72 (10.6%) were CNVs that were interpreted as likely benign either because they were inherited from a phenotypically normal parent (66) or they were de novo (6) but had been previously observed in our aCGH database of over 28,000 clinical cases and phenotypically normal individuals. Of the 57/682 (8.4%) cases in which clinically significant genomic imbalances were detected, 26/432 were detected on our 44K clinical BCMV6 or BAC arrays (6% detection frequency) and 31/250 were detected on our higher-resolution 105K BCMV7 array (12.4% detection frequency). A subset of 88 cases with an indication of abnormal ultrasound was studied using our BCMV7 array, with a resulting detection rate of 21.6%. Conclusion: aCGH testing yields highly accurate results on both direct CVS/AF samples and cultured cells, with a rapid turn around time averaging 6 days. aCGH maximizes the possibility of detecting unbalanced genomic rearrangements, including submicroscopic copy number changes below the resolution of standard karyotype. Since the introduction of our higher-resolution BCMV7 clinical prenatal array, we have observed an approximately 2-fold increase in our detection rate for clinically significant unbalanced genomic rearrangements.

1261/W

A novel *de novo* deletion of the chromosome 12q23.1q23.2 in a patient with short stature, dysphagia, severe microcephaly and developmental delay. *W. Burdo-Hartman[1], H. Toriello[1], J. Koops[1], M. Gorre[2], S. Gunn[2], K. Hovanes[2].* 1) Spectrum Health, Grand Rapids, MI; 2) CombiMatrix Diagnostics, Irvine, CA.

The patient is a four-year-old girl who was first evaluated for significant hypotonia, global developmental delay and microcephaly at the age of nine months. She was born at 34 weeks of gestation via cesarean section due to breech presentation and was 3 pounds and 2 ounces at delivery. She was kept in the NICU for 5 weeks. Her mother smoked during pregnancy and was on insulin due to insulin-dependent diabetes. At the age of 12 months, her weight was below 3rd percentile. She received a Nissen fundoplication and a gastrostomy tube. She has been fed mainly with her G-tube since then. She was able to sit by herself at the age of 16 months and walked at the age of 21 months. At 30 months, she had cognitive and language score within the normal limits but her expressive language was at 25 months. She had fine and gross motor delays which suggested the diagnosis of developmental coordination disorder. She is a poor sleeper and has breathing concerns including snoring. She also coughs and gags when she runs. At the last visit, she was 4 years 4 months of age. Her weight, height, and head circumference were in 25th to 50th, 10th and less than 2nd percentile respectively. Oligonucleotide array comparative genomic hybridization analysis revealed an approximately 4.9 Mb deletion of the 12q23.1-q23.2 region. FISH analysis confirmed the deletion in the proband and parental analysis suggested a *de novo* event. The 12q deletion encompasses 33 annotated genes including a number of disorder-related genes such as *PAH, SLC17A8, SLC25A3, IGF1, TMPO, SYCP3, ASCL1* and *GNPTAB*. This patient's short stature and microcephaly is likely due to haploinsufficiency of *IGF1* gene as reported previously in patients with *IGF1* mutations. The *ASCL1* deletion is likely the cause of the patient's breathing problems since intragenic deletion of this gene is associated with congenital central hypoventilation syndrome. Haploinsufficieny of *PMCH* may be related to her dysphagia based on the recent studies of *PMCH* (-/-) rats. Missense mutation of *SLC17A8* gene is associated with autosomal dominant deafness, however, no hearing problems were reported in our patient. Haploinsufficiencies of the other disorder-related genes have not been correlated with a specific phenotype. This report summarizes a novel deletion encompassing a number of disorder-related genes and the genotype/phenotype correlation.

1262/W

Detection and quantification of mosaic isodisomy in Beckwith-Wiedemann patients in the Clinical CytoGenomics Laboratory. *L.K. Conlin[1], B.D. Thiel[1], S. Mulchandani[1], J.M. Kalish[2], K.E. Nichols[3], J.A. Biegel[1,2], C.A. Stanley[4], L.M. Ernst[5], N.B. Spinner[1], M.A. Deardorff[2].* 1) Department of Pathology and Laboratory Medicine, The Children's Hospital of Philadelphia, Philadelphia, PA; 2) Division of Genetics, The Children's Hospital of Philadelphia, Philadelphia, PA; 3) Division of Oncology, The Children's Hospital of Philadelphia, Philadelphia, PA; 4) Division of Endocrinology, The Children's Hospital of Philadelphia, Philadelphia, PA; 5) Department of Pathology, Northwestern University, Chicago, IL.

Historically, cytogenetic abnormalities, such as inversions and duplications involving 11p, have been reported in a small percentage (1-2%) of patients with Beckwith-Wiedemann syndrome (BWS). The majority of BWS patients present with methylation abnormalities, detectable by molecular methods. With the advent of SNP array analysis in the cytogenetics laboratory, we have been able to expand our detection of pathogenic findings in BWS patients to include paternal uniparental isodisomy. To date, we have identified mosaic loss of heterozygosity (LOH) of 11p in 9 of 43 patients (21%) referred for BWS, hemihypertrophy, hypoglycemia, and/or congenital hyperinsulism. Mosaic LOH was detected by identification of abnormal genotype frequencies in combination with normal probe intensities, and was quantified using a mathematical model developed in our laboratory. Samples from multiple tissues were tested in 4 patients, with LOH detected in all but one sample. Our testing was more sensitive than methylation testing, as SNP array analysis allowed for quantification of the level of mosaicism down to 5%. In addition, the extent of the LOH along the chromosome was determined for each patient. All patients had LOH involving the IGF2, H19, and CDKN1C loci at 11p15.5, while 3 patients had LOH of the Wilms tumor loci at 11p13. In total, 7 patients presented with LOH involving only 11p, suggesting mitotic recombination as a mechanism of formation. One patient presented with whole chromosome LOH, suggesting a mechanism of mitotic nondisjunction. A final patient was found to have mosaic genome-wide LOH and a 46,XX karyotype, indicating a subset of cells with complete uniparental isodisomy. Using SNP genotyping data, we were able to verify that this cell line showed isodisomy of the paternal genome, consistent with a fertilization error resulting in androgenetic chimerism. The use of array technology in the cytogenetics laboratory has enormously enhanced our ability to detect small genomic deletions and duplications, and additionally, to identify isodisomy. In BWS, this technology is useful for finding low levels of mosaicism for loss of heterozygosity, and in determining parent-of-origin. The high resolution allows for identification of breakpoints and precise gene involvement in the isodisomy. In summary, the robustness of the genome-wide SNP assay provides the ability to diagnose unexpected etiologies and characterize uniparental disomy in BWS.

1263/W

Pathogenic copy number variants detected by array-based comparative genomic hybridization (aCGH) in fetuses with malformations and a normal karyotype. *G. D'Amours[1,3,6], G. Mathonnet[1], R. Fetni[2,3,4], F. Tihy[1,3,5,6], S. Nizard[1,5,6], J. Michaud[1,3,5,6], E. Lemyre[1,3,5,6].* 1) Service de génétique, CHU Sainte-Justine, Montréal, Canada; 2) Pathologie, CHU Sainte-Justine, Montréal, Canada; 3) Centre de recherche, CHU Sainte-Justine, Montréal, Canada; 4) Pathologie et biologie cellulaire, Université de Montréal, Montréal, Canada; 5) Pédiatrie, Université de Montréal, Montréal, Canada; 6) Faculté de médecine, Université de Montréal, Montréal, Canada.

Array-based comparative genomic hybridization (aCGH) has been increasingly used in the clinical setting, and has even replaced conventional karyotyping as the first-tier test in patients with mental retardation and/or multiple congenital malformations. Only a few studies have investigated its use in the prenatal setting, and even fewer have focused on fetuses with malformations. We report copy number variants (CNV) detected by aCGH in a cohort of 50 fetuses with major malformations diagnosed by ultrasound. Most cases were tested after termination of pregnancy. Pathogenic CNVs were identified in 7 fetuses (14%), and CNVs of uncertain clinical significance in 3 (6%). The proportion of CNVs of uncertain clinical significance reported here is comparable to what has been reported in similar cohorts (0-12%), while the proportion of clinically significant results is slightly higher (2-10%). This difference might be explained by the lower resolution and the more targeted coverage of the arrays used for those studies. Cerebral, cardiac and renal abnormalities were the malformations most often found in fetuses with a pathogenic CNV.

Our results confirm the relevance of aCGH as a useful prenatal diagnostic tool, as it allowed a larger proportion of fetal malformations to be explained, thus improving the associated genetic counseling. However, clinical interpretation difficulties, linked with CNVs of uncertain clinical significance, prompt for caution when ordering aCGH in the prenatal setting, especially during ongoing pregnancies.

1264/W

Non-contiguous de novo chromosome 1q43 and 1q44 segmental losses detected in a patient with developmental delay by array CGH and FISH techniques. *G. Ghaffari[1], B. Gray[1], A. Bent-Williams[1], S. Link[1], R. Zori[1,2].* 1) University of Florida, Department of pathology Cytogenetics Laboratory, 4800 SW 35th Dr, Gainesville, FL; 2) University of Florida, Department of Pediatric, Box 100296, Gainesville, FL.

Microarray analysis is now the preferred method for clarification of chromosome deletions and duplications in situations where routine cytogenetic study is inconclusive. Here we report a 3 year and 7 month old boy with developmental delay and muscular hypotonia. He was visually attentive, with significant motor delays, he was not able to sit up independently and was not saying any words at 3 years 7 months of age. He was a normal-appearing child without obvious facial dysmorphism. His height was 85.8 cm, weight 12.6 kg, and head circumference 47.5cm, which was in 3rd percentile. He had no minor or major anomalies of the extremities. Chest, abdomen, and genitalia were normal. His muscle mass felt fairly good but he clearly was hypotonic both in upper and lower extremities. Chromosomal studies revealed a normal karyotype. Comprehensive genetic analyses using microarray-based comparative genomic hybridization (aCGH) analysis on patient DNA revealed two non-contiguous distinct deletions in 1q43 and 1q44 of 1.6 and 1.0 Mb in size respectively and 2.5 Mb apart. aCGH results were confirmed by conducting metaphase FISH using BAC probes RP11-80B9 from 1q43 for 1.6 Mb deletion, RP11-212E221 from 1q43 for 2.5 Mb undeleted , and RP11-156E8 from 1q44 for 1.0 Mb deleted region. Parental FISH studies using the above BAC probes confirmed a de novo origin of deletions. Further investigations are needed to determine the mechanism of formation for the above changes. Within the deleted regions based upon our finding 18 refseq gene are located, including FMN2, GREMLIN2, OPN3, KMO, FH, and ADSS, those genes play important roles in G-protein signaling , hydroxylation of L-tryptophan, krebs cycle, cytoskeletal organization, and purine nucleotide biosynthesis. This is a novel Non-contiguous de novo change not reported to-date. The 1.0 Mb deletion at 1q44 has an overlap with the smallest common region of 360kb, in which has been reported by Van Bone et al (2007) in nine out of the 10 patient with corpus callosum.

1265/W

Clinical Dilemma in Interpretation of CGH Microarray Results: Illustration of a case with a copy number gain and loss inherited from each parent. *A. Pandya, V. Kirkland, K. Withrow, J. Hiemenga.* Dept. of Human & Molecular Genetics, Med Col Virginia, VCU, Richmond, VA.

Microarray-based comparative genomic hybridization (array CGH) is a powerful molecular cytogenetic tool increasingly utilized in clinical practice to detect genomic imbalances which help to explain the clinical presentation in a child with multiple anomaly syndromes and/or cognitive delay. Current standard practice includes performing parental studies in follow up when a variant of unknown significance is identified in the proband. Although this practice is helpful in interpreting the pathogenic nature of a genomic imbalance, it often poses a dilemma in certain situations as illustrated by our case. We present a 20 month old Caucasian infant who presented with hypotonia, moderate cognitive delay, minimal coarse facial features and large body size, with subsequent failure to thrive. He developed a seizures after his initial evaluation. Routine cytogenetic studies, LIT1 and SNRPN methylation testing were negative. CGH microarray studies through Athena Diagnostics (105K Panel) revealed 1) loss of 20q13.33 region with a 1000Kb deletion which is paternal in origin and 2) gain of material at 22q13.31, about 490Kb in size which is maternally inherited. Both the parents are asymptomatic and hence these changes of unknown clinical significance are thought to represent a benign polymorphism. However the proband has several features which overlap with the clinical presentation in a few cases reported with 20q13.33 subtelomeric deletion. Additional clinical details of the case with genes of interest in the affected chromosomal regions and its comparison to the few reported cases in the literature will be presented. This case illustrates the uncertainty posed with regard to ascribing genotype-phenoytpe correlation and pathogenic significance to a laboratory finding in light of parental studies. Additional reports of unusual cases and publicly available databases with clinical copy number variants will help resolve such dilemmas and enhance clinical care.

1266/W

Frequency of aneuploidy in sperm cells of smoking males. *C. Pereira[1], M.S. Juchniuk de Vozzi[1], S.A. Santos[1], M.A.C. Vasconcelos[2], C.C.P. Paz[1], L. Martelli[1].* 1) Department of Genetics, FMRP- University of Sao Paulo, Ribeirão Preto, SP, Brazil; 2) Department of Gynecology and Obstetrics, FMRP- University of São Paulo, Ribeirão Preto, SP, Brazil.

The chromosomal aneuploidies in sperm are directly related to infertility, increasing the risk of birth defects and fetal losses. Although advanced maternal age still remains as one of the few well established risks, other factors have been identified as potential contributors to the increase of aneuploidy, including alcoholism, smoking and occupational exposure to pesticides. In this study, using interphase FISH analysis, we have evaluated the frequency of disomy of chromosomes 3, 13, 21, 22, X and Y sperm in ten male smokers compared with a control group of seven fertile men, nonsmokers. We analyzed aneusomies of these chromosomes by dividing in two hybridization sets; 13/21/22 and 3/X/Y. All the members of both groups presented a normal karyotype. The overall frequency of disomies showed a significant increase in the smoking group when compared to the control group, ($p<0.0001$) in both hybridization sets. When the analysis was made for each chromosome individually, the error in meiosis I to sex chromosome complement (disomy XY) and disomies involving chromosome 3 (disomy 33) showed a significant increase in smokers ($p<0.0001$ and $p<0.01$ respectively). The frequency of diploidy arising from an error in meiosis I (diploidy XY33) increased in the smoking group ($p<0.0002$). When the error was in meiosis II, only diploid YY33 complement was increased in smoker subset ($p<0.0167$). Our results suggest an association between cigarette smoking and incomplete reduction in meiosis I. Comparison of semen characteristics between the two groups found higher frequency of abnormal morphology in the smoking group ($p<0.0365$), showing that these factors may also be affected by the habit of smoking.There was no statistically significant difference between the frequency of disomies, when smokers were divided into those who smoked less and more than 20 cigarettes per day. Molecular cytogenetic investigation of germ cells is an essential tool for the elucidation of the meiotic segregation mechanisms, allowing the identification of possible factors responsible for infertility.

1267/W

Submicroscopic Interstitial Deletion 3q22.3-q23 in a Patient with Blepharophimosis-Ptosis-Epicanthus Inversus Syndrome (BPES) and Microcephaly, Development Delay and Growth Retardation: Further Characterized by SNP Array Analysis. *C.A. VENEGAS[1,2], L.M ZEPEDA[1,2], F. FERNANDEZ[2], J. BERUMEN[3], S.E KOFMAN[1,2].* 1) Servicio de Genética, Hospital General de México, Mexico City, DF, Mexico; 2) Facultad de Medicina, UNAM; 3) Servicio de Medicina Genómica, Hospital General de México.

Blepharophimosis, ptosis and epicanthus inversus syndrome [BPES; OMIM# 110100] is a rare autosomal dominant disorder caused by mutations in the FOXL2 gene, located in 3q23. Haploinsufficiency of FOXL2 gene in typical BPES patients can occur by intragenic mutations (~72%), microdeletion (6kb to <1.9Mb) (14%) and cytogenetic rearrangement (2%). To our knowledge only five cases with a submicroscopic interstitial deletion (2.7Mb to <4Mb) of 3q23 have been described (Beysen D et al., Am. J Hum Genet.77:205-218, 2005) and a defined clinical phenotype has not been established yet. We report the clinical and molecular findings in a 5-year-old male with de novo interstitial deletion on 3q22.3-q23. Our patient has typically BPES with additionally facial anomalies, microcephaly, psychomotor and growth retardation. Conventional cytogenetic analysis performed in the child and parents did not reveal abnormalities. 500K SNP array analysis showed an interstitial deletion in 3q. Physical position of the deletion was from [139,017,148 to 142,106,194] and the size of the deletion was ~3.09 Mb. We compare the clinical and molecular findings in our patient with previously reported cases and hypothesize that our case has a contiguous deletion gene syndrome and that the non-BPES-associated anomalies (microcephaly, psychomotor and growth retardation) result from the deletion of FOXL2 and the genes telomeric to it. Additional studies on patients carrying microdeletions are required to shed light on genotype-phenotype correlations for these deletions encompassing FOXL2. Acknowledgements: CONACyT (2006-C01-13947).

1268/W

SNP Array in clinical diagnostics: experience from 309 cases. *J. Wiszniewska[1], S-H.L. Kang[1], A. Patel[1], P.A. Ward[1], S. Al Masri[1], C.A. Bacino[1,2], H-G. Bock[3], M. Descartes[4], B.H. Graham[1], L. Potocki[1,2], F. Probst[1], F. Scaglia[1,2], M.F. Wangler[1], D.R. Witt[5], T. McDowell[5], L-J. Wong[1], S-W. Cheung[1], C.M. Eng[1].* 1) Dept Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Texas Children's Hospital, Houston, TX; 3) Dept of Pediatrics, University of Mississippi Medical Center, Jackson, MS; 4) Dept of Genetics, University of Alabama, Birmingham, AL; 5) Dept of Genetics, Kaiser Permanente, San Jose, CA.

Chromosomal Microarray Analysis (CMA) is a widely available method for the detection of genomic gains and losses, and a useful tool to help establish a clinical diagnosis. High density single nucleotide polymorphism (SNP) arrays have been recently implemented for clinical diagnostics and allow for the detection of copy neutral changes such as contiguous regions of absence of heterozygosity (AOH) in addition to copy number variation. AOH limited to single chromosomes may indicate uniparental disomy (UPD) that can cause clinically recognizable phenotypes if an AOH block contains imprinted genes or mutated autosomal recessive alleles. The detection of multiple contiguous regions of copy neutral AOH that occurs through identity by descent may help to elucidate genetic syndromes due to recessive conditions in consanguineous families. Baylor Medical Genetics Laboratories has provided clinical testing for over 300 cases using the Illumina Human610-Quad SNP array. We have detected clinically significant CNVs in ~10% of cases, and CNVs of uncertain clinical significance in 11% of cases. Notably, at least one contiguous region of AOH greater than 5Mb was detected in 27.5% of samples. Five patients evidenced UPD. Two had UPD of chromosome 15, the others had UPD of either chromosome 1,11, or 22. Several cases from consanguineous families for whom pedigree information was available had an extent of AOH that was consistent with the estimated coefficient of inbreeding, including seven cases where the proportion of AOH was consistent with mating between first degree relatives. In six patients, the block of AOH was present in a region containing autosomal recessive genes or loci related to the indicated clinical findings. Our experience shows that the CMA - SNP array is a comprehensive clinical tool that can detect common microdeletion/microduplication syndromes, novel copy number variants, and UPD. Interestingly, this method is also being applied as a tool to narrow down the regions of interest in patients from consanguineous families as a "positional" approach to locate potential disease-causing genes.

1269/W

Pure trisomy of distal long arm of chromosome 15(q24-qter) detected by SKY. *K. Abe, I.M.P.O. Rizzo, L.M. Formigli, M. Schneider, C.E. Speck-Martins.* Pat Molecular, Lab de Citogenetica, Rede SARAH-Hosp de Reabil, Brasilia DF, Brazil.

Introduction. There are only few reports of distal 15q duplications with no concomitant monosomy for another chromosome segment. In order to contribute to the clinical characterization of 15q24-qter trisomy/duplication phenotype, we report some additional clinical findings on the patient with pure trisomy 15q24-qter. Cytogenetic analysis showed additional material on 21p. Spectral karyotyping (SKY) showed an extra copy of the chromosome 15q transposed to chromosome 21p. Clinical report. The patient, female, first twin of three amnion, was born at 34 weeks because was affected by oligohydramnion. The two others were normal. At birth, she had respiratory distress, jaundice, arterial channel persistence and atrial septal defect, development delay, hypotonia, abnormal head shape, increase in OFC, dysfagia, chronic respiratory distress with choanal atresia, and pubic hair. MRI and CT-scan of the brain revealed supratentorial hydrocephalus and sagittal synostosis. Imaging analyses showed stomach malrotation and ovarian cysts. Clinical evaluation at 9 months showed normal growth, synophrys, short palpebral fissures, telecanthus, high nasal bridge, defined philtrum, thin upper lip, flat occiput, prominent forehead, camptodactyly of the third finger, deep crease on feet, heart murmur, and hypotonia with development delay. At 2 years and 3 months, she had an extremely delay in cognitive, motor, and behavioral development. Results and Discussion. After SKY, reevaluating G band analysis we could conclude that the patient had a duplication of 15q24-qter with no assotiated monosomy. In previously reported cases, unusual prenatal overgrowth, tall stature, macrocephaly, and craniosynostosis have been described as distal 15q trisomy syndrome, although they also had monosomic components. By contrast, similar trisomy without concomitant monossomy has been reported to patients with ptosis, small size, developmental delay, and no craniosynostosis or overgrouth. In addition, case of a child with autistic disorder, seizure, posnatal overgrowth, and a minor brain malformation has been described to 15q25.2-qter. Based on the cases reported to date, some of features as above quoted are controversial. Further studies could help to delineate a pattern of abnormalities to patients with trisomy of terminal segment of chromosome 15. Since the patient reported here had no concomitant monosomy, we can reasonably assume that her abnormal phenotype results from those duplicated segment.

1270/W

Marker chromosome 13 with an apparent neocentromere detected by array CGH in a fetus with MCA. *r. habibian, a. hajianpour, j. Szymznska, f. wang, q.q. huang, j.c. wang.* cytogenetics laboratory, genzyme, monrovia, CA.

Amniocentesis was performed on a 31 years old woman at gestational age of 19 weeks due to abnormal fetal ultrasound findings, including enlarged stomach, postaxial polydactily of upper extremities, and nuchal fold thickening. Cytogenetic analysis revealed a de novo supernumerary medium size marker chromosome. FISH analysis using alpha satellite centromeric probes specific for chromosomes X, Y, 18 and acrocentric chromosomes excluded these chromosomes as the origin of the marker. Array CGH analysis revealed two copy gain of 24.2 Mb from terminal long arm of chromosome 13 segment 13q31.3-qter. FISH confirmed the marker to be an inverted duplication of the 13q31.3-q34 region in 30 out of 30 cells examined (100%). As the marker was stable and did not show hybridization with D13Z1/D21Z1 probe specific for the centromere of chromosomes 13 and 21, formation of a neocentromere is speculated. The pregnancy was terminated. This case illustrates the importance of aCGH as an adjunct test to conventional cytogenetics for the characterization of marker chromosomes in abnormal pregnancies and to help parents make informed decision.

1271/W

Cytogenetic biomonitoring of residents of a distillery unit. *K. Kaur, G. Kaur, G. Gandhi.* Department of Human Genetics, Guru Nanak Dev University, Amritsar, India.

Alcohol distilleries discharge highly coloured effluents which tend to be highly noxious in terms of odour, causing water and soil pollution and may alter the pH of the soil and thereby affect agricultural crops. Rather treatment of effluents by standard practices may potentiate the outcomes and hence requires the continuous evaluation of environmental health and the environmental contribution to the burden of human disease. Human Biomonitoring studies have gained significance with genetic biomarkers as useful tools for the early assessment of exposure to occupational/environmental pollution. The objective of the present study was to investigate the genomic integrity of some people residing near and/or working in a distillery unit at Hamira, Punjab. Samples of peripheral blood were evaluated using the single cell gel electrophoresis assay (comet assay) and the micronucleus (MN) assay. The exposed group (n = 50; 25males, 25 females; 20-40 y) was compared to a reference group (n = 50) of subjects located far from industrial units and matched for age, sex and socio-economic status. The standard Cytokinesis block MN assay revealed significant increase for aneugenicity/clastogenicity. DNA damaging effect as functions of DNA migration length, per cent tail DNA, tail moment and Olive tail moment was also significant. Statistical analysis (Student's t-test) revealed high significance ($p<0.001$) for tail length and micronuclei frequency values compared for the exposed (12.87+0.29μm, 0.62±0.06%) and control (5.13+0.21 μm, 0.13±0.15%) groups. Thus, indicating that individuals residing near/working in distillery have more chromosomal and DNA damage as compared to controls. The statistical comparison for both parameters on exposed males (14.00±0.47 μm, 1.07±0.65 %) and females (11.67±0.16 μm, 0.31±0.10%) revealed greater genetic damage in males than in females. Keywords: Cytokinesis block micronucleus assay, Comet assay, Peripheral blood lymphocytes, Binucleated cells, DNA migration.

1272/W

Clinically relevant microdeletions encompassing long-range gene expression regulatory elements as a cause of human genetic disease. *F.M. Mikhail, N.H. Robin, J.C. Henegan, K.D. Rutledge, E.J. Lose, A.J. Carroll.* Department of Genetics, University of Alabama at Birmingham, Birmingham, AL.

The routine use of array CGH has enabled the detection of numerous clinically relevant submicroscopic copy number variations (CNVs) throughout the human genome. However, determining the significance of a CNV in an extragenic region close to clinically significant gene(s) has proven to be difficult. The expression pattern of developmental and tissue-specific genes is regulated not only by the core promoter but also by multiple *cis*-acting distant genomic elements that are crucial for spatiotemporally correct gene expression. These regulatory elements can be as distant as 1.0 Mb in either direction from the gene transcription unit. Here we report three cases that illustrate this problem. In each case, array CGH (ISCA 4x44k and 2x105k Agilent oligo-arrays) revealed a microdeletion thought to encompass long-range gene regulatory/enhancer elements for proximal clinically significant gene(s) relevant to these patients' phenotypes. Patient 1 presented with neonatal respiratory distress, congenital hypothyroidism, and abnormal frequent movements. He was shown to carry a *de novo* ~280 kb deletion at 14q13.3 located ~80 kb downstream of the *NKX2-1* gene, which encodes the thyroid transcription factor 1 (TTF1). Haplonsufficiency of *NKX2-1* has been reported to cause the same phenotype seen in our patient. Patient 2 presented with bilateral sensorineural hearing loss. He was shown to carry an ~1.3 Mb extragenic deletion at 13q31.1q31.2 flanked by the *SLITRK5* and *SLITRK6* genes, which encode integral membrane proteins that are expressed predominantly in neural tissues. *SLITRK6* deficient mice have been shown to have disorganized innervation and neural loss in the inner ear. Patient 3 presented with autistic features and language delay. He was shown to carry an ~1.0 Mb extragenic deletion at 2q14.3 located ~450 kb upstream of the *CNTNAP5* gene, which encodes a member of the Neurexin family. *CNTNAP5* has been linked to autism and language delays. No benign CNVs spanning these regions have been reported in the DGVs. A comparative sequence analysis of these deleted regions using the UCSC Multiz Alignment and Conservation track showed multiple highly conserved elements between the human sequence and other species. In conclusion, this report supports the notion that some CNVs can cause human genetic disease by transcriptional misregulation of nearby genes, and underscores the need for careful interpretation of CNVs in extragenic regions.

1273/W

Utilization of high density oligonucleotide array to analyze patients with developmental delay and congential anomalies. *M. Nimmakayalu[1], H. Major[1], Q. Qian[1], O.A. Shchelochkov[1], B. Darbro[3], R. Van Rheeden[1], D. Hulseberg[1], V.C. Sheffield[1], P.L. Nagy[2], S.R. Patil[1].* 1) Med Genetics, Pediatrics, Univ Iowa, Iowa City, IA 52242; 2) Department of Pathology, Columbia University Medical Center, New York City, NY 10032; 3) Department of Pathology University of Iowa, Iowa City IA 52242.

Array comparative genomic hybridization (aCGH) is a powerful tool that provides high resolution whole genome analysis of copy number variations and can reveal submicroscopic deletions and duplications. We present aCGH data on 937 patients referred for developmental delay/mental retardation (DD/MR) and/or congenital anomalies using a high-density 385K oligonucleotide array (Roche NimbleGen). We found genomic copy number variations (CNVs) in approximately 22% (214/937) of the examined cases. Of these 214 patients, 49% (104/214) were referred for evaluation of DD/MR, 31% (67/214) for congenital anomalies, and 19% (40/214) for other diagnoses. Of the genomic alterations detected, 25% (53/214) represented known clinical syndromes, for example del(22)(q11.2) which was present in 6% (12/214) of the cases. The remaining 75% (161/214) consisted of the recently described CNVs including del(16)(p11.2) which was present in 3% (7/161) of the cases, as well as CNVs of uncertain clinical significance. Parental aCGH analyses of 58 probands with CNVs (200 kb - 1.5 Mb) showed that the majority of them were inherited (37% maternal vs. 63% paternal). Our cohort also includes patients with cytogenetically balanced rearrangements in which aCGH detected subtle imbalances at the breakpoints in ~30 % of the cases. We used FISH and higher density array to further characterize clinical significance of the de novo cases and cases with interchromosomal and intrachromosomal rearrangements. We provide frequency estimates of the common and newly identified CNVs in the samples submitted to the diagnostic lab.

1274/W

Mechanism of XXY/XXXY: Maternal MII/Post Zygotic X Chromosome Non-Disjunction and Homozygosity Identified SNP Microarray Analysis. *J.H. Tepperberg[1], S. Schwartz[1], P.R Papenhausen[1], A.N. Injac[2], I.K. Gadi[1], V. Jaswaney[1], J. Neely[1], H. Risheg[3], E. Keitges[3], R. Pasion[1], J. Smith[4], R. Potluri[4], R. Burnside[1].* 1) Dept Cytogenetics, Lab Corp America, Res Triangle Pk, NC; 2) Baylor College of Medicine, Dept Pediatrics. One Baylor Plaza, MS BCM 320 Houston, TX 77030; 3) Dept Cytogenetics, Dynagene/LabCorp America, Seattle WA; 4) Dept Cytogenetics, Dynagene/LabCorp America, Houston, TX.

The frequency of 47,XXY Klinefelter syndrome (KS), is reported to be ~1:1000 male births with ~50% involving the maternal X chromosome [MI non-disjunction (47.0%), MII (29.0%) or post zygotic mitotic (PZM) nondisjunction (15.7%)], and ~50% due to paternal MI nondisjunction. The 48,XXXY KS is much less common than XXY KS with an estimated frequency of 1:8500. XXY/XXXY mosaicism is even rarer, requiring two independent nondisjunction events. We report a 12.1 year old male referred for microarray testing due to DD, hypotonia, and inattentiveness in which XXY/XXXY chromosome mosaicism was detected. Formation of this particular mosaicism is difficult to elucidate and could result from various complex maternal or paternal meiotic and mitotic nondisjunction pathways. SNP genotyping microarray analysis offers an easier, more informative alternative method of assessing the origin of extra X chromosomes than the traditional method using DNA polymorphic markers in which samples from parents and proband are needed. Microarray analysis showed a copy number gain of ~2.8 consistent with poly X mosaicism. FISH was performed using alpha satellite X and Y probes and confirmed 80% of the metaphases XXXY and 20% XXY, while interphase analysis showed 56/100 nuclei with XXXY hybridization signals and 44% with an XXY hybridization pattern. No XY cell line was observed. The SNP genotyping array also showed complete homozygosity for all three X chromosomes indicating that all were genetically identical. Therefore, the X aneusomy must have originated from an MII error followed by a PZM nondisjunction error. Paternal origin is less probable, requiring meiotic MI and MII nondisjunction errors to produce an XXY gamete, with subsequent PZM nondisjunction error following fertilization with a nullisomic X egg. A more plausible supposition is maternal MII nondisjunction error producing an XXY fetus with subsequent PZM error producing three theoretical cell lines, XY/XXY/XXXY, although no XY cell line was observed. This result demonstrates a sophisticated utility for SNP based copy number/genotyping microarray testing in patients with X chromosome aneuploidy (and autosomal aneuploidy and UPD), as well as providing easier insight into the meiotic and post zygotic mechanistic origin of X chromosome nondisjunction. Although no XY cell line was observed in the blood sample, it is conceivable that an XY cell exists in another tissue. Buccal smear FISH would address this hypothesis.

1275/W

Recombinant chromosome 7 in a mosaic triple X/Turner patient. *E.K. Torgbe[1], F. Valdez[1], S. Iyer[1], R. Garcia[1], P. Pichurin[2], M. Suterwala[3], P. Koduru[1], S. Dallaire[4], T. Appleberry[4], C. Tirado[1].* 1) Pathology, University of Texas Southwestern, Dallas, Te; 2) Genetics, The University of Texas Southwestern Medical Center at Dallas, Dallas, TX; 3) Pediatrics, Baylor College of medicine, Department of Pediatrics; 4) Perkin Elmer Laboratories, Waltham, Massachusetss.

Trisomy X syndrome occurs at a rate of 1/1000 and presents with no obvious physical features. Individuals with this syndrome may be at an increased risk for mild speech and motor delay as well as learning disabilities. However, they have normal secondary sexual development with minimal impairment in their reproductive abilities. They may also have an increased risk for psychologic problems during their lifetime. A small proportion of patients with Turner syndrome (3-4%) are mosaic for a triple X (47,XXX) cell line. In general, patients with a lower proportion of the 45,X cell line show fewer Turner syndrome features. Terminal 7q deletions present with variable phenotype depending on the size of the deletion. These includes cleft palate, microcephaly, syndactily of fingers and toes, cardiac malformations and hyperplasia of Lagerhans' cells. Herein, we present a newborn with pedal edema, microcephaly, micrognathia, slightly down slanting palpebral fissures, ventricular septal defect, patent ductus arteriosus, and a unilateral dysplastic kidney. Conventional cytogenetics and FISH revealed a mosaic karyotype: 47,XXX,add(7)(q32)[18]/45,X,add(7)(q32)[2]. Chromosome analysis of the mother showed a normal female karyotype. Chromosome analysis of the father showed an abnormal but apparently balanced karyotype due to two overlapping paracentric inversions in the long arm of chromosome 7. His karyotype was described as 46,XY,der(7)inv(7)(q22.1-q34)inv(7)(q32q36). This result was confirmed by FISH and array CGH with BACs specific for 7q36.1, 7q36.2 and 7q36.3 bands (RP11-728K20, RP11-80J22 and RP11-58F7). FISH using the same BACS on the proband showed deletion from 7q36.1 to 7q36.3 in the rec(7) chromosome, therefore her karyotype was described as: 47,XXX,rec(7)del(q35q36.3)inv(7)(q22.1q34)inv(7)(q32q36)[18]/ 45,X,rec(7)del(q35q36.3)inv(7)(q22.1q34)inv(7)(q32q36)[2]. Genetic counseling was recommended for this patient and her parents regarding planned future pregnancies.

1276/W

Coexistence of different genomic mutations in three mentally retarded subjects: further evidence that a genetic double trouble can explain atypical phenotypes. *M. Zollino[1], G. Marangi[1], D. Orteschi[1], M. Murdolo[1], M.C. Stefanini[2,3], G. Vento[4], M.G. Pomponi[1], S. Lattante[1], G. Neri[1].* 1) Dept Medical Genetics, Univ Catt Sacro Cuore, Rome, Italy; 2) Dept Child Neuropsychiatry, Univ Catt Sacro Cuore, Rome, Italy; 3) Don Carlo Gnocchi Foundation, Rome, Italy; 4) Dept Pediatrics, Univ Catt Sacro Cuore, Rome, Italy.

During routinary genetic diagnosis for mental retardation or neuromuscolar disorders we observed:1) a DMD gene mutation causing Duchenne muscolar distrophy in a 8-year-old boy who also had severe mental retardation;2) a 47,XXY chromosome constitution in a newborn who was referred for hypotonia and minor facial anomalies and 3) a full mutation of the FMR1 gene in an 18-year-old male who was severely mental retarded and also had minor physical anomalies. Since the clinical manifestations in each of these patients exceeded those typical of their diagnosed conditions, we decided to perform a further study of the cases by array-CGH (Agilent 4x44k, NCBI 36 hg18 probes alignment). We found the following CNVs not reported in the Database of Genomic Variants: 1) a 220 kb deletion of chromosome region 11q14.3 (89508377...89730095) in association with the DMD gene mutation (parents are under investigation); 2) a dn deletion of 2 Mb in 2q37.3, (240561565...242690037) in association with the 47,XXY chromosome constitution (a-CGH revealed the additional copy of the X chromosome as well); 3) a deletion of 500 kb in 2p25.3 (1135059...1649356), in association with the full FMR1 mutation. Genotype-phenotype correlations are discussed. These cases represent further examples of genetic double troubles resulting in complex phenotypes. Overall, they stress the importance of considering additional DNA testing in patients with known mutations who have unusual phenotypes.

1277/W

Validations of array-CGH results and definition of CNVs categories in mental retardation. *D. Orteschi[1], G. Marangi[1], M. Murdolo[1], S. Lattante[1], S. Ricciardi[1], V. Romanelli[2], O. Gabrielli[3], D. Battaglia[4], E. Mercuri[4], M.C. Stefanini[4,5], G. Zampino[6], C. Leoni[6], F. Gurrieri[1], P. Chiurazzi[1], R. Lecce[1], M.E. Grimaldi[1], G. Neri[1], M. Zollino[1].* 1) Dept Medical Genetics, Univ Catt Sacro Cuore, Rome, Rome, Italy; 2) INGEMM, Instituto de Genética Médica y Molecular, IdiPAZ-Hospital Universitario La Paz, Universidad Autónoma de Madrid, Madrid, Spain; 3) Institute of Maternal-Infantile Sciences, Polytechnic University of Marche, Ancona, Italy; 4) Dept Child Neuropsychiatry, Univ Catt Sacro Cuore, Roma, Italy; 5) Don Carlo Gnocchi Foundation, Rome, Italy; 6) Dept Pediatrics, Univ Catt Sacro Cuore, Rome, Italy.

Array-CGH is proving to be a powerful tool enabling the detection of submicroscopic chromosomal imbalances associated with mental retardation and physical anomalies. However the pathogenic relevance of several CNVs can be uncertain, thus a validation of the a-CGH results is needed for proper genetic diagnosis and counseling. We performed a-CGH (Agilent 4x44k or 244K) in a total of 550 subjects with mental retardation and minor physical anomalies, who mostly had apparently normal chromosomes on conventional cytogenetics. A total of 186 CNVs were detected, that were grouped in the following categories: 1) pathogenic (total 96/550, 17.5 %). This category was defined by the following criteria: de novo occurrence; association with known syndromic conditions or sized > 2 Mb; gene content analysis consistent with the clinical features. Of them, 6 were susceptibility factors, that were usually associated with mental retardation of variable degree; 2) polymorphic CNVs, that were all inherited from a healthy parent (total 75/550, 14%). Of them, 57 were common polymorphisms, as reported in the Database of Genomic Variants, 18 were rare, not reported in the Database.; 3) CNVs of uncertain significance (total 15/550, 3%). Based on genotype-phenotype correlation analysis of pathogenic CNVs, we found that predictive of a causative role were also: deletion event, disproportion of growth parameters, peculiar facial characteristics, hands and feet abnormalities, hypotonia, subtle brain abnormalities, major malformations and cryptorchidism. A checklist of the most consistent clinical signs is suggested, as useful tool in validating a-CGH results of uncertain significance, along with gene content analysis. With respect to familial data, in some instances we found that a carrier parent, first referred as healthy, actually had a variable expressivity of the child genetic condition. A detailed clinical analysis of parents is suggested in assessing the pathogenic role of inherited CNVs. Finally, rare polymorphic CNVs are considered to act as cofactors in causing complex phenotypes, as inferred from their occurrence in association with different genomic mutations in several occasions. This last observation seems to confirm the recently suggested two-hit model for severe developmental delay.

1278/W

Xp22.31 duplication: the role of recombination and secondary CNVs. *E. Rajcan-Separovic[1], R. Wildin[2], J. Eichmeyer[2], R. Jiang[3], B. DuPont[4], L. Arbour[5], B. Kamien[6], S. Martell[1], E. Strong[1], C. Harvard[1], Y. Qiao[1], J. Holden[7], S. Lewis[3], W. Robinson[3].* 1) Dept Pathology, Child & Family Res Inst, Vancouver, BC, Canada; 2) St. Luke's Regional Medical Center, Boise, USA; 3) Dept. Medical Genetics, UBC, Vancouver, BC, Canada; 4) Greenwood Genetic Center, Greenwood, USA; 5) Medical Genetics Dept, Victoria General Hospital, Victoria, Canada; 6) Royal Brisbane and Women's Hospital Health Service District, Herston, Australia; 7) Depts Psychiatry & Physiology, Queen's University, Kingston, Ontario, Canada.

Recent studies show that Xp22.31 duplication occurs in ~ 0.4% subjects with intellectual disability (ID) and 0.15% of controls, suggesting the possibility of its role as a risk factor in ID (Li et al., 2010). In our cohort of 435 subjects with ID, the Xp22.31 duplication (Xpdup) was detected in 3 males (0.7% of cases) either by qPCR or whole genome array screening. We performed skewed X inactivation and chromosome X allele transmission analysis using a panel of 8 microsatellite probes in two multigenerational families from this cohort and in one family recruited subsequently. In all three families the male proband had a maternally transmitted Xp22.31 duplication. The duplications ranged from ~0.5 to 1.5 Mb. In 2/3 male probands with Xpdup an additional chromosome change was noted upon whole genome array analysis-de novo gain of 19p13.3 and de novo loss of 11p15.1. All normal female Xpdup carriers (8) showed skewed X inactivation with values >80%(>90% in 7/8 cases). However, the Xpdup chromosome was the preferentially inactive chromosome in only 4 of these cases, based on the AR allele transmission analysis and haplotype segregation. The Xpdup in the male probands was always a recombinant chromosome, with recombination occurring between the Xpdup carrying and non-carrying X chromosomes in their mothers or grandmothers or both. We therefore propose that the inconsistent pattern of inactivation of Xpdup carrying chromosome, may suggest that in some instances skewing is due to the presence of a deleterious chromosome X mutation that is unrelated to the Xp duplication. In support of this hypothesis, two affected male cousins of one of the probands with Xp22.31 duplication, neither of which had the Xp dup, showed an Xq21.1 duplication (Xqdup) in the X-linked ID gene MAGT1 detected by a chromosome X specific array. The Xqdup was subsequently also detected in the proband with Xpdup using the chromosome X array. Further haplotype and chromosome X specific array analysis showed that the Xqdup chromosome was inherited from the great-grandmother and was preferentially inactive in all Xqdup female carriers, regardless of whether they had the Xpdup or not. Investigations of the presence of deleterious CNVs/mutations on chromosome X in affected subjects from families with Xpdup, as well as the search for secondary CNVs at the whole genome level in carriers of Xpdup, may shed more light on the clinical and biological variability associated with this duplication.

1279/W

ASSESSING THE PHENOTYPIC EFFECTS OF IMMP2L AND DOCK4 DETECTED BY WHOLE GENOME SNP ARRAY. *H. Risheg[1], E. Keitges[1], R.D. Burnside[2], I. Gadi[2], V. Jaswaney[2], R. Pasion[2], S. Schwartz[2], J. Tepperberg[2], P. Papenhausen[2].* 1) LabCorp/Dynacare, Seattle, Washington; 2) LabCorp, Research Triangle Park, North Carolina.

Several copy number variants within putative autism genes have been implicated in autism susceptibility. Recently, copy number variations of the *IMMP2L* gene and the adjacent *DOCK4* gene have been identified as possible risk factors for autism spectrum disorder (ASD), speech and language disorder, Tourette syndrome, and dyslexia. To investigate this phenomenon, we performed a retrospective analysis on a population of ~15,000 patients referred for SNP microarray analysis for the detection of duplications or deletions of *IMMP2L* and/or *DOCK4*. We identified 169 patients with >50kb deletions or duplications of *IMMP2L* and *DOCK4*. Of these, 164 had intragenic deletions of *IMMP2L* only , one had a 174 kb partial deletion of both *IMMP2L* and *DOCK4* , three had intragenic *DOCK4* deletions only, and one had a *DOCK4* duplication. No duplications of the *IMMP2L* gene were identified within our cohort. Detailed clinical information was obtained and assessed on a subgroup of 32 *IMMP2L* deletion patients and 5 *DOCK4* deletion/duplication patients. Of the 32 *IMMP2L* microdeletions, 13 deletions were localized within intron 3, and 19 deletions involved exons 1, 2 or 3. Furthermore, secondary changes were seen in 4/32 individuals (2 patients with structural chromosome abnormalities of other chromosomes; and 2 patients with a duplication or deletions of other chromosomes of varying size). Of eight cases with available parental follow-up, all were determined to be inherited (3 maternally inherited; 5 paternally inherited). The most common phenotypic features in this cohort were developmental delay, speech delay, and autism. The three individuals with deletions of *DOCK4* had multiple congenital anomalies and mental retardation. Parental follow-up was available on two of the three patients(1 maternally inherited; 1 paternally inherited). The single *DOCK4* duplication was paternally inherited, and was associated with a complex de novo rearrangement. The findings in our initial 37 *IMMP2L* and *DOCK4* cohort suggest a possible involvement of this region with developmental delay. Since all of the deletions or duplications of IMMP2L or DOCK4 were inherited, additional research is warranted focusing on secondary changes within the genome that might include possible modifier regions.

1280/W

Intragenic ANCO-1 duplication in two brothers affected by complex malformation syndrome. *D. Rusconi[1], C. Castronovo[1], M. Crippa[1], S. Russo[1], C. Gervasini[2], A. Cereda[3], A. Selicorni[3], L. Larizza[1,2], P. Finelli[1,4].* 1) Laboratorio di Citogenetica Medica e Genetica Molecolare, Istituto Auxologico Italiano, Milano, Italy; 2) Divisione di Genetica Medica, Dipartimento di Medicina, Chirurgia e Odontoiatria, Facoltà di Medicina e Chirurgia, Ospedale San Paolo, Università degli Studi di Milano, Milano, Italy; 3) Ambulatorio di Genetica Clinica Pediatrica, Clinica Pediatrica, Ospedale San Gerardo, Monza, Italy; 4) Dipartimento di Biologia e Genetica per le Scienze Mediche, Università degli Studi di Milano, Milano, Italy.

Genome-wide analysis in large cohorts of patients with mental retardation and/or multiple congenital anomalies (MR/MCA), showing a not recognizable phenotype, has led to identify a number of novel microdeletion/microduplication syndromes. We present two brothers sharing facial dysmorphisms, nasal voice, growth retardation, microcephaly and moderate mental retardation. In both patients a congenital atrioventricular canal defect was diagnosed and surgically corrected. The male patient also displayed a third degree vesicoureteral reflux whereas in the female proband a left ureterocele in double calico pielico district was diagnosed. The mother showed a milder phenotype characterized only by slight dysmorphisms and a nasal voice. High resolution array-CGH analysis identified in the two siblings a ~60 kb microduplication in 16q24.3, involving the ANCO-1 gene, also known as ANKRD11, which was not found in the parents by the same technique. The microduplication, confirmed by BAC i-FISH experiments, interrupts ANCO-1, likely resulting in its haploinsufficiency. On the basis of the mother's mild phenotype a low-level mosaic condition of the duplication was suspected. In order to verify this hypothesis, i -FISH was also carried out on the parents' peripheral blood lymphocytes. Indeed, a mosaicism of about 5% was identified in the mother and excluded in the father. Microsatellite analysis confirmed the maternal origin of the duplication. Recently, microdeletions at 16q24.3, varying in size but with a common overlapping region involving ANCO-1 and ZNF778 genes, have been described. It has been proposed that the haploinsufficiency of ANCO-1 and ZNF778 may be responsible for a distinctive syndrome mainly characterized by autism spectrum disorder, cognitive impairment, facial dysmorphisms and brain abnormalities. We suggest that our patients' phenotype, milder than those reported, is likely due to the only involvement of ANCO-1, resulting in distinctive craniofacial dysmorphisms, growth retardation and cognitive impairment but not autistic traits. Further investigations of patients with ANCO-1 anomalies are needed to correlate the presence of major malformations in organs derived from the same embryonic leaflet, such as cardiac and renal anomalies, to the single ANCO-1 haploinsufficiency.

1281/W

Targeted Array for Autism Spectrum Disorders. *G.A. Toruner, D. Streck, R. Kurvathi.* Institute of Genomic Medicine, UMDNJ-NJ Medical School, Newark, NJ.

Aim: Clinical validation of a targeted array called AutChip 1.0 **Background:** There is an ongoing debate about the use targeted or genome wide arrays in diagnostic genetic testing. Our hypothesis was an array targeting recurrent cytogenetic aberrations in autism spectrum disorders would have a good diagnostic yield and low rate detection of copy number variants of unknown significance. **Materials and Methods:** We designed a custom array on Agilent 44K platform targeting 33 loci. Using that array, fifty-one independent banked DNA samples obtained from Autism Genetics Resource Exchange and Simons Simplex Collections were analyzed. When a copy number variation was detected, the clinical significance of that finding was ascertained using a portal provided by International Standard Cytogenomic Array Consortium. **Results:** No copy number variants were detected in twenty-seven (53%) of the samples. Nine samples (17%) have pathogenic copy number variants. Fourteen samples (%) have benign copy number and one sample (2%) have a copy number variation of unknown significance. **Conclusion:** AutChip 1.0 has a reasonable diagnostic yield and low rate of equivocal results. Targeted array designs should be considered during array-CGH testing for autism spectrum disorders.

1282/W

Analysis workflow for array-based CNV genotyping. *A. Tsalenko[1], N. Sampas[1], A. Ben-Dor[2], Z. Yakhini[2], R. Navon[2], P. Sudmant[3], C.D. Campbell[3], E. Eichler[3,4], L. Bruhn[1].* 1) Agilent Laboratories, 5301 Stevens Creek Blvd., Santa Clara, CA; 2) Agilent Laboratories, 94 Em Hamoshavot Road, Petach-Tikva, Israel; 3) Department of Genome Sciences, University of Washington, Seattle, WA; 4) Howard Hughes Medical Institute, University of Washington, Seattle, WA.

Copy Number Variation (CNV) is now recognized as a prevalent form of structural variation in the genome contributing to human genetic variability. Methods for genotyping samples over known CNV regions (CNVRs) provide a basis for exploring CNV association to Mendelian and complex diseases. We present a data analysis pipeline that starts with raw two-color hybridization data and a list of CNVRs, and ends with the assigning of absolute discrete copy numbers or copy number estimates for all samples for each CNVR. The workflow includes sample clustering and copy number assignment utilizing both the measured signal intensities and their ratios. We then evaluate population specificity of each CNVR using minimum hyper geometric statistics (Eden et.al., PLOS Comp Bio, 2007) in both low and high copy number regions. We apply the pipeline to a recently published array CGH dataset for 447 distinct HapMap samples (Conrad et. al., Nature 2009) hybridized to an Agilent Human CNV array with 105K probes targeting ~11,000 loci against a common pooled reference. For each CNVR, we first compared the variability of the single channel signal intensities in the cohort to the variability of single channel signal intensities of the reference. Based on this comparison, we estimate that only 44% of the regions are polymorphic, and 56% are non-polymorphic. For each polymorphic CNVR, we applied a t-test based clustering approach to distributions of median log2ratio values as measured across the cohort, followed by a discrete copy number fitting procedure applied to these log2ratios and to corresponding median single channel intensities. We observe that 55% of these regions could be fitted robustly to discrete copy numbers. Regions that do not robustly cluster are typically associated with high sample signals, corresponding to high-copy number states. A significant fraction of polymorphic regions are population specific with more than 30% of them having more than four copies on average among HapMap samples. We compare our results with Conrad et.al., and to copy number estimates for a subset of these samples from 1000 genomes sequencing data. In more than 77% of regions absolute discrete array-based copy number assignments agree with sequencing-based copy numbers in more than 80% of the samples. This analysis demonstrates the utility of combining single intensity and ratio measurements for array-based copy number profiling in the context of various association studies.

1283/W

Infertile sperm cells exemplify telomeres dysfunction and genetic instability parameters. *T. Biron-Shental[1,4], A. Shulman[1,4], M. Yonish[2,3], L. Goldberg-Bittman[2,3], M.D. Fejgin[1,2,4], A. Amiel[2,3].* 1) Obstetrics and gynecology, Meir Medical Center, Kfar Saba, Israel; 2) Genetic Institute, Meir Medical Center, Kfar Saba, Israel; 3) Faculty of Life Sciences, Bar Ilan University, Ramat Gan, Israel; 4) Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel.

Objective: Telomeres are nucleoprotein complexes that maintain the stability of chromosomal ends. They are shortened with aging and by environmental injury and may form aggregates. Telomere capture can stabilize chromosome breakage. Some parameters of genetic instability include aneuploidy and telomeres dysfunction. Aim: In this study, we evaluated aneuploidy, telomeres length, aggregates and capture, as genetic instability markers, in human sperm of fertile and subfertile men. Materials & Methods: Sperm cells retrieved from 16 men with sub-fertility that required in-vitro fertilization and from 10 fertile men. 200-500 cells were assessed from each participant. Using FISH techniques, the following parameters of genetic instability were evaluated in both groups: random aneuploidy, telomeres length, telomeres aggregates and telomere capture. Results: We found significantly higher rates of spontaneous aneuploidy among sub-fertile compared to fertile sperm cells, in somatic chromosomes (P=0.006) and in the sex chromosomes (P= 0.04). Although telomeres length was not different between the groups, the amount of telomeres aggregates was significantly higher in the sub-fertile group (P<0.001). The rates of telomere capture were also higher among the sub-fertile sperm cells (P=0.007). Conclusions: Sub-fertile sperm cells may have shortened telomeres that are elongated by the alternative pathway of telomere capture. The genetic instability parameters of increased aneuploidy and dysfunctional telomeres may imply on sperm fertilization ability.

1284/W

Characterization of inversion polymorphisms in genomic disorders. *O. Migita[1], M.A. Joseph-George[1], R. Wong[1], Y. He[1], A.W. Pang[1], J.R. MacDonald[1], D. Pinto[1], C.R. Marshall[1], R. Weksberg[2], S.W. Scherer[1,2].* 1) The Centre for Applied Genomics, the Hospital for Sick Children, Toronto, ON, Canada; 2) Genetics & Genome Biology, the Hospital for Sick Children, Toronto, ON, Canada.

Structural variants such as copy number variants (CNVs) and copy neutral variants (i.e. inversions and translocations) are recognized as important contributors to underlying mechanisms of human disease. It has been reported that structural variants encompass more nucleotides than SNPs in the human genome, and it has been suggested that segmental duplications and/or low copy repeats (LCRs) are favored sites mediating chromosomal rearrangements. Our understanding of sub-microscopic copy neutral structural variants is however, rather limited in terms of contribution to genomic instability in CNV biogenesis.

Williams-Beuren Syndrome and Sotos syndrome are genomic disorders associated with deletion variants, which are flanked by LCRs. Intriguingly, with Sotos syndrome half of Japanese patients have 1.9Mb deletion at 5q35, but only 10% of non-Japanese have the same deletions. Osborne et al. (2001) reported that ~30% of parents of Williams-Beuren Syndrome individuals carry an inversion polymorphism on chromosome 7q11.23. Visser et al. (2005) found an inversion polymorphism at the NSD1 gene on chromosome 5q35 in all parents of Sotos syndrome patients. Building on these and other studies, we hypothesize that inversions can cause higher CNV formation rates.

To further determine the genomic architecture in Sotos syndrome we tested for the presence of inversions in 8 non-Japanese families (European, African and Asian populations from HapMap). In our preliminary data, we have found no inversion polymorphisms at the NSD1 locus, which is consistent with the fact that non-Japanese Sotos syndrome cases have less deletions involving NSD1. We are further characterizing this region and others, which will provide new insights about the population genetics of inversions and allow evaluating their contribution to chromosomal disorders.

1285/W

Visualizing sister chromatid segregation during metaphase by nanoscale imaging. *P.K. Rogan[1], W. Khan[1], J.H.M. Knoll[1], S.M. Tadayyon[2], P.R. Norton[2], R. Chisholm[3], L.J. Johnston[3].* 1) Schulich Sch Med & Dentistry, University of Western Ontario, London, ON, Canada; 2) Department of Chemistry, University of Western Ontario; 3) Steacie Institute, National Research Council of Canada, Ottawa.

At metaphase, chromosome segregation initiates processes that reorganize DNA sequences that have previously replicated and form kinetichore structures attached to the spindle through a microtubule network. This network is disintegrated by colchicine treatment; however these structures remain essentially unaltered. We have investigated formation of these structures and sister chromatid segregation at nanometer scale resolution. We have developed protocols to relate fluorescent images of FISH probes on the topography of human metaphase chromosomes. Correlated fluorescence in situ hybridization (FISH) with atomic force and near field scanning microscopy (AFM, NSOM) can demonstrate the spatial relationships between centromeric DNA and epigenetic structures associated with these sequences on the surface of chromosome 17. Optical and topographic images of multicopy centromeric probes hybridized to chromosomes have been obtained independently in the UWO and NRC facilities. Generally, probe signals of similar fluorescence intensities correspond to the highest features at the centromeric locations of sister chromatids. Image processing procedures quantify probe context, relative to neighboring 30 nm chromatin fiber bundles imaged by AFM. Kinetochore structures are the highest prominences (typically 100 nm above background) found at the centromere and are present on all metaphase chromosomes. These peaks are formed early in metaphase, and are usually present as pair (or sometimes triplet) structures, and correspond to kinetichore attachment sites. Interestingly, segregation of sister chromatids is not coordinated with the formation of these structures within individual homologous chromosomes, occurring after the formation of these structures. By the end of metaphase, the locations of the segregated sister chromatids are coincident with the locations of these structural features. The integrated area and intensities of FISH probes on different homologs can differ by up to ~20% in the same cell, as expected for length polymorphisms in alphoid heterochromatin. The timing of epigenetic mark formation and DNA segregation are not synchronized between homologs, or between cells. We propose that asynchronous segregation of previously replicated sister chromosomes may account for differences in the hybridization patterns for multicopy and single copy probes in different cells from the same individual.

1286/W

Identification of a new gene involved in IHH by positional cloning of an IHH patient with t(8;10)(q13;p13). *H. Kim[1], R. Ullmann[2], H. Ropers[2], V. Kalscheuer[2], L. Layman[1].* 1) Dept OB/GYN, Medical College of Georgia, Augusta, GA. USA; 2) Dept of Human Molecular Genetics, Max Planck Institute for Molecular Genetics, Berlin, Germany.

Patients with idiopathic hypogonadotropic hypogonadism (IHH) present with absent puberty due to a hypothalamic-pituitary defect, and may be either normosmic (nIHH) or anosmic (Kallmann syndrome/KS). Although mutations in genes such as FGFR1, KAL1, CHD7, and PROKR2 constitute the most commonly encountered etiology in IHH/KS, the molecular basis for most patients remains unknown. Approximately 1/2,000 liveborns possess a de novo balanced translocation, and about 6% of these patients manifest the phenotype of a significant, developmental disorder. These rare, but exceedingly informative patients provide the unique opportunity to identify new genes causing birth defects. We were able to ascertain a male patient with adult onset of IHH who has an apparent balanced chromosome translocation 46, XY,t(8;10)(q13;p13), which affords the opportunity to identify a gene important in pubertal development. Array Comparative Genome Hybridization (CGH) analysis of patient DNA was consistent with a balanced translocation and excluded Copy Number Variation (CNV) as the cause of the IHH phenotype. We hypothesize that a new IHH gene is disrupted or dysregulated by one breakpoint. By performing FISH we revised the real chromosome 10 translocation breakpoint as 10p11.23-10p12.1, narrowing the breakpoint to 3.6 Mb containing 29 known genes between RP11-990I14 and RP11-747L3. The breakpoint of 8q13 was revised to 8q12.1-12.3 and was refined to 6.3 Mb region containing 33 genes between RP11- 826G18 and RP11-59E4. Thus further refining of both breakpoint regions in our balanced translocation patient by array painting is warranted to identify a new causative gene for IHH/KS.

1287/W

Deletions of Xp Provide Evidence for the Role of *HCCS* in Congenital Diaphragmatic Hernia. *D.M. Pearson[1], K. Qidwai[1], G. Simpson Patel[2], B.R. Pober[3,4], L.L. Immken[2], S.W. Cheung[1], D.A. Scott[1].* 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Specially for Children, Austin, TX; 3) Department of Pediatrics, Mass General Hospital for Children, Boston, MA; 4) Department of Surgery, Children's Hospital, Boston, MA.

Microphthalmia with linear skin defects (MLS) is a rare, congenital, X-linked dominant syndrome most commonly caused by terminal deletions of Xp. The Holocytochrome C-Type Synthase gene (*HCCS*) is located on Xp22.2 and inactivation of this gene has been implicated as the cause of many of the characteristic findings in MLS including linear skin defects, microphthalmia and other ocular anomalies, cardiac anomalies, and mild to severe mental retardation. Inactivation of *HCCS* is usually lethal in 46,XY males but several MLS males with Xp;Yp translocations involving the SRY gene have been reported. We report a male infant, prenatally diagnosed with congenital diaphragmatic hernia (CDH), who carried a de novo unbalanced translocation between Xp and Yp—46,X,der(X)t(X;Y)(p22.2;p11.2). After birth he was found to have additional anomalies consistent with MLS including microphthalmia, linear skin defects, bilateral ventriculomegaly, agenesis of the corpus callosum, a small phallus, and right cryptorchidism. Array comparative genome hybridization (aCGH) analysis confirmed that the deleted portion of Xp included the *HCCS* gene. Review of data from this case, and of all other Xp deletions associated with CDH, suggest that disruption of *HCCS* may be sufficient to cause CDH and that another gene(s) located distal to the STS may also contribute to the development of CDH in these patients.

1288/W

Identification of cryptic genomics aberrations in patients with idiopathic mental retardation using MLPA and array-CGH methods. *S. Berker Karauzum[1], D. Yasar Sirin[1], M. Ozcan Caliskan[2], E. Mihci[3], S. Tacoy[3], G. Luleci[1].* 1) Akdeniz University, Medical Faculty, Department of Medical Biology & Genetics; 2) Department of Pathology; 3) Department of Pediatric Genetics, Antalya, Turkey.

The cryptic subtelomeric chromosomal rearrangements are found 5 to 7% of cases with moderate-severe Idiopathic Mental Retardation(IMR) and %1 of cases with mild IMR. The Fluorescence In Situ Hybridization (FISH) using chromosome-specific subtelomeric probes is commonly used for detection of the subtelomeric chromosomal rearrangements. Because the FISH technique is very expensive and time consuming, alternative methods are being used for detection of these rearrangements. One of the these methods is MLPA (multiplex ligation dependent probe amplification) which is a semi-quantitative method that aims to detect copy number chances at genomic levels in a test DNA compared a control DNA. In this study, we aimed to compare the diagnostic capacity of MLPA and subtelomeric FISH methods for cryptic subtelomeric rearrangements and detection an addition rearrangements by array-CGH. In 100 patients with dismorphic features and IMR who had normal karyotype by conventional cytogenetic analysis and subtelomeric FISH analysis, MLPA were used. Although no subtelomeric rearrangements were observed using either MLPA or FISH techniques, the deletions and/or duplications were found in 38(67 %) of 56 patients using array-CGH methods. We propose that the use of array-CGH can be a reliable whole genome screening method to detect of the copy number changes as shown in this study.

1289/W

MRNET TP7 - Summary of high resolution molecular karyotyping of patients with mental retardation -identification of regions containing candidate genes. *K. Hackmann, N. Tyshchenko, T.N. Neuhann, E.M. Gerlach, S. Tinschert, E. Schrock.* Inst. fuer Klinische Genetik, TU Dresden Medizinische Fakultät Carl Gustav Carus, Dresden, Germany.

The German Mental Retardation Network (MRNET) is a nationwide consortium that aims for elucidation of genetic causes of intellectual disability (ID). Currently, ten centers (TP="Teilprojekt") are involved. Participating patients are carefully examined in the Clinical Genetics departments of each center. Eligible causes like Fragile X syndrome or RETT syndrome as well as other phenotypically noticeable conditions are excluded by standard diagnostics before chromosome analysis is performed. If this examination does not reveal any major genomic rearrangements array CGH is carried out. We started off with Agilent's array CGH platform utilizing the standard 244k chip and subsequently upgraded to the 1M technology employing standard 2x400k arrays. Every suspicious copy number variation (CNV) is validated by FISH analysis, customized array CGH, quantitative PCR, or quantitative genotyping in patients as well as in parents to see if a certain variation is of familial origin or arose de novo. All obtained data sets are fed into a database that is accessible for every participating center to carry out comparisons to distinguish between unpublished common CNVs and CNVs that may contain candidate genes. Disease causing mutations that have been confirmed as plausible reason of a patient's phenotype will be further investigated for functional analysis for example in animal models. Out of 117 patients we found 16 de novo mutations of which five have not been described to date and 27 patients without any suspicious CNVs. All remaining patients carry common and/or familial rearrangements of unclear impact. Candidate loci we are currently investigating include one locus with four genes for frontotemporoparietal polymycrogyria and one gene for perisylvian polymicrogyria, one candidate gene for Shprintzen-Goldberg syndrome/ Loeys Dietz syndrome type 1, one gene for microcephaly as well as several other genes for non-syndromal intellectual disability. One family with autosomal dominant presentation of ID and unremarkable array CGH will be put to exome sequencing on a next generation sequencing device.

1290/W

CNV-WebStore: Online CNV Analysis, Storage and Interpretation. *F. Kooy, G. Vandeweyer, E. Reyniers, N. Van der Aa, L. Rooms.* Dept Medical Genetics, Univiversity and University Hospital , Antwerp, Belgium.

Microarray technology allows the analysis of genomic aberrations at an ever increasing resolution, making functional interpretation of these vast amounts of data the main bottleneck in rou-tine usage of high resolution platforms such as the Illumina BeadArray platform, and emphasising the need of a centralised and easy to use CNV data management system. We present an online platform to streamline the processing and downstream analysis of microarray data, tailored towards but not limited to the Illumina BeadArray platform. Provided tools include data analysis, data visualisation, gene prioritisation, automated qPCR primer design and linking data to several genome browsers and databases. To gain insight in the practical resolution of the BeadArray platform, we validated and integrated into the platform an approach to reliably detect rare Copy Number Variations (CNVs) covered by 3 consecutive probes. The presented approach combines three published Hidden Markov Method based algorithms. Up to one third of the small CNVs detected by the presented platform disrupted known coding sequence, stressing the importance of centralised, easy to use functional interpretation tools. The website is freely available for non-commercial use at http://medgen.ua.ac.be/cnv. It is implemented in PHP and Apache with support for all major browser types.

1291/W

A small duplication of 9q22.3-p23 in a patient with 9p-duplication syndrome suggests critical region and a candidate gene for mental retardation and digital anomalies. *X. Li[1], M. Sifry-Platt[2], A. Grix[2].* 1) Dept Genetics, Kaiser Permanente San Jose Medical Ctr, San Jose, CA; 2) Dept Genetics, Kaiser Permanente Sacramento Medical Ctr, Sacramento, CA.

The 9p-duplication syndrome is a clinically recognizable genomic disorder with growth and mental retardation, short fingers and toes with hypoplastic nails, and characteristic craniofacial appearances including microbrachcephaly, deep and wide-set eyes, bulbous nose, and low-set ears. Most of the reported 150 cases with partial or complete 9p duplications were derived from unbalanced translocations and had a concurrent deletion of another chromosome. Isolated tandem duplications of 9p are uncommon. The critical region of the 9p duplication syndrome has recently been delineated to approximately 2.6 Mb (12.8-15.4 Mb) at 9p22.3-9p23 (Zou et al 2009). We report here that a patient with the 9p-duplication syndrome has the smallest de novo isolated duplication of 9p22.3-p23. The patient is a 10 years old male with mental retardation but a normal stature, microcephaly, broad nasal root, conductive or mixed hearing loss, superiorly protruding and mildly posteriorly rotated ears, bifid uvula, and digital anomalies including hypoplastic middle and distal phalanges on the 5th fingers with radial clinodactyly, hypoplastic distal phalanges on the 2nd digits, thumbs mildly hypoplastic with good thenar muscle mass, left foot with cavo-varus position with hypoplastic toenail, left 2nd toe distal phalange mildly bulbous with small nail, right hallux with creased nail, and mild 2/3 toe syndactyly. Array comparative genomic hybridization using the Agilent 8x60K human whole genome microarray designed by the International Standard Cytogenomic Array (ISCA) Consortium revealed an interstitial genomic copy gain of 2.21Mb from 9p22.3-p23 at genomic nucleotide position 12,377,640 - 14,586,920. The affected region of 9p contains 3 OMIM genes, TYRP1, MPDZ, and NFIB, and 2 transcripts, C9orf150 and AK123194 (UCSC HG Build 19). The Nuclear Factor I/B (NFIB) gene belongs to a transcriptional factor family also composed of NFIX and NFIC. Brain malformation (including neocortical callosal dysgenesis) and skeletal defects have been observed in Nfi-deficient mice (Driller et al, 2007, Piper et al, 2009, Pérez-Casellas et al, 2009). Therefore, we proposed here that the NFIB is a candidate gene and its haploinsufficiency is associated with mental retardation and digital anomalies in 9p-duplication syndrome.

1292/W

A 16q24.3 Microdeletion Involving Only the ANKRD11 Gene in a Male with Autism. *M.G. Butler, E.L. Youngs, J.A. Hellings.* Departments of Psychiatry & Behavioral Sciences and Pediatrics, University of Kansas Medical Center, Kansas City, KS 66160.

The 16q24.3 microdeletion syndrome has been recognized only recently with one previous report involving four patients with autism and variable cognitive impairment and each having interstitial deletions of this cytogenetic region (Willemsen et al., 2010). We report another example of a subject involving a microdeletion of the 16q24.3 band with atypical autism and mental retardation. He was the product of a full term gestation following a difficult delivery and a calcified placenta. During infancy and early childhood, developmental delays, recurrent infections, self-injury and anxiety were noted. He was nonverbal, but did use several basic signs. Because of his developmental delays and behavior problems, he had undergone two brain MRIs which were normal and a normal chromosome analysis. On physical exam at 17 years of age, his height, weight and head circumference were < 3rd centile. He had two disorganized hair whorls, bilaterally attached ear lobes, long eye lashes, and a high-arched palate with dental crowding. Plantar creases were noted bilaterally. He had cutis marmorata of his feet and Raynaud's phenomenon (mother with same finding) noted on his hands (right greater than left). Chromosomal microarray analysis performed on our subject identified a small 180 kb deletion of chromosome 16q24.3 band (located at 87.92 to 88.10 Mb from pter) containing the ankyrin repeat domain-containing protein 11 (ANKRD11) gene, a proposed candidate gene for autism (Marshall et al., 2008). ANKRD11 is a member of the ankyrin repeat-containing cofactors that interacts with p160 nuclear receptor coactivators and inhibits ligand-dependent transcriptional activation. The four previously reported subjects with 16q24.3 deletions had deletions of varying size, but had an approximate 90 kb region of overlap (located at about 87.80 to 87.90 from pter) involving ANKRD11 and zinc finger 778 (ZNF778) genes with characteristics including autism spectrum disorder, variable cognitive impairment, facial dysmorphisms and brain anomalies. These two genes were reported as candidates for the autism phenotype in their subjects. The 16q24.3 microdeletion in our subject was more distally positioned compared with the smallest region of overlap in the four previously reported subjects and involved only the ANKRD11 gene further supporting a role for this gene in the clinical presentation of autism.

1293/W

A novel 4p16.3 microduplication characterized by aCGH with new phenotypic features. *A.B. Cyr, M. Nimmakayalu, H. Major, S.R. Patil, K. Keppler-Noreuil, O.A. Shchelochkov.* Division of Medical Genetics, Department of Pediatrics, University of Iowa Hospitals and Clinics, Iowa City, IA.

Trisomy 4p syndrome is a heterogeneous disorder defined by large duplications of the short arm of chromosome 4. The syndrome presents clinically with growth retardation, delayed psychomotor development, and an array of craniofacial and skeletal malformations including microcephaly, prominent glabella, short neck, and low set ears. The majority of the cases reported to date have large duplications of several megabases making precise genotype-phenotype correlations difficult. Reports of smaller microduplications in this region have been rare. We present a 16-month old boy diagnosed with microduplication of chromosome 4p16.3, who, while having some characteristic findings of trisomy 4p syndrome, presents with unique features of macrocephaly, normal growth parameters and irregular iris pigmentation/heterochromia. Chromosome studies conducted on the patient were normal. The array CGH showed a 506kb duplication at 4p16.3. The parental aCGH studies were normal, indicating a de novo microduplication. The purposes of this study are to: 1) report a case with the smallest duplication of chromosome 4p; 2) compare this case to other similar cases in the literature; 3) provide a more specific template for genotype-phenotype comparisons; and 4) to discuss involvement of dosage dependent genes accounting for the clinical phenotype.

1294/W

Clinical Report of a 4q35.2 Microdeletion Involving the MTNR1A, FAT1 and F11 Genes. *R.S. Henkhaus, E.L. Youngs, J.A. Hellings, M.G. Butler.* Departments of Psychiatry & Behavioral Sciences and Pediatrics, University of Kansas Medical Center, Kansas City, KS 66160.

Our subject was diagnosed in early childhood with a variety of conditions, including autism, ADHD, OCD, mild mental retardation, PTSD, self-injury, pica, aggression, easy bruisability and irregular sleep patterns. High resolution chromosome and fragile X DNA analyses were reportedly normal. He was born following a 33 week gestation complicated by maternal diabetes, alcohol used during the first trimester and premature rupture of membranes. He has been successfully treated medically to control his behavior, but other symptoms such as easy bruisability and irregular sleep patterns have persisted. Genetics evaluation at 12 years, 9 months showed normal height, weight and head circumference with flattening of the right occiput, coarse hair and a disorganized single posterior hair whorl. He had malar hypoplasia, relative hypertelorism, a short nose with anteverted nares, a smooth and flat philtrum, a thin upper lip, a broad mouth and a high-arched palate. He had 5th finger clinodactyly, hyperflexible fingers and shallow nail beds. He had short 5th toes bilaterally with malalignment. There was a large ecchymotic area (6 X 8 cm) on right lateral knee area not related to recognized trauma. A chromosome microarray analysis revealed a 1.2 Mb deletion of chromosome band 4q35.2 (located at 187.47 to 188.66 Mb from pter). This deletion contains three genes: Melatonin receptor 1A (MTNR1A); Fat tumor suppressor, Drosophila, homolog of 1 (FAT1); and the blood coagulation factor XI (F11). MTNR1A is a G-protein coupled receptor that plays a role in the regulation of sleep patterns and the circadian clock. The FAT1 gene is a trans-membrane protein that regulates cell-cell adhesion and is expressed in embryonic stem cells and neuronal tissues. Mutations and deletions in the FAT1 gene have been linked to developmental delay and autism. The F11 gene was partially deleted (at the 3' end), likely contributing to his easy bruisability. Several prior reports of chromosome 4q deletions have shown facial and digital dysmorphism, congenital heart defects (e.g., VSD), cleft palate, and mental retardation and one recent report of an autistic individual with a 4q35.1-35.2 deletion, including the area deleted in our subject. Further investigations of this chromosomal region and the expression of genes contained therein may give insights into new causes and therapies for autism and other developmental disorders.

1295/W

Challenges in establishing the clinical implications of microarray findings of uncertain clinical significance. *D.L. Pickering, B.J. Dave, D.M. Golden, J. Stevens, E. Griess, W.G. Sanger.* Munroe Meyer Institute, University of Nebraska Medical Center, Omaha, NE.

Chromosomal microarray has a significantly higher diagnostic yield than conventional cytogenetics among patients with unexplained developmental delay and/or multiple congenital anomalies and has become the first tier testing for this patient cohort. A substantial number of investigations, however, result in novel copy number changes of uncertain clinical significance presenting a formidable challenge in establishing the clinical implications of these findings. For most cases, the standard testing algorithm involves parental studies to determine inheritance patterns. Customarily, the copy number change is interpreted as 'benign' if the aberration is inherited from an 'apparently' normal parent. Our report focuses on cases with initial microarray findings of uncertain clinical significance and subsequent classification of these cases after parental studies. In the past 2 years, our laboratory has performed 2540 post-natal microarray investigations utilizing a customized 44,000 oligonucleotide array. We detected a significant copy number change in 551/2540 (21.6%) cases and 400/2540 (15.7%) were reported as causative for the patient's phenotype. In 151/2540 (5.9%) cases, the copy number change was associated with uncertain clinical significance. The majority of these aberrations were in regions of genome previously not associated with a known syndrome, contained one or more genes and showed little or no overlap with the public and internal databases. All cases had a normal karyotype and harbored cryptic deletions or duplications ranging in size from 94Kb - 2.27Mb. Parental studies were accomplished in 54 of these 151 cases; 3 were *de novo* aberrations and in 51 cases, the abnormality was inherited. Of the 51 familial studies, 38 (74.6%) had alterations that were classified benign in the proband following parental studies, 6 studies (11.7%) remained uncertain and 7 (13.7%) familial aberrations were subsequently determined to be of probable clinical relevance in the proband because the "carrier" parent and/or other family member(s) had a clinical history of a condition similar to the child. A total of 25.4% of our cases were classified as either uncertain or abnormal after parental studies. Thus, our report underscores the importance of collecting precise family history and accurate clinical information in addition to detailed communication with the provider when interpreting inherited copy number changes of uncertain clinical significance.

1296/W

Characterization of previously identified chromosomal abnormalities by high-resolution SNP array analysis. *S. Zimmerman, K. Sund, S. Rudnik, L. Bao, T. Smolarek.* Human Gen, Cincinnati Children's Hosp Med, Cincinnati, OH.

Genome-wide single nucleotide polymorphism (SNP) microarrays provide high resolution copy number information. SNP microarray was used to further characterize previously identified cytogenetic abnormalities to determine whether any significant additional information would be gained. Of approximately 1,667 individuals analyzed by SNP microarray, approximately 56 (3.4%) had a previously identified cytogenetic abnormality detected by karyotype and/or FISH analysis. A chromosomal imbalance was identified in 50 cases, of which clinically significant genetic results were identified in 26 (52%).The chromosomal breakpoints were refined or identified potential pathogenic genes within the imbalance in 24 cases (48%). Two patients had an apparently balanced translocation with an imbalance at the breakpoints and one balanced translocation was identified with an additional clinically significant abnormality. Additional material of unknown origin identified the chromosomes involved in four patients, and the breakpoints were refined in five patients with duplications and 20 patients with. Breakpoints were defined in seven patients with an unbalanced translocations and marker chromosomes (material of unknown origin) were identified in two patients.. Further characterization of complex abnormalities were completed in nine patients. No imbalances were detected in the remaining six patients, which included two patients with an apparently balanced tranlocation inversion, and two inversions. The remaining two patients were phenotypic male or female. These results emphasize the value of combining conventional cytogenetic methods with SNP microarray analysis to better define previously known cytogenetic abnormalities.

1297/W

TULIP1 (RALGAPA1) haploinsufficiency with brain development delay. *K. Shimojima[1], Y. Komoike[2], J. Tohyama[3], S. Takahashi[2], M. Paez[1], E. Nakagawa[4], Y. Goto[4], K. Ohno[5], M. Ohtsu[6], H. Oguni[7], M. Osawa[7], T. Higashinakagawa[2], T. Yamamoto[1].* 1) Institute for Integrated Medical Sciences, Tokyo Women's Med Univ, Shinjuku Ward, Japan; 2) Department of Biology, School of Education, Waseda University, Tokyo, Japan; 3) Department of Pediatrics, Nishi-Niigata Chuo National Hospital, Niigata, Japan; 4) Department of Mental Retardation and Birth Defect Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan; 5) Division of Child Neurology, Institute of Neurological Sciences, Faculty of Medicine, Tottori University, Yonago, Japan; 6) Department of Pediatrics, Saiseikai Yokohamashi Nanbu Hospital, Yokohama, Japan; 7) Department of Pediatrics, Tokyo Women's Medical University, Tokyo, Japan.

A novel microdeletion of 14q13.1q13.3 was identified in a patient with developmental delay and intractable epilepsy. The 2.2-Mb deletion included 15 genes, of which TULIP1 (approved gene symbol: RALGAPA1)was the only gene highly expressed in the brain. Western blotting revealed reduced amount of TULIP1 in cell lysates derived from immortalized lymphocytes of the patient, suggesting the association between TULIP1 haploinsufficiency and the patient's phenotype, then 140 patients were screened for TULIP1 mutations and four missense mutations were identified. Although all four missense mutations were common with parents, reduced TULIP1 was observed in the cell lysates with a P297T mutation identified in a conserved region among species. A full-length homolog of human TULIP1 was identified in zebrafish with 72% identity to human. Tulip1 was highly expressed in zebrafish brain, and knockdown of which resulted in brain developmental delay. Therefore, we suggest that TULIP1 is a candidate gene for developmental delay.

1298/W

A functional analysis of GABARAP on 17p13.1 by knockdown zebrafish. *T. Yamamoto[1], Y. Komoike[2], K. Shimojima[1], J-S. Liang[1], Y. Fujii[3], Y. Maegaki[3], M. Osawa[4], S. Fujii[5], T. Higashinakagawa[5].* 1) Institute for Integrated Medic, Tokyo Women's Med Univ, Tokyo, Japan; 2) Department of Hygiene and Public Health I, Tokyo Women's Med Univ, Tokyo, Japan; 3) Division of Child Neurology, Institute of Neurological Sciences, Faculty of Medicine, Tottori University, Yonago, Japan; 4) Department of Pediatrics, Tokyo Women's Medical University, Tokyo, Japan; 5) Department of Biology, School of Education, Waseda University, Tokyo, Japan.

Array-based comparative genomic hybridization identified a 2.3-Mb microdeletion of 17p13.2p13.1 in a boy presenting with moderate mental retardation, intractable epilepsy and dysmorphic features. This deletion region was overlapped with the previously proposed shortest region overlapped for microdeletion of 17p13.1 in patients with mental retardation, microcephaly, microretrognathia and abnormal magnetic resonance imaging (MRI) findings of cerebral white matter, in which at least 17 known genes are included. Among them, DLG4/PSD95, GPS2, GABARAP and KCTD11 have a function in neuronal development. Because of the functional importance, we paid attention to DLG4/PSD95 and GABARAP, and analyzed zebrafish in which the zebrafish homolog of human DLG4/PSD95 and GABARAP was knocked down and found that gabarap knockdown resulted in small head and hypoplastic mandible. This finding would be similar to the common findings of the patients with 17p13.1 deletions. Although there were no pathogenic mutations in DLG4/PSD95 or GABARAP in a cohort study with 142 patients with idiopathic developmental delay with/without epilepsy, further studies would be required for genes included in this region.

1299/W

CGH+SNP microarrays for simultaneous detection of copy number and copy-neutral variations. *P. Costa, B. Curry, B. Peter, P. Anderson, N. Sampas, S. Giles, A. Ashutosh, J. Ghosh, D. Roberts, A. De Witte.* Agilent Technologies, Santa Clara , CA.

Microarray-based comparative genomic hybridization (aCGH) is a powerful tool used in the detection of DNA copy number variations. The analysis of single nucleotide polymorphisms (SNPs) can complement DNA copy number (CN) measurements to enable identification of copy-neutral changes such as loss/absence of heterozygosity (LOH/AOH) and uniparental disomy (UPD). A set of SNP probes was included in the Agilent SurePrint G3 Human CGH platform, in addition to CGH probes, to enable simultaneous copy number and LOH detection. Using a standard enzymatic labeling workflow, copy number and copy-neutral variations were assessed in a cohort of cytogenetic samples with previously established conditions. Prior to labeling, the genomic DNA was restriction digested with AluI and RsaI, to allow the identification of the SNPs located in the enzymes' recognition sites. After hybridization to CGH+SNP microarrays, the data were analyzed using a novel algorithm implemented in Agilent's Genomic Workbench software. Several LOH regions were identified, from 3 Mb to entire chromosome arms. In two cases, the detected UPDs were found associated with samples known to have Prader Willi and Angelman syndromes. In an additional case, numerous copy-neutral LOH regions were observed throughout the genome, associated with parental consanguinity. SNPs were also accurately determined in genomes bearing amplified and deleted regions in one of the chromosomal copies. Genomic variations including allelic gains and losses, LOH and UPD aberrations were confidently determined by the CGH+SNP platform.

1300/W

SNP MICROARRAY ALLELE DIFFERENTIATION IN 4-COPY NUMBER INTERVALS. *P. Papenhausen, S. Schwartz, R. Burnside, I. Gadi, V. Jaswaney, C. Kelly, R. Pasion, J. Tepperberg.* Dept Cytogenetics, Labcorp America, Res Triangle Park, NC.

Over the past several years the utility of array technology in delineating copy number change has become well established. Genotyping microarrays have major advantages for the detection of UPD, parent of origin and consanguinity, while providing high resolution copy number analysis. However, what is not often highlighted is that the allele designation also permits the elucidation of the mechanism of genomic errors. Segmental triplications have presented G-band diagnostic problems, as well as in microarrays and often FISH. Allele ratios in 31 cases of confirmed four copy intervals offer insight into the underlying etiology. These cases may be divided into five groups with all but group four showing three copies in one homologue: 1) twelve cases that yielded an allele pattern of homozygous (HZ) alleles mixed with 3:1 ratio heterozygotes , 2) eleven cases with homozygosity and both 3:1 and 2:2 ratio heterozygotes, 3) two cases which showed only HZ alleles, 4) two cases with duplications of both homologues and 5) four cases with exclusive 2:2 ratio heterozygosity. Metaphase/interphase FISH analysis revealed that the largest 12 case subset with 3:1 allele heterozygotes represents a triplication of a single parental homologue. Two of the 11 cases in group 2 shared the same distal base pair site adjacent to a large LCR at 5q13.2 and two others were PWS and VCF region triplications, suggesting a NHR correlation and involvement of both parental homologues in this group. In one of two cases in group 4, FISH showed two copies of the 16p11.2 microduplication on each homolog and the SNP array further showed that the interval was within one of many long runs of homozygosity in the proband, consistent with first degree consanguinity and inheritance from both parents. Group 5 has the most intriguing pattern, consisting of four cases with the restricted AABB heterozygosity pattern that is consistent with duplication of both homologues. However, bicolor FISH confirmed a single homolog triplication with the middle copy inverted all four which also demonstrated unbroken allele homozygosity from the distal end of the triplication to the telomere, the hallmark of mitotic recombination. These 4 cases offer compelling evidence for a mitotic recombination mechanism that adds a third copy to the duplication from the other homologue, creating the unique AABB triplication pattern while generating homozygosity from the distal end of the triplication through to the telomere.

1301/W

Increased Telomere Shortening as a Potential Biomarker for Mild Cognitive Impairment and Dementia in Down syndrome. *E. Jenkins[1], L. Ye[1], M. Velinov[1], D. Pang[2], S. Krinsky-McHale[2], W. Zigman[2], N. Schupf[3,4], W. Silverman[5,6].* 1) Dept Hum Genetics, NYS Inst Basic Res Dev Disab, Staten Island, NY; 2) Dept Psychol, NYS Inst Basic Res Dev Disab, Staten Island, NY; 3) Taub Inst Res Alzheim Dis and Aging Brain, Columbia Univ, New York, NY; 4) Depts Epidem and Psychol, Columbia University, New York, NY; 5) Dept Behav Psychol, Kennedy-Krieger Inst, Baltimore, MD; 6) Dept Psychiatry Behav Med, Johns Hopkins Univ Sch Med, Baltimore, MD.

Using quantitative telomere protein nucleic acid FISH analysis, we have shown that people with Down syndrome and dementia (N=15) or mild cognitive impairment (MCI)(N=6) had consistently shorter telomeres (chromosome ends consisting of highly conserved TTAGGG repeats) than age and sex-matched peers (total N = 42; Jenkins et al., 2006, 2008a). “Manual counts” of total fluorescence telomere signals showed increased signal loss for 9 of 9 individuals with DS and dementia as well as 4 of 4 with MCI. Including age- and sex-matched controls, 26 individuals were analyzed for “total telomere loss” (Jenkins et al., 2008b). Employing centromere 2 fluorescence as a “standard,” we calculated the ratio of telomere/centromere 2 fluorescence in telomeres from 9 more individuals (5 females and 4 males and controls) with MCI using PNA probes for both the telomere and the chromosome 2 centromere. This measure generally resulted in greater statistically significant differences between cases and controls versus the PNA telomere light intensities method alone. In addition, it was possible to analyze one of the 9 age- and sex-matched pairs, where one woman of the pair developed MCI over the course of follow-up and one remained cognitively healthy. For the woman with MCI, the light intensity was significantly higher in the earlier sample (p<.005) while the telomere length remained relatively constant from one time to the next for control samples (ps<.23, .33) but were strikingly longer than the telomeres in samples from the woman with MCI (p<.000000). Mean duration of follow-up was 5.4 years. The rate of change was 5.7 light intensity units/year in the woman who developed MCI, while the rate of change was 3.4 light intensity units/year in the woman who remained cognitively healthy. Until now, our analyses of individuals with and without dementia/MCI have been cross-sectional. This is the first longitudinal demonstration of increased telomere shortening “pre-clinically.” Only additional longitudinal research will confirm these exciting results so that telomere shortening may be established as a biomarker indicating increased risk for MCI/dementia. Early detection is especially important because it would allow more timely initiation of future treatments and interventions to minimize central nervous system damage. Supported in part by NYS-OMRDD, Alzh. Assoc. grants IIRG-07-60558, IIRG-99-1598, IIRG-96-077; by NIH grants P01HD35897, R01HD37425, R01AG014673, and R01AG14771.

1302/W

Correlation between interstitial telomeres, chromosomal instability and mosaicism. *M. Roy-Tourangeau[1,2], W. Dridi[3], N. Lemieux[1,2], R. Fetni[1,2].* 1) Pathology and cell biology, University of Montreal, 2900 boul. Édouard-Montpetit, Montreal (Quebec), Canada, H3T 1J4; 2) Pathology and research center, CHU Sainte-Justine, 3175 Côte Sainte-Catherine, Montreal (Quebec), Canada, H3T 1C5; 3) Faculty of Medical Applied sciences, Jazan University, Po Box 114, Royaume de l’Arabie Saoudite.

Telomeres are nucleoprotein complexes localized at chromosomes extremities to maintain the genomic integrity and stability. We propose that the aberrant presence of interstitial telomeres in break points of chromosomal translocations could induce a fragile site like chromosomal instability. To verify our hypothesis, we used Fluorescent In Situ Hybridization (FISH) to look for the presence of interstitial telomeres in patients bearing chromosomal translocations with breakpoints within the terminal bands. We used probes targeting telomeric and sub telomeric sequences of two chromosomal translocations: t(3;3)(p26;q27) and t(14;21)(q24.3;q22.3). Breakpoints and gaps are co-localized with translocation breakpoints and interstitial telomeres. Comparison of the frequency of normal cells and gaps was carried out on cells cultured in RPM-I 1640 with and without BrdU and in 199 media. Our results confirm our proposed hypothesis that the interstitial telomeres confer a chromosomal instability of derivative chromosomes, leading to mosaicism especially in cultures after exposure to BrdU. The role of telomeric sequences as a cause of the observed chromosomal mosaicism is discussed.

1303/W

A normal appearing child with developmental delay and uniparental maternal disomy for all of chromosome 21 including a region of isodisomy. *J.I. Estrada Veras, T.C. Markello, C.J. Tifft.* National Institutes of Health, National Human Genome Research Institute, Bethesda, MD.

Uniparental disomy for chromosome 21 is an uncommon finding rarely reported in the literature. There is no published evidence for imprinting of Chromosome 21. Previous reports of these individuals describe a phenotype that includes the normal range. We investigated the observation of a region of anomalous continuous homozygosity reported from a commercial micro array laboratory. This array was ordered by a local pediatrician for a 4 year old boy with language and neuro-cognitive delays. This individual was the product of an uncomplicated pregnancy with no evidence of a prenatal aneuploidy. He was born full term via vaginal delivery at 3.5kg and had no perinatal complications or dysmophic features noted in the newborn nursery exam. His first year of life was without documentation of delays in early developmental milestones. Cognitive and speech delays were first recognized at the 15 month well child evaluation. His progression has been delayed but continuous and currently he is enrolled on a special education program where he receives speech and occupational therapy. The parents are bilingual and non-consanguineous. To further investigate this case we reanalyzed the child and both parents on a high density genotyping array (Illumina Ominiquad IM). The results revealed 736 SNPs that were consistent with Maternal disomy on chromosome 21 only. The fluorescent intensity at all SNPs showed two alleles at each locus. For a region on chromosome 21 from 20,995,444 to 35,526,209 (bands 21q21.1 to 21q22.12) there is a region of uniparental isodisomy which is 14.53Mb long and contains 53 genes from C21orf131 to RUNX1. We recently reported a patient with Chediak Higashi Syndrome due to Paternal UPD on chromosome 1. That syndrome was due to uniparental isodisomy of a paternal LYST gene that had a single point mutation(Manoli et. al. AJMG(2010) part A,152A: 1474). We are currently in the process of performing whole exome sequencing though the entire region of uniparental isodisomy on Chromosome 21 for both the mother and child. We will identify and further characterize any homozygous mutations in this region that may be involved in this child’s neurodevelopmental delay.

1304/W

Exploration of genes related to X-linked mental retardation (XLMR) by BAC-based X-tiling array. *S. Honda[1], S. Hayashi[1], J. Kobayashi[1], I. Imoto[1], E. Nakagawa[2], Y. Goto[2], J. Inazawa[1].* 1) Dept Molec Cytogenetics, Medical Research Inst, Tokyo, Japan; 2) Dept. of Mental Retardation and Birth Defect Research, Natl. Inst. of Neurosci., Natl. Center of Neurol. and Psychiat.

An estimated 10-12% of mental retardation (MR) is caused by mutation on the chromosome X. Although >90 X-linked mental retardation (XLMR) genes have been identified so far, many other XLMR genes have not been identified. Here, we examined copy number variations (CNVs) by array CGH using BAC-based X-tiling array in patients with MR from 171 families with at least one affected male. CNVs have been considered candidate pathogenic CNVs (pCNVs) when CNVs containing known XLMR genes or not registered in Database of Genomic Variants (http://projects.tcag.ca/variation/) were segregated with MR in the family. This results showed that pCNVs were detected in 13 families (7.6%) and inherited from mothers in 12 families other than unavailable one family. Among them, 8 families had pCNVs involved in known XLMR genes: dup(X)(q28) including *MECP2* detected in 5 families, dup(X)(p11.23) including *FTSJ1* and *PQBP1*, del(X)(p11.23) including *SHROOM4* and del(X)(q24) including *UBE2A* detected in one family respectively. In one family having dup(X)(q28), dup(X)(q21.1) containing *ATRX* was detected simultaneously. On the other hand, pCNVs detected in other 5 families were novel: dup(X)(p22.2) including *MSL3* and part of *FRMPD4*, dup(X)(q21.1) including *HDX*, del(X)(q24) at non-coding region detected in one family respectively, and identical complicated genomic rearrangement involved in Xp22.2 and Xp21.3 containing *NHS*, *REPS2* and *IL1RAPL1* detected in non-consanguineous two families. We identified these breakpoint sequences and performed screening in the general population from ethnic group. As a result, the genomic rearrangement was detected in Japanese (male3; female4/1000), Mongolia (female1/435) and Chinese Korean (male1/200), suggesting that the genomic rearrangement was occurred in a founder in Mongol and introduced into Japan.

1305/T

Examining the role of Rere in inner ear development and hearing loss. *H. Zaveri[1], B. Kim[1], M. Justice[1], B. Lee[1], J. Oghalai[2], D. Scott[1].* 1) Molec & Human Gen, Baylor College Med, Houston, TX; 2) Otolaryngology-Head & Neck Surgery, Baylor college of Medicine, Houston, TX.

The most common telomeric deletion in humans is monosomy 1p36 which affects 1 in 5000 newborns. This deletion has a spectrum of phenotypes which includes mental retardation, developmental delay, and hearing loss. One of the key regulatory genes that reside in the proximal region of chromosome 1p is Atrophin 2 which is also known as RERE due to the presence of Arg-Glu repeat motifs. RERE acts as a nuclear receptor coregulator and is required for normal embryogenesis. In zebrafish, reductions in Rerea expression lead to inner ear anomalies—including fused otoliths and abnormal semicircular canals—and diminished microphonic potentials. Since Rere is conserved from fly to human, RERE may play a similar role in the development of the inner ear in mammals. Mice that are homozygous for the Rere null allele openmind (Rereom/om) are not a useful model for hearing loss because they die at E9.5 with open neural tube and cardiac failure. To overcome this, we have generated an allelic series of Rere deficient mouse bearing different combinations of the om allele and a hypomorphic allele, eye3. In contrast to Rereom/om mice, a portion of Rereom/eye3 mice live into adulthood. Rere om/eye3 mice have an inappropriate startle response to a 108 dB burst at 19.9 kHz emitted from a click box—a common preliminary test for severe hearing loss. Rereom/eye3 mice also fared poorly in tests requiring normal balance and coordination. We are presently confirming and characterizing these phenotypes using distortion product otoacoustic emissions (DPOAE), auditory brainstem evoked response (ABER) testing, and micro CT analysis. We are also making a conditional Rere knock out mouse which will be used to identify the molecular mechanisms by which Rere contributes to normal inner ear development. This will also allow us to bypass the high mortality rate caused by the wide spectrum of phenotypes.

1306/T

Determining the expression pattern of NPAS3 during human brain development. *P. Gould[1], K. So[2], C. Hawkins[3], D. Kamnasaran[4].* 1) CHAUQ Hôpital de l'Enfant-Jésus, Québec, PQ, Canada; 2) PRP laboratory, Laboratory Medicine Program, University Health Network, Toronto, ON, Canada; 3) Division of Neuropathology, Hospital for Sick Children, Toronto, ON,Canada; 4) Department of Pediatrics, Laval University, Québec, PQ, Canada.

The NPAS3 gene belongs to the neuronal PAS transcription factor gene family: basic helix-loop-helix Period-aryl hydrocarbon receptor-single minded (bHLH-PAS); genes which have diverse roles including cancer, development and neuro-behavior. We previously cloned the human NPAS3 gene, mapping to human chromosome 14 and is among one of the largest genes in the human genome. Unfortunately to date, the function of NPAS3 in humans is still relatively unknown. Prior work by us and others had discovered some precursory findings on the developmental expression pattern of NPAS3 in mice. We now determined the expression of NPAS3 in the developing human nervous system from first trimester human fetuses towards adulthood, using immunohistochemistry. During the first trimester, we identified very confined expression of NPAS3 in the developing nervous system, but with some evidence of expression also noted in the developing gut, lungs and oro-pharyngeal cavity. Expression is very strong at this developmental stage especially in the eppendymal cells of the ventricular zones in the developing cerebrum. However, the expression continues to progressively emanate into the outer cortex of the developing fetal brain as the brain matures. In this manner, expression expands into many cell types including choroid plexus epithelial cells, astrocytes, oligodendrocytes and neurons. In fact, no expression was noted in endothelial cells of blood vessels and in smooth muscle cells in the developing brain. Interestingly in early childhood and even in adult brains, NPAS3 expression co-localizes with calretinin expressing neurons. Furthermore, in the brains of the fetuses, children and adults, NPAS3 expression is very strong in the neurogenesis centres, including in the dentate gyrus of the hippocampus, the subventricular zone and in the external granular layer of the cerebellum. Overall, the expression pattern in developing human nervous system appeared to be similarly conserved with the developing murine brain. In summary, findings from this study will assist in better understanding the role of the NPAS3 gene in human nervous system diseases.

1307/T

VANGL2 gene mutations in human Neural Tube Defects. *Z. Kibar[1], P. De Marco[2], S. Salem[3], C. Bosoi[1], E. Pauwels[3], E. Merello[2], A. Bassuk[4], P. Gros[3], V. Capra[2].* 1) Obstetrics & Gynecology, Univ Montreal, Montreal, QC, Canada; 2) U.O. Neurochirurgia, Instituto G. Gaslini, Genova, Italy; 3) Department of Biochemistry, McGill University, Montreal, QC, Canada; 4) Department of Pediatrics, University of Iowa, Iowa City, Iowa, USA.

Neural tube defects (NTDs) are congenital malformations resulting from failure of neurulation. Recent years have witnessed a breakthrough in elucidating the role of planar cell polarity (PCP) pathway in neurulation and how molecular lesions in this pathway lead to NTDs in animal models and humans. PCP is controlled by the non canonical Frizzled/Dishevelled signalling pathway that involves a number of additional core genes, including Stbm/Vang, Flamingo, Prickle, and Diego. The Loop-tail (Lp) mouse that develops a severe form of NTDs called craniorachischisis provided the first line of evidence for involvement of the PCP pathway in NTDs in mammals .Two independent alleles of Lp carry two missense mutations in the Vangl2 gene that is the mammalian homolog of the Drosophila Stbm/Vang gene. .Vangl2 has another homologue Vangl1 that shares similar structures and biochemical activities. We previously identified 8 missense mutations in VANGL1 in human NTDs that were most likely pathogenic based on genetic and functional data. In this study, we screened the human VANGL2 gene in a cohort of 673 patients and we identified 6 novel missense in 7 patients affected with open and closed forms of isolated NTDs. All these mutations were missense heterozygous and were not present in 1362 analyzed controls.The VANGL2 variants, p.Arg135Trp, p.Arg177His, p.Arg270His, affect absolutely conserved aa residues while the three VANGL2 variants, p.Leu242Val, p.Thr247Met and p.Arg482His, affect highly conserved residues across evolution. Four of these mutations were predicted to be deleterious to protein function using bioinformatics tools. The frequency of VANGL2 mutations predicted to be pathogenic in this study (7 in 673 or 1%) is comparable to what we detected in its homologue VANGL1 (0.9%) These results support a role for VANGL2 as a genetic risk factor in a fraction of NTD patients.

1308/T

A transcriptional atlas of human brain development. *E. Lein[1], A. Bernard[1], A. Bongaarts[1], T. Chen[2], G. Coppola[3], C. Dang[1], E. Deelman[4], O. Evgrafov[2], B. Fischl[5], M. Gerstein[6], D. Geschwind[3], J. Hohmann[1], S. Horvath[3], T. Hyde[7], A. Jones[1], H. Kang[6], Y. Kawasawa[6], J. Kleinman[7], P. Levitt[2], S. Mane[6], J. Noonan[6], G. Sedmak[8], E. Shen[1], K. Smith[1], A. Stevens[5], S. Sunkin[1], P. Wohnoutka[1], M. Hawrylycz[1], J. Knowles[2], N. Sestan[6].* 1) Allen Institute for Brain Science, Seattle, WA; 2) USC, Los Angeles, CA; 3) UCLA, Los Angeles, CA; 4) Information Sciences Institute, USC, Los Angeles, CA; 5) Massachusetts General Hospital, Harvard Medical School, Boston, MA; 6) Yale University, New Haven, CT; 7) NIMH, Bethesda, MD; 8) Croatian Institute for Brain Research, Zagreb, Croatia.

Although the human genome sequence has been available for over a decade, similar systematic efforts to map detailed gene expression patterns in human brain development have been lacking. To fill this void, we describe here an ARRA-funded, consortium-based project aimed at creating a unique multimodal transcriptional atlas of the pre- and postnatal developing human brain as a publicly accessible online resource for the neuroscience, genome and medical research communities. This resource will integrate transcriptomic, cellular resolution histology and imaging data in the context of human brain development through an online portal for viewing, searching and mining of spatiotemporal gene expression patterns. Specifically, the project aims to profile the transcriptome of a series of cortical and subcortical brain regions in males and females across eleven pre- and postnatal developmental stages from very early neocortical development (4-7 post-conceptual weeks) through adulthood (n=6/stage, 8-16 structures/stage, ~960 samples total). Transcriptome analysis is performed using Illumina RNA-seq methodology to allow a highly quantitative analysis of gene, exon and splice junction usage in specific brain regions. To complement these moderate resolution transcriptome data, a large scale in situ hybridization (ISH) data set is being generated to analyze cellular distributions using an industrial scale histology platform. ISH gene selection was biased towards genes associated with human neurological and neuropsychiatric disorders, as well as a portion matching the NIH Blueprint NHP Atlas (www.blueprintnhpatlas.org) to allow a direct comparison of gene expression patterns between human and non-human primate expression model systems across postnatal brain development. Finally de novo MRI, DTI and histology reference atlases spanning human brain development are being created to provide a neuroanatomical and neurodevelopmental context for understanding spatiotemporally regulated transcriptional programs. The final product, integrating these large-scale data sets with tools for data visualization and analysis, aims to create a lasting resource for relating specific transcriptional programs to processes of human brain development and a normative data set for understanding the genetic basis of neuropsychiatric disease. The transcriptional atlas of human brain development will be publicly accessible through the Allen Institute for Brain Science portal (www.brain-map.org).

1309/T

Tubulinopathies are associated to neuroblasts migration deficit and axonal guidance disturbances in human brains. *K. Poirier[1], J. Martinovic[2], M. Bonnieres[2], M. Ossondo[3], V. Cayol[4], T. Attie-Bitach[2], A. Benachi[5], J. Chelly[1], M. Vekemans[2], F. Encha-Razavi[2].* 1) Institut Cochin - INSERM Unité 1016, CHU Cochin, Paris, France; 2) Unité de Génétique du Développement Embryo-F=9Ctal, Groupe Hospitalier Necker-IPP. Université Paris Descartes. INSERM U781; 3) CHU de Fort de France; 4) Service Gynécologie-Obstétrique, Groupe Hospitalier Necker-IPP Paris; 5) Service Gynécologie-Obstétrique, Hôpital A. Béclère, Clamart.

Microtubules are important components of the eukaryote cytoskeleton. They are crucial for many cell functions, including axon formation, cell shape, motility, intracellular trafficking and mitosis. The main components of microtubules are different isoforms of alpha and beta tubulins, which are often cell-type specific. The recent identification of numerous mutations in genes that encode different alpha- and beta-tubulin isoforms in relation to congenital neurological disorders has raised the question of the impact of microtubules deficit on brain development. Here we report a de novo heterozygous mutation in the TUBA1A (p.K326N) gene observed in a 23 weeks-gestation fetus. The fetus presented on neuropathological examination, a microlissencephalic brain with unusual cytoarchitectonic anomalies, dominated by an arrest of neuroblasts migration and major axonal guidance disturbances. The cerebellum was hypoplastic poorly cellular and devoid of lamination. The present study supports the hypothesis that tubulin genes are required for normal brain development.

1310/T

Mutations in centrosomal protein CEP152 in primary microcephaly families linked to MCPH4. *M. Samuels[1, 2, 3], D. Guernsey[3], H. Jiang[3], J. Hussin[1], M. Arnold[5], K. Bouyakdan[1], S. Perry[3], T. Babineau-Sturk[6], J. Beis[6], N. Dumas[3], S. Evans[3], M. Ferghuson[6], M. Matsuoka[3], C. Macgillivray[3, 4], M. Nightingale[3], L. Patry[1], A. Rideout[6], A. Thomas[6], A. Orr[4], I. Hoffman[5], J. Michaud[1], P. Awadalla[1], D. Meek[7], M. Ludman[6, 8].* 1) Centre de Recherche du CHU Ste-Justine, Montreal, PQ, Canada; 2) Deparment of Medicine, University of Montreal, Montreal, PQ, Canada; 3) Department of Pathology, Dalhousie University, Halifax, NS, Canada; 4) Department of Ophthalmology and Visual Sciences, Dalhousie University, Halifax, NS, Canada; 5) German Cancer Research Center, Heidelberg, Germany; 6) IWK Health Sciences Centre, Halifax, NS, Canada; 7) Dalhousie University, Halifax, NS, Canada; 8) Department of Pediatrics, Dalhousie University, Halifax, NS, Canada.

Primary microcephaly is a rare condition in which brain size is substantially diminished without other syndromic abnormalities. Seven autosomal loci have been genetically mapped, and the underlying causal genes have been identified for five of these. The known genes play roles in mitosis and/or cell division. We ascertained three families from a Maritime Canadian subpopulation, each with one microcephalic child having head circumference between five and seven standard deviations below the mean (two girls, one boy). There were no other dysmorphic signs and height and weight were normal. One girl showed cognitive impairment with both verbal and performance scores below 1st percentile, but with visual motor skills up to 4th percentile. The other girl excelled at reading and kept up to her peers academically until age 11 years. MRI scan was performed for one girl, showing markedly reduced brain size with mild enlargement of the posterior horns of the lateral ventricles. The gyral pattern was simplified in keeping with the small brain size but the cortex appeared of normal thickness. Homozygosity analysis in two families using genome-wide dense SNP genotyping supported linkage to the published MCPH4 locus on chromosome 15q21.1. Sequencing of coding exons of candidate genes in the interval identified a non-conservative amino acid change in a highly conserved residue of the centrosomal protein CEP152. The affected children in these two families were both homozygous for this missense variant. The third affected child was compound heterozygous for the missense mutation plus a second, premature termination mutation, truncating a third of the protein and preventing its localization to centrosomes in transfected cells. CEP152 is the putative mammalian ortholog of Drosphila asterless, mutations in which affect mitosis in the fly. Published data from zebrafish are also consistent with a role of CEP152 in centrosome function. By RT-PCR, CEP152 is expressed in embryonic mouse brain, similar to other MCPH genes. Like some other MCPH genes, CEP152 shows signatures of positive selection in the human lineage. CEP152 is a strong candidate for the causal gene underlying MCPH4, and may be an important gene in the evolution of human brain size. This represents the sixth novel gene discovered as part of the Genome Canada/Genome Atlantic project, the Atlantic Medical Genetics and Genomics Initiative (AMGGI).

1311/T

The Role of *Semaphorin 3D* in Hirschsprung disease (HSCR): From GWAS to Functional Analyses in Model Systems. *S.M. Arnold[1], T.A. Heanue[2], S.L. Bessling[1], G.M. Burzynski[1], V. Pachnis[2], A.S. McCallion[1], A. Chakravarti[1].* 1) Inst Gen Med, Johns Hopkins Univ Sch Med, Baltimore, MD; 2) Div Mol Neurobiol, Natl Inst Med Res, London, UK.

Hirschsprung disease (HSCR) is a multifactorial neurocristopathy of the enteric nervous system. The receptor tyrosine kinase *RET* plays a key role in all forms of HSCR and interacts with other genes to produce a range of phenotypes. By genome-wide association studies in 220 affected trios and a replication set of 430 trios, we identified, beyond a known enhancer polymorphism in *RET*, a polymorphism (rs12707682) at 7q21.11 with significant association with HSCR ($p=1.03x10^{-7}$). This marker resides in the vicinity of several Semaphorin Family 3 members (downstream of *SEMA3D*; upstream of *SEMA3A*, *SEMA3E*, and *SEMA3C*). To identify the culprit gene, we sought functional insight through model systems. Morpholino knock down of orthologous genes in zebrafish identified *sema3d* and *sema3c* as the prime candidates, with increasing concentrations of each morpholino producing a proportional loss of innervation along the digestive tract at days 4-6 post-fertilization. In addition, the morpholino for *sema3d*, at a concentration too weak to generate a gut phenotype, worked synergistically with a *ret* morpholino (also at a sub-phenotypic concentration) to eliminate intestinal innervation. This effect was also seen when the *sema3c* morpholino was coinjected with the *ret* morpholino but required higher, modest phenotype-producing input concentrations for both. These results support the hypothesis that additional HSCR genes exist to modify the effect of *RET* and mirrors our previous work in mice showing a synergistic relationship of *Ret* and *Ednrb* on HSCR. *In situ* hybridization in mice similarly supports these data. Of the four candidates, temporal and spatial expression of *Sema3d* most closely resembled that of *Ret*: both were present in the enteric neural crest-derived cells (ENCCs) at E11.5 and in the myenteric plexus at E15.5 and P3. *Sema3c* was the only other message evident in the developing ENS (confined to E15.5) while *Sema3e* was detected in the ENS only at P3, and *Sema3a* showed no ENS expression at any stage. In addition, analysis of $Ret^{k-/k-}$ mice at E15.5 showed an absence of *Sema3d* and *Sema3c* expression in the myenteric plexus of aganglionic intestines and severe reduction of both in the partially ganglionated stomach, but no change in *Sema3a* or *Sema3e* expression in these tissues, confirming an ENS role for the former and no role for the latter. Taken in concert, these data suggest that *SEMA3D* is the most likely *RET* modifier and HSCR candidate at 7q21.11.

1312/T

CRISPLD2 and Neural Crest Cell Migration. *B.T. Chiquet[1,2], E. Swindell[1], R. Henry[1], L. DeVault[1], A. Burt[3], J.B. Mulliken[4], S. Stal[5], M. Warman[4,6], S.H. Blanton[3], J.T. Hecht[1].* 1) Dept Pediatrics, Univ Texas Med Sch, Houston, Houston, TX; 2) Univ Texas Dental Branch, Houston, TX; 3) Univ Miami Miller School of Medicine, Miami, FL; 4) Children's Hospital, Boston, MA; 5) Texas Children's Hospital, Houston, TX; 6) Howard Hughes Medical Institute, Boston, MA.

Craniofacial development is a tightly regulated process that involves the complex orchestration of genetic and environmental factors, including interaction of cell growth, growth factors and receptors, the convergence and fusion of the facial and palatal processes, apoptosis, and adequate nutrient supply. The tissues that form the craniofacies are derived from cells that migrate from the neural tube and direct craniofacial patterning. Perturbation of neural crest cell (NCC) migration can cause abnormal craniofacial development, such as nonsyndromic cleft lip with or without cleft palate (NSCLP). NSCLP is a common, complex birth defect that is caused by genetic and environmental factors. We have previously identified association between NSCLP and variants in the CRISPLD2 (cysteine rich secretory protein LCCL domain containing 2) gene and genes in the folate gene pathway. The CRISPLD2 protein contains more cysteine residues than comparably sized proteins and the folate gene pathway produces endogenous cysteines. In this study, we tested for gene interactions between the CRISPLD2 gene and genes in the folate gene pathway and identified 19 interactions in our nonHispanic white dataset (0.0005£p£0.01) and 11 interactions in our Hispanic dataset (0.002£p£0.01). Of these findings, the most significant interactions were with genes in the methionine arm of the folate gene pathway, which is responsible for producing homocysteine and cysteine. Homocysteine has previously been shown to regulate neural crest cell (NCC) derived tissues. Interestingly, our recent zebrafish studies have shown that knockdown of CRISPLD2 during embryogenesis alters Dlx2 staining of NCCs and the zebrafish have altered cartilage structures of the craniofacies. Taken together, these studies suggest a potential function of CRISPLD2 in NCC migration that alteration of normal CRISPLD2 during development can contribute to clefting etiology.

1313/T

Gene expression profiling of developing human hearts. *L. Larsen[1], K.D. Ajbro[1], M. Bak[1], M.L. Vestergaard[1], K. Møllgård[2], K. Lage[3], C.A. Clement[4], S.T. Christensen[4], E. Bendsen[5], N. Tommerup[1].* 1) Wilhelm Johannsen Centre for Functional Genome Research, Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen, Denmark; 2) Department of Cellular and Molecular Medicine, University of Copenhagen, Blegdamsvej 3, DK-2200 Copenhagen, Denmark; 3) NNF Center for Protein Research, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark; 4) Department of Biology, University of Copenhagen, Universitetsparken 13, DK-2100 Copenhagen, Denmark; 5) Fertility Clinic, Department of Obstetrics and Gynaecology, University Hospital of Odense, Odense, Denmark.

Development of the human heart starts approximately two weeks post fertilization (PF) with formation of the cardiac crescent and the subsequent formation and looping of the primitive heart tube in week 3-4. These events are followed by extensive tissue remodeling which takes place in week 5-9 and includes septation of the atrium and ventricles, development of trabeculae within the ventricles, valve development and separation of the outflow tract. Development of the heart involves spatial and temporal regulation of an unknown number of genes. In order to identify genes which are temporally regulated during heart development, we used deep-sequencing based expression analysis to study the gene expression profile in human developing hearts. Using Illumina's digital gene expression assay we analyzed total RNA extracted from twelve different hearts (four groups of three hearts at ages around day 41, 48, 59 and 67 PF). Differentially expressed genes were identified using student's T-test. The set of differentially expressed genes are enriched for genes encoding sarcomeric proteins and genes known to cause heart defects when deleted in mice. Data quality was validated by real-time quantitative RT-PCR analysis of randomly selected differentially expressed genes. For 79% of the genes the gene expression profiles were replicated by this independent method. We conclude that expression profiling based on deep-sequencing analysis is useful for identification of temporal differentially expressed genes within the developing human heart.

1314/T

Shox2 mediates Tbx5 activity by regulating Bmp4 in the pacemaker region of the developing heart. *S. Puskaric[1], S. Schmitteckert[1], A.D. Mori[2], A. Glaser[1], K.U. Schneider[1], B.G. Bruneau[2], R.J. Blaschke[1], H. Steinbeisser[1], G. Rappold[1].* 1) Institute of Human Genetics, Heidelberg, Germany; 2) Gladstone Institute for Cardiovascular Disease, San Francisco, US.

Heart formation requires a highly balanced network of transcriptional activation of genes. The homeodomain transcription factor Shox2 is essential for the formation of the sinoatrial valves and for the development of the pacemaking system. The elucidation of molecular mechanisms underlying the development of pacemaker tissue has gained clinical interest as defects in its patterning can be related to atrial arrhythmias. We have analysed putative targets of Shox2 and identified the *Bmp4* gene as a direct target. Shox2 interacts directly with the *Bmp4* promoter in ChIP assays and activates transcription in luciferase reporter assays. In addition, ectopic expression of *Shox2* in *Xenopus* embryos stimulates transcription of the *Bmp4* gene and silencing of *Shox2* in cardiomyocytes leads to a reduction in *Bmp4* expression. In *Tbx5*$^{del/+}$ mice, a model for Holt-Oram syndrome, and *Shox2*$^{-/-}$ mice we show that the T-box transcription factor Tbx5 is a regulator for *Shox2* expression in the inflow tract and that *Bmp4* is regulated by Shox2 in this compartment of the embryonic heart. In addition, we could show that Tbx5 acts cooperatively with Nkx2.5 to regulate *Shox2* and *Bmp4* expression. This work establishes a link between Tbx5, Shox2 and Bmp4 in the pacemaker region of the developing heart and thus contributes to the unravelling of the intricate interplay between the heart-specific transcriptional machinery and developmental signalling pathways.

1315/T

CRELD1 is Required for Vascular Maturation and Craniofacial Development. *J.K. Redig[1], D. Babcock[2], B. Reshey[2], C.L. Maslen[1, 2].* 1) Molecular and Medical Genetics; 2) Cardiovascular Medicine, Oregon Health & Science University, Portland OR.

CRELD1, a member of the TGFβ superfamily, is a cell surface protein of unknown function. We previously demonstrated that heterozygous missense mutations in *CRELD1* are associated with cardiac atrioventricular septal defects (AVSD), establishing *CRELD1* as a genetic risk factor for AVSD. Creation and characterization of a constitutive Creld1-knockout mouse model has provided insight into the role of CRELD1 during development. Lack of Creld1 results in embryonic lethality with *Creld1*$^{-/-}$ embryos dying before embryonic day 11.5 (E11.5). One striking feature is that there is a failure of vascular maturation. Although a primitive vascular plexus forms, the vascular system does not continue on with higher-order branching morphogenesis. This lack of a mature vasculature is apparent in the *Creld1*$^{-/-}$ embryos and their yolk sacs by approximately E10.0. Branching morphogenesis is a VEGF-dependent process, and we had previously demonstrated that Creld1-deficient cells are hyper-responsive to VEGF, which may provide a mechanism to explain why vascular development is arrested in the absence of Creld1. Until approximately E10.5 the *Creld1*$^{-/-}$ embryos are morphologically indistinguishable from their wild type and heterozygous littermates. At that point they begin to lag in development and exhibit gross morphological defects. There is considerable apoptosis in the null embryos, particularly in the head and branchial arches and some regions of the heart. Accordingly, the null embryos have significant developmental defects that appear to be a result of programmed cell death as opposed to necrosis due to vascular insufficiency. This includes a failure of development of the forebrain and abnormal facial development. In addition, the tissue along the dorsal midline fissure appears to be grossly disorganized which might disrupt midline fusion if development continued. Consequently, in some respects the cranial phenotype is reminiscent of holoprosencephaly. The heart, which is still beating, has a thin myocardium with decreased trabeculation of the single common ventricle. Significantly, the atrioventricular endocardial cushions show considerable cell loss due to apoptosis, which is consistent with the proposed role of CRELD1 in the cause of AVSD. Overall, the phenotype suggests that CRELD1 is involved in the development of neural crest derived structures as well as a playing a specific role in cardiovascular development, which is likely linked to an interaction with VEGF.

1316/T

Fibulin-4 is required for the development of the notochord and the cardiovascular system. *Z. Urban[1,2], A.B. Maxfield[2], S.M. Khatri[1], R.P. Mecham[3], E.M. Joseph[4].* 1) Department of Human Genetics, University of Pittsburgh, Graduate School of Public Health, Pittsburgh, PA; 2) Department of Pediatrics, Washington University School of Medicine, St. Louis, MO; 3) Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO; 4) Massachusetts General Hospital, Charlestown, MA.

Homozygous mutation in fibulin-4 (*FBLN4*, *EFEMP2*) cause a recessive cutis laxa syndrome associated with vascular tortuosity and aortic aneurysms. To elucidate the molecular mechanisms of this novel disease, a zebrafish model was chosen for study. In silico analysis of the zebrafish genome demonstrated that human *EFEMP2* has two paralogues in the zebrafish genome, *efemp2a* and *efemp2b*. Whole mount in situ hybrization analysis of zebrafish *efemp2a* and *efemp2b* displays a dynamic expression pattern during embryogenesis associated with the segmentation of the lateral mesoderm and neural crest migration. Expression of *efemp2a* is then detectable in the bulbus arteriosus (outflow tract) of the developing heart around 101 hours post fertilization. Zebrafish embryos injected with morpholinos designed to knock down the expression *efemp2a* and *efemp2b* where shorter than controls, displayed delayed development of segmental vasculature and circulation, developed thinner notochords as well as disorganized ventral mesenchyme at 24 hours post fertilization. At 2 days post fertilization, embryos injected with *efemp2a* specific morpholinos did not develop a swim bladder and showed enlargement of the heart associated with valvular insufficiency. Knockdown of *efemp2b* resulted in circulation failure with pooling of the blood in the caudal region suggesting obstructive vascular disease. Immunoblot analysis showed progressive proteolytic degradation of both efemp2a and efemp2b during the course of segmentation. This degradative process coincided with a peak in pSMAD2 levels. Losartan treatment rescued vascular abnormalities in both *efemp2a* and *efemp2b* knockdown animals. Our results suggest that efemp2 inhibits the release of transforming growth factor-beta from the extracellular matrix, but allows developmentally regulated release through proteolysis.

1317/T

Change in Visual Acuity in Albinism in the Early School Years. *J.M. Dijkstal, A.M. Holleschau, S.S. Cooley, R.A. King, C.G. Summers.* University of Minnesota, Department of Ophthalmology, Minneapolis, MN.

Introduction and Purpose: Albinism is a genetic disorder in which biosynthesis of melanin pigment is absent or reduced. Affected persons typically have reduced vision, nystagmus, iris transillumination, and foveal hypoplasia. We sought to determine if binocular best-corrected visual acuity (BCVA) improves in the early school years, and if this is related to type of albinism, ocular pigment, and/or appearance of the macula.

Methods: This retrospective chart review was conducted at the University of Minnesota with IRB approval and included persons with albinism seen from 1984-2010. Diagnosis was made by a geneticist and a pediatric ophthalmologist based on clinical phenotype and detection of a gene mutation known to cause albinism, and was supported by misrouting with VEP, a normal ERG, and the absence of platelet dense bodies in Hermansky Pudlak syndrome. We included patients with ophthalmologic visits at ages 5.5 to <9 years (Visit A) and 9.5 to <14 years (Visit B), with Visit A and B being separated by ≥2.5 years. Patients who had surgery for nystagmus were excluded. We recorded type of albinism, binocular BCVA, glasses wear, grades of iris pigment and macular transparency, and presence or absence of an annular reflex and melanin in the macula.

Results: Mean age of 65 subjects was 6.9 years at Visit A and 11.8 years at Visit B ($p<0.001$). Mean BCVA was 20/84 at Visit A and 20/61 at Visit B ($p<0.001$). Subgroup analysis for OCA1B and OCA2 also found BCVA improvement from 20/68 to 20/51 ($p=0.003$) and 20/79 to 20/63 ($p=0.018$), respectively. Iris grade correlated moderately with change in BCVA ($r=0.329$, $p<0.001$). Weak correlations were found between improvement in BCVA and glasses wear, macular grade, and macular melanin and/or an annular reflex. A moderate correlation was found between BCVA and iris grade ($r=0.559$, $p<0.001$) and the presence of macular melanin ($r=0.402$, $p<0.001$) at Visit A. BCVA also moderately correlated with iris grade at Visit B ($r=0.499$, $p<0.001$).

Conclusions: BCVA often improves in albinism in the early school years and this should be included in counseling. The etiology is unknown but may be related to change in nystagmus, use of precise null point, developmental maturation, and/or some of the ocular characteristics evaluated in this study.

1318/T

Deciphering the connection between cilia and cell signaling in the dorsal neural tube using the mouse mutant, *Arl13b^{hnn}*. *V. Horner, T. Caspary.* Department of Human Genetics, Emory University, Atlanta, GA.

Human disease states that disrupt cilia have long supported the importance of this ubiquitous cellular organelle; however, renewed interest in cilia-related genes was sparked by the discovery that cilia are essential for cellular signaling during development. Joubert syndrome is the most commonly inherited cerebellar malformation syndrome with a prevalence of approximately 1:100,000 and is known to be due to defects in cilia. To better understand the relationship between cilia, cell signaling, and disease, we study a small GTPase implicated in all three. In humans, hypomorphic mutations in *Arl13b* lead to the classical form of Joubert syndrome. In mice, lack of Arl13b disrupts the structure of the ciliary axoneme, resulting in abnormally short cilia. The ciliary defect in *Arl13b^{hnn}* mutants disrupts the gradient of Sonic Hedgehog (Shh) activity during development, leading to ventral neural tube patterning defects. Here we show that the *Arl13b^{hnn}* mutation also disrupts dorsal neural tube patterning. In the dorsal neural tube, transforming growth factor-β (TGFβ) and Wnt signaling pathways specify dorsal cell types. The dorsal phenotype in *Arl13b^{hnn}* raises the possibility that the signaling pathways involved in dorsal cell specification are disrupted in *Arl13b^{hnn}* mutants. Here, we show that Wnt signaling is normal in the *Arl13b^{hnn}* dorsal neural tube, but BMP signaling is disrupted. Further, we demonstrate that Arl13b is not directly required in the dorsal neural tube for BMP signaling; instead aberrant Shh signaling in the ventral neural tube prevents cells in the dorsal neural tube from responding to BMP signals. However, unlike other mutants where abnormal Shh signaling disrupts dorsal neural tube patterning, BMP ligand expression is normal in the *Arl13b^{hnn}* roof plate. Therefore, our results propose a novel role of Shh signaling in modulating the BMP pathway downstream of BMP ligands.

1319/T

BBS8 interacts with a component of the Wnt/planar cell polarity (PCP) pathway to establish left-right asymmetry in zebrafish. *H.L. May-Simera[1], M. Kai[4], V. Hernandez[2], D.P.S. Osborn[2], M. Tada[3], M. Kelley[1], P. Beales[2].* 1) NIDCD, National Section on Developmental Neuroscience, National Institute on Deafness and other Communication Disorders, National Institutes of Health, Bethesda, Maryland, USA of Health, Bethesda, MD; 2) Molecular Medicine Unit, Institute of Child Health, University College London, WC1N 1EH, UK; 3) Department of Anatomy and Developmental Biology, University College London, WC1E 6BT, UK; 4) National Institute for Basic Biology, Okazaki, 444-8585, Japan.

Laterality defects such as situs inversus are not uncommonly encountered in humans either in isolation or as part of another syndrome, but can have devastating developmental consequences. The events that break symmetry during early embryogenesis are highly conserved amongst vertebrates and involve the establishment of unidirectional flow by cilia within an organising centre such as the node in mammals or Kupffer's vesicle (KV) in teleosts. Disruption of this flow can lead to failure to successfully establish left-right asymmetry. The correct apical-posterior cellular position of each node/KV cilium is critical for its optimal radial movement which serves to sweep fluid (and morphogens) in the same direction as its neighbours. Planar cell polarity (PCP) is an important conserved process that governs ciliary position and posterior tilt; however the underlying mechanism by which this occurs remains unclear. Here we show that Bbs8, a ciliary/basal body protein important for intraciliary/flagellar transport and the core PCP protein Vangl2 are required for establishment and maintenance of left-right asymmetry during early embryogenesis in zebrafish. We discovered that loss of bbs8 and vangl2 results in laterality defects due to cilia disruption at the KV. We discovered that perturbation of cell polarity following abrogation of vangl2 causes nuclear mislocalisation, defective centrosome/basal body migration and apical docking. These data suggest that bbs8 and vangl2 act synergistically on cell polarization to establish and maintain the appropriate length and number of cilia in the KV and thereby facilitate correct LR asymmetry.

1320/T

N-Ethyl-N-Nitrosurea mutagenesis identifies a hypomorphic allele at the *Looptail* locus. *M. Guyot[1], F. Kharfallah[1], M.J. Justice[2], Z. Kibar[1].* 1) CHU Sainte Justine and Department of Obstetrics and Gynecology, University of Montreal, Montreal, Quebec, Canada; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas.

The *Looptail* (*Lp*) mouse represents a well-established model for the study of neural tube defects (NTDs), the most common and severe malformations of the central nervous system in humans. *Lp* heterozygous mice are characterized by a looped-tail appearance while homozygous embryos suffer from a severe form of NTDs called craniorachischisis where the neural tube remains open throughout the hindbrain and spinal cord region. *Lp* homozygous embryos also display severe inner ear polarity defects manifested by disorganization of the stereociliary bundles of hair cells of the organ of Corti. In two previously described *Lp* alleles, *Lp* and *Lp^{m1Jus}*, the gene mutated was identified as *Vangl2* that forms part of the non-canonical Frizzled-Dishevelled or planar cell polarity pathway. In this study, we describe a novel *Lp* allele called *Ska^{m17Jus}* that was generated by N-Ethyl-N-Nitrosurea (ENU) mutagenesis at the Mouse Mutagenesis and Phenotyping Center for Developmental Defects in Texas Medical Center in Houston. Sequence analysis of *Vangl2* in *Ska^{m17Jus}* identified one disease-specific mutation, p.Arg259Leu, that was absent in parental strains, C57BL/6J Tyr-/Tyr- Brd (albino) and 129SvEv, and in 28 other inbred strains. Interestingly, and as compared to the two other *Lp* alleles, this mutation segregated in a recessive manner where all heterozygous *Ska^{m17Jus}* /+ mice analyzed appeared normal and homozygous *Ska^{m17Jus}* / *Ska^{m17Jus}* had a looped-tail appearance at a penetrance of 46%. In addition, 71% of homozygous females had imperforate vagina. Examination of homozygous *Ska^{m17Jus}*/*Ska^{m17Jus}* embryos revealed spina bifida in 13% but no severe craniorachischisis. These embryos also showed modest inner ear polarity defects. Complementation studies demonstrated the severe craniorachischisis in 62% and spina bifida in 12% of *Ska^{m17Jus}*/+; *Lp*/+ compound heterozygous embryos. This study suggests that *Ska^{m17Jus}* represents a new hypomorphic allele of *Lp*, providing an important tool for molecular and cellular studies of the underlying pathogenic mechanisms.

1321/T

Evidence of adenomyosis in fetuses : Another emerging piece in resolving the endometriosis puzzle. *J. Martinovic[1,2], D. Rambeau[3], J. Gogusev[3], C. Bergeron[1], A. Benachi[5].* 1) Unit of Fetal Pathology, Department of Pathology, Laboratory Cerba, St Ouen L'Aumone, France; 2) AP-HP, Unit of Fetal Pathology, Department of Genetics, Antoine Béclère Hospital, Paris, France; 3) Gynecology and Obstetrics Office, 5 rue de la Pompe, Paris, France; 4) Institut Cochin, UM3, Genomics and Epigenetics of Infertility and Placental Diseases, Paris, France; 5) AP-HP, Department of Gynecology and Obstetrics, Antoine Béclère Hospital, Paris, France.

BACKGROUND: Endometriosis affects approximately 10% of the female population in their reproductive years and represents one of the most common human diseases. The most widely accepted theory of origin is Sampson's theory of reflux menstruation. Sampson's classification of heterotopic endometrial tissue is based on pathogenesis : 1) "direct or primary endometriosis" [adenomyosis]; 2) "peritoneal or implantation endometriosis"; 3) "transplantation endometriosis"; 4) "metastatic endometriosis"; and 5) "developmentally misplaced endometrial tissue". METHODS: In order to test the hypothesis of congenital adenomyosis ("developmentally misplaced endometrial tissue"), in 2000, we designed a prospective study of uteri in fetuses terminated spontaneously or for lethal malformations. Histology with systematic serial sections were performed in 420 fetal uteri. FINDINGS: Adenomyosis was observed in a total of 10 fetuses aged from 19 to 37 weeks. There was no increase in the threshold toward adenomyosis in the fetuses presenting malformations of the uro-genital system. INTERPRETATION: These data suggest that adenomyosis might be present from the fetal stage, with possible genetic backgrounds in some cases. Furthermore, our results enhance a novel etiopathogenetic concept of adenomyosis as a developmental defect of differentiation or migration of the mullerian duct system during embryogenesis.

1322/T

RET mediated gene expression profiling of ENS precursors. *R.M.W. Hofstra[1], Y. Sribudiani[1], R.S.N. Fehrmann[1], G. Burzynski[1], M. Metzger[2], J. Osinga[1], G.J. te Meerman[1], A.J. Burns[2], N. Thapar[2].* 1) Genetics, University Medical Center Groningen, Groningen, Groningen; 2) Gastroenterology and Neural Development Units, Institute of Child Health, University College London, London, United Kingdom.

Activation of the RET receptor is believed to trigger many important signal transduction routes crucial for the migration, proliferation and differentiation of the neural crest stem cells (NCSCs) that are responsible for the formation of the enteric nervous system (ENS), the intrinsic innervation of the gastrointestinal tract. To identify pathways and genes involved in/or triggered by RET activation, we performed gene expression profiling using Microarray Affymetrix GEneChip Mouse Genome 430 2.0 platform on GDNF (the RET ligand) stimulated and non-stimulated NCSCs. For this we isolated YFP labeled-NCSCs from embryonic mouse guts (E14.5) of Wnt1-Cre/LoxP/R26-YFP transgenic mice. The YFP-positive NCSCs were isolated by FACS sorting and short term cultured in selection media with or without GDNF. The data were analyzed by Gene Set Enrichment Analysis (GSEA) and single-gene analysis methods. Not only does this approach provide information on GDNF-induced RET signaling in NCSCs, it may also be helpful in identifying candidate genes for Hirschsprung disease (HSCR), a congenital disorder characterized by a lack of neurons in the distal part of the colon, which is caused by aberrant migration, proliferation or differentiation of NCSCs. Pathways that were up-regulated during GDNF stimulation were those important for ATP-synthesis, cholesterol-synthesis and lipid metabolism. Several important signaling pathways for ENS development and more generally, embryonic development were found to be significantly down-regulated upon GDNF stimulation. These included the Notch, TGF-β/Smad and Wnt signaling pathways. Genes involved in adherens Junctions formation were down-regulated which could lead to a loss of cell-cell contact, a process crucial for cell migration. Genes involved in the cell growth arrest and apoptosis were down-regulated, which nicely correlates with the observed proliferation of the stimulated cells. By single-gene analysis, we identified 72 genes that were differentially expressed between NCSCs treated with GDNF and untreated cells after Bonferroni multiple testing correction ($p < 0.05$). Among these were one known HSCR gene, ECE1, and 6 other genes located in the HSCR susceptibility loci (3p21, 9q31 and 16q23). In summary, here we provide a profile of pathways and genes that are expressed in ENS precursors and regulated by the RET signaling. These data might prove helpful in identifying possible candidate HSCR predisposing genes.

1323/T

The role of *Interferon regulatory factor 6* (Irf6) in the development of the placenta. *A.L. Smith[1], M. Meister[4], M. Hoinski[2], M. Dunnwald[4], D. Hoffmann[5], B. Yang[6], B.C. Schutte[1,2,3].* 1) Genetics Program, Michigan State University, East Lansing, MI; 2) Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI; 3) Pediatrics and Human Development, Michigan State University, East Lansing, MI; 4) Department of Pediatrics, The University of Iowa, Iowa City, IA; 5) Anatomy and Cell Biology, The University of Iowa, Iowa City, IA; 6) Obstetrics and Gynecology, The University of Iowa, Iowa City, IA.

IRF6 is necessary for craniofacial, limb, and skin development in humans and mice. Recent studies in other animal models suggest that *IRF6* also has a role in the development of extra-embryonic tissue. Specifically, Irf6-deficient zebrafish (Danio rario) and frog (Xenopus laevis) show defects in their primary surface epithelium (Sabel, Dev. Biol. 2009), the extra-embryonic cells that surround the early embryo. In mammals, the extra-embryonic tissues are derived from trophectoderm and develop into the placenta. Previous studies show that Irf6 is expressed in murine placenta. We hypothesize that *Irf6* is expressed in the trophectoderm and is required for proper development of the placenta. Wildtype and *Irf6*-deficient placentas were collected at embryonic day 14.5 (E14.5) and 17.5 (E17.5) from matings between mice heterozygous for an *Irf6* null allele. Placental sections were stained with Hematoxylin and Eosin and Periodic Acid Schiff (PAS) for histological analysis and to identify trophoblast glycogen cells, and immuno-stained with Irf6 antibodies to detect Irf6 expression. In human placenta, we observed that Irf6 is expressed in the syncytiotrophoblasts that surround the placental villae. In placenta from E14.5 wild type mice, we observed Irf6 in the full range of differentiated trophoblasts of the junctional zone, which includes spongiotrophoblasts, giant cells, and trophoblast glycogen cells, as well as the decidua and the uterine lining. In *Irf6*-deficient placentas Irf6 expression is unaffected only in the decidua and uterine epithelium, as the both of these tissues are maternally derived. Placentas deficient for *Irf6* have more clusters of trophoblast glycogen cells in the labyrinth zone than wildtype placentas at E14.5 ($n=4$, $p=0.03$). Preliminary data also suggests that excess trophoblast glycogen cell clusters in the labyrinth zone of *Irf6*-deficient placentas persists through E17.5. The expression data and the morphological abnormalities suggest that *Irf6* has a role in the development of the placenta in addition to its known role in craniofacial development. The correlation between expression of IRF6 in human and murine placenta will allow future analysis into the conserved role of this gene in early development. These observations may be relevant clinically because DNA variation in *IRF6* contributes risk to non-syndromic cleft lip and palate. Future studies will test whether this DNA variation also affects development of the placenta.

1324/T

Expression of Lox-1, a gene activating inflammatory pathways both in atherosclerosis than in cancer, and of its alternative splicing isoforms during mouse development. *L. Vecchione[1], L. Diano[1], L. Campagnolo[2], L. Rocchi[1], G. Siracusa[2], J.L. Mehta[3], G. Novelli[1,3,4], F. Amati[1].* 1) Department of Biopathology and Diagnostic Imaging, Tor Vergata University of Rome; 2) Department of Public Health and Cell Biology, Tor Vergata University of Rome; 3) Department of Internal Medicine, University of Arkansas for Medical Sciences; 4) PTV Medical Hospital Tor Vergata University.

LOX-1 (Lectin-like oxidized low-density lipoprotein receptor-1) is the primary endothelial receptor of oxidized LDL (oxLDL). Both in vitro and in vivo experiments have shown this receptor to be important in the initiation of atherosclerosis and to be upregulated by pro-atherogenic factors, e.g. shear stress, tumor necrosis factor (TNF-α) and oxLDL itself. Recently, it has been demonstrated that LOX-1 is important for maintaining the transformed state in different cancer cell lines and for tumor growth, suggesting that it acts in a molecular pathway connecting cancer and atherosclerosis. Despite the amount of data on LOX-1 function in adult tissues, very little is known about its expression and localization during embryo development. We analysed the mRNA expression level of Lox-1 at various developmental stages from 4.5dpc (days post coitum) to 18.5dpc by RT-PCR and quantitative real time PCR (qRT-PCR). Lox-1 is expressed in all the developmental stages we analysed with the highest levels at 7.5dpc, 8.5dpc and 9.5dpc, critical time points in mouse cardiogenesis. Two novel alternative splicing isoforms were detected in all the developmental stages analysed. The first, named Lox-1D2D5, lacks exons from 2 to 5 while the second, Lox-1D3D5, lacks exons from 3 to 5. Both isoforms retain the C-type lectin domain, that is the functional domain for the oxLDL binding, highly conserved among species. Both isoforms seem to be specific of embryonic and foetal stages since we don't find them in mouse adult tissues. By immunofluorescence assays, we showed a plasma membrane localization for Lox-1 but not for the isoforms, thus suggesting that Lox-1 isoforms may not have the capacity to recruit the receptor to the plasma membranes than the Lox-1 full length. Whole-mount in situ hybridization performed on 9.5dpc mouse embryos, revealed Lox-1 expression mainly in the heart and portions of the vasculature. Both isoforms co-localize with Lox-1 full length. So our expression data suggest a possible involvement of Lox-1 (and probably of its alternative splicing isoforms) in the processes of murine cardiogenesis and angiogenesis.

1325/T

Mouse models reveal the role of SOX7 in the development of diaphragmatic hernias and cardiovascular malformations associated with recurrent 8p23.1 deletions. *M. Wat[1], M. Garcia[2], Y. Chen[1,4], Z. Yu[1], R.J. Schwartz[3], B. Lee[1,4], M.E. Dickinson[2], D.A. Scott[1].* 1) Molec & Human Gen, Baylor College Med, Houston, TX; 2) Molec Physiology & Biophysics, Baylor College of Medicine, Houston, TX; 3) Biology and Biochemistry, University of Houston, Houston, TX; 4) Howard Hughes Medical Institute.

Recurrent interstitial deletions of a region of 8p23.1 flanked by low copy repeats are associated with a spectrum of anomalies that can include congenital diaphragmatic hernia (CDH) and cardiovascular malformations (CVMs). We hypothesize that haploinsufficiency of *SOX7* contributes to the development of these phenotypes. To test this hypothesis we created *Sox7*$^{+/-}$ mice. Approximately 15% of these mice have retrosternal CDH in which a portion of the liver and gallbladder have herniated into the thoracic cavity and are encased in a thin membrane. This type of retrosternal sac hernia is identical to that seen in some patients with 8p23.1 deletions and suggests that *SOX7* is required for normal diaphragm development. *Sox7*$^{-/-}$ mice die in utero around E10.5, with the majority exhibiting enlarged pericardial sacs and failure of yolk sac vascular remodeling. Several *Sox7*$^{-/-}$ mutants displayed hypoplastic hearts whereas others had hearts morphologically similar to those of wild-type littermates. To determine if these cardiovascular phenotypes were primarily due to abnormalities in cardiac development, we conditionally ablated *Sox7* in cardiac tissues using an *Nkx2.5-Cre*. *Sox7*$^{-/flox}$*;Nkx2.5* $^{Cre/+}$ mice were recovered in Mendelian ratios and did not exhibit cardiovascular anomalies. This suggests that the effects of *Sox7* on cardiac function may be caused primarily by a defect in vascular development. This is consistent with the high level of *Sox7* expression seen in the developing vasculature.

1326/T

Tracing the derivation of embryonic stem cells from the inner cell mass by single cell RNA-Seq analysis. *K. Lao[1], T. Tang[2], C. Barbacioru[1], S. Bao[2], C. Lee[2], E. Nordman[1], X. Wang[1], M. Surani[2].* 1) Molec Cell Biol, Applied Biosystems, part of Life Technology, Foster City, CA; 2) Wellcome Trust/Cancer Research UK Gurdon Institute of Cancer and Developmental Biology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QN, UK.

The molecular mechanism underlying the transition from the inner cell mass (ICM) of blastocysts to pluripotent embryonic stem cells (ESC) is not fully understood. This is partly because of the apparent heterogeneity amongst a small group of cells, which poses difficulties in investigating this question. Using single cell RNA-Seq transcriptome analysis at the resolution of single cells, we have analyzed the dynamic molecular network within individual cells from the ICM outgrowth and the established ESC. This study has identified molecular changes that accompany this transition. Our study shows that key genes that confer the property of self-renewal are up regulated as ICM cells progress to ESC. We also detected very significant global changes of transcript variants from individual genes, amongst which the general metabolism genes are strongly over-represented. Furthermore, there was a global increase in the expression of repressive epigenetic regulators with a concomitant decrease in gene activators. The unique ESC epigenotype may thus be sustained while retaining an inherent plasticity for differentiation. Moreover, changes in miRNAs result in one set that targets early differentiation genes, and the second set targets ESC specific pluripotency genes to maintain a delicate balance between pluripotency and a capacity for rapid differentiation. In conclusion, our study provides insight into the dynamic and systematic molecular changes that occur during cell fate decisions from identical cells. During the conversion of ICM cells to ESCs, there is an evident arrest of a normal developmental program, which is subverted in vitro in favor of a dynamically stable status for unrestricted self-renewal while retaining the full potential to undergo differentiation into all the diverse cell types. We demonstrate how both the retention of the expression of a full set of master genes allows inheritance of a key property of the ICM, namely pluripotency, while the up regulation and down regulation of other crucial genes permits exit from a normal developmental program, which at the same time confers the key property of unlimited self-renewal. Changes in epigenetic regulators apparently allow for the stable transmission and robust maintenance of the newly acquired epigenotype in ESCs between exceptionally fast cell generations.

1327/T

Genetic background of *Prop1* mutants provides remarkable protection against hypothyroidism induced hearing impairment. *S.A. Camper[1], Q. Fang[1], A.M. Giordimaina[1], M. Mustapha[1,2], D.F. Dolan[1], T.J. Jones[1], A.H. Mortensen[1], M.T. Fleming[1], Y. Raphael[1], K.R. Johnson[3].* 1) Dept Human Genetics, Univ Michigan Med Sch, Ann Arbor, MI; 2) Stanford University, Palo Alto, CA; 3) The Jackson Laboratory, Bar Harbor, ME.

Hearing impairment occurs in 1/2000 births and ~1/2 are genetic. Hypothyroidism is a cause of genetic and environmentally induced deafness. Congenital hypothyroidism affects 1/3500 children, causing growth insufficiency, mental impairment, and permanent hearing deficits if untreated. Maternal hypothyroidism and environmental thyrotoxin exposure can impair children's hearing. The sensitivity of cochlear development and function to thyroid hormone (TH) mandates understanding TH action and identification of TH dependent genes. There are several examples of such genes that are relevant to genetic deafness: i.e. Prestin (*SLC26A5*, *DFNB61*) and *KCNQ4* (*DFNA2A*). The *Prop1* and *Pou1f1* mutant mice carry mutations in different pituitary transcription factors, each resulting in the inability to produce pituitary thyrotropin and lack of detectable serum TH. Despite the same TH deficit, these mutants have very different hearing abilities: *Prop1* mutants are mildly affected, while *Pou1f1* mutants are completely deaf. Intercrosses and genetic mapping studies show that this difference is attributable to the genetic backgrounds. Using embryo transfer we discovered that factors intrinsic to the fetus are the major contributor to this difference, not maternal effects. There are multiple, permanent abnormalities in the cochlear development of *Pou1f1* mutants that contribute to their profound deafness. We analyzed *Prop1* mutants to identify processes that are protected by their genetic background. Outer hair cell (OHC) function develops slowly. It depends on Prestin and KCNQ4 expression, which is normal in mature *Prop1* mutants. The endocochlear potential and KCNJ10 expression in the stria vascularis are normal in mature *Prop1* mutants. Hypothyroidism affects innervation and synapse remodeling *Pou1f1* mutants, but no differences in neurofilament or synaptophysin staining are evident in *Prop1* mutants. The synaptic vesicle protein otoferlin (*DFNB9*) normally shifts expression from OHC to IHC as temporary afferent fibers beneath the OHC regress in the first postnatal week. *Prop1* mutants exhibit persistent, abnormal expression of otoferlin in apical OHC, possibly indicating incomplete regression of afferent fibers. Thus, the genetic background of *Prop1* mutants is remarkably protective for most functions except otoferlin expression in the cochlear apex. The *Prop1* mutant is an attractive model for identifying the genes that protect against deafness due to hypothyroidism.

1328/T

Cellular suppression of *Lgl1* induces tropoelastin expression and augments response to lipopolysaccharide (LPS). *L. Montermini[1], I. Mandeville[1], N.B. Sweezey[2], F. Kaplan[1,3,4].* 1) Montreal Children's Hospital Research Institute, Montreal , Quebec, Canada; 2) Hospital for Sick Children, Toronto, ON, Canada; 3) Dept.of Pediatrics, McGill University, Montreal,PQ, Canada; 4) Dept.of Biology, McGill University, Montreal,PQ, Canada.

Background: *Lgl1* (also known as crispld2), modulates lung branching and alveogenesis. Absence of *Lgl1* in null mice is embryonic lethal. Postnatal *Lgl1* $^{+/-}$ mice show impaired lung maturation and features common to human BPD including distal airspace enlargement, disruption of elastin fibers, elevated tropoelastin (TE) and increase in pro-inflammatory cytokines. All these features are exacerbated by hyperoxia. Native Lgl1 binds LPS. Recombinant Lgl1 inhibits LPS binding to target cells and suppresses LPS-induced cytokine production. Hypothesis: *In vitro* deficiency of *Lgl1* confers increased susceptibility to LPS-induced inflammatory cytokine production and induces elastin expression in developing lung fibroblasts. Methods: Plasmids encoding *Lgl1* shRNAs were used to stably suppress *Lgl1* expression in NIH3T3 and Human Fetal Lung fibroblasts (HFL). Specificity and potency of the targeting sequence were validated by qRT-PCR and Western Blot. Cells lines expressing stable clones were assessed for TE expression by qRT-PCR and WB. NIH3T3 fibroblasts were exposed to 1μg/mL of LPS for 6-24hrs and assessed for interleukin (IL) 6 expression by qRT-PCR. Results: In NIH3T3 and HFL cells lines that stably expressed an *Lgl1* shRNA, *Lgl1* mRNA and protein was suppressed by 80%. In NIH3T3-Lgl1^{KD} and HFL-Lgl1^{KD} cells, *Lgl1* suppression was associated with markedly reduced cell growth. An increase in TE mRNA and elastin protein was observed in NIH3T3-Lgl1^{KD} and HFL-Lgl1^{KD} cells respectively. LPS-exposed NIH3T3-Lgl1^{KD} cells had significantly elevated levels of IL-6 compared to PBS-exposed NIH3T3-Lgl1^{KD} controls. Conclusion: Our results suggest that *Lgl1* is involved in TE mRNA regulation. *Lgl1*, secreted by fibroblasts adjacent to epithelium may protect these cells from endotoxin-induced inflammation. The *in vitro* properties of *Lgl1* support the hypothesis that it is a molecular regulator of perinatal inflammation and of downstream effects on alveogenesis.

1329/T

A *Sox10* sensitized mouse ENU mutagenesis screen: uncovering pathways in development and disease. *D.E. Watkins-Chow[1], K.E. Leeds[1], R. Mullen[1], D.L. Silver[1], K. Buac[1], H.W. Hwang[1], I. Matera[2], S.K. Loftus[1], D.M. Larson[1], A. Incao[1], W.J. Pavan[1].* 1) Genetic Disease Research Branch, NHGRI, NIH, Bethesda, MD; 2) Laboratorio di Genetica Molecolare, Istituto G. Gaslini, Genova, Italy.

Melanocytes are specialized, neural crest-derived cells responsible for pigment production in the skin. Genetic interference of neural crest development can present as altered pigmentation in skin and/or hair and can be associated with debilitating diseases (neurocristopathies) including deafness, blindness, cleft lip, congenital megacolon, and albinism. Disruption of genes that regulate pigmentation can also affect a diverse array of tissues, due to inherent pleiotropic roles of genes that have been co-opted for function in non-neural crest-derived structures. We have used a *Sox10* sensitized mouse ENU mutagenesis screen to uncover previously uncharacterized pathways in melanocyte development, to provide insights into development of additional organ systems, and to generate models relevant for dissecting human disease etiology. This screen identifies mutations that increase the phenotypic severity of *Sox10* haploinsufficient mice (*Sox10$^{LacZ/+}$*) that carry a mutation in a transcription factor essential for melanocyte development. From analysis of 600 pedigrees, we have identified five dominant modifiers of the *Sox10* phenotype (*Mos1-5*) and four recessive modifiers of the embryonic *Sox10^{LacZ}* expression pattern (*msp1-4*). The causative mutations associated with these modifiers affect genes involved in a variety of functions including hedgehog, neuregulin, and semaphorin signaling as well as ribosomal and RNA binding proteins. Comparative analysis of melanoblast development confirms that these mutations affect different time points in development and that different mechanisms lead to the observed melanoblast phenotypes. Further characterization of genes identified in the screen will contribute to our understanding of human genome function, provide additional disease models for human neurocristopathies and identify additional candidate pathways for melanoma progression.

1330/T

Studying Molecular Bases of Muscular Dystrophies in *Drosophila* Model System. *D. Lyalin, N. Nakamura, K. Koles, V. Panin.* Dept of Biochemistry-Biophysics, Texas A&M Univ, College Station, TX.

O-mannosylation is a posttranslational modification of proteins with *O*-linked mannose attached to serine or threonine residues of protein backbones. This unusual type of glycosylation is thought to be important for several aspects of cell interactions, including cell adhesion, migration, and interaction with extracellular matrix. POMT1 and POMT2 are the two *O*-mannosyltransferase genes that have been described in mammalian genomes to date. Notably, mutations in these genes have been linked to dystroglycanopathies, including the Walker-Warburg syndrome, a severe form of human muscular dystrophy. One of few known targets of *O*-mannosylation in humans is *Dystroglycan* (*Dg*), and its abnormal glycosylation underlies developmental and physiological defects in a number of muscular dystrophies.

Drosophila rt and *tw* genes encode homologs of mammalian POMT1 and POMT2 *O*-mannosyltransferases, respectively. Mutations in these genes result in “rotated abdomen” phenotype, a clockwise rotation of abdominal segments. It is not known what causes the rotation of the abdomen in *Drosophila*. We studied temporal requirements for both genes and spatial requirements of *tw* for the abdomen development. We found that abdomen rotation phenotype could be rescued by *tw* or *rt* expression during an unusually big “window” of developmental stages. The results of these experiments will be discussed from the perspective of using *Drosophila* as a model system for the investigation of molecular and genetic mechanisms of *O*-mannosylation and would help to shed light on underlying cause of muscular dystrophies in humans.

1331/T

Patterns of sensory processing and their relationship to the neurobehavioral phenotype of children with Smith-Magenis syndrome (SMS). *R.S. Morse[1], H. Hildenbrand[2], A.C.M. Smith[1].* 1) NHGRI/Office of the Clinical Director, NHGRI, NIHOCD, NHGRI/NIH, Bethesda, MD; 2) Rehabilitative Medicine Dept, NIH, Bethesda, MD.

Smith- Magenis Syndrome (SMS) is a rare (1/15,000) microdeletion syndrome of chromosome 17 p11.2 associated with a specific pattern of physical, developmental and behavioral characteristics. The neurobehavioral phenotype is distinct and complex, characterized by high rates of outbursts/tantrums, attention-seeking, impulsivity, aggression, hyperactivity, distractibility, toileting difficulties, stereotypies (repetitive or self-stimulatory behaviors), sleep disturbance and self-injurious behaviors (Dykens & Smith, 1998). We suspect that underlying sensory processing deficits contribute to previously observed maladaptive behaviors, social difficulties and functional deficits that characterize the syndrome. This cross-disciplinary study investigates the relationship between sensory modulation and maladaptive behaviors characteristic of SMS using two validated instruments derived from the disciplines of occupational therapy and behavioral psychology. The Sensory Profile (SP) Caregiver Questionnaire and Child Behavior Checklist (CBCL) were collected from 25 parents of individuals (13F/12M; ages 3-25yrs; mean 7.16 yrs) with a confirmed SMS diagnosis. Results: Clinically meaningful low scores for one or more of the SP quadrants were documented in 24/25 (96%) of SMS subjects, indicative of sensory processing/modulation problems (Registration = 22; Seeking = 18; Sensitivity = 17; Avoiding = 21). Only one individual scored within the normal limits for all four SP quadrants (male; 3 years of age). On the CBCL, 20/25 (80%) SMS subjects had clinically significant high total scores for maladaptive behavior. Spearman Correlation Coefficients revealed statistically significant inverse relationships ($p=<0.05$) between the SP quadrant scores and the total scores on the CBCL. These results support the hypothesis that in individuals with SMS sensory modulation difficulties are associated with maladaptive behavior and functioning. Further examination of the relationship between patterns of sensory processing and maladaptive behaviors may expand the behavior management approach and contribute to improved social participation and daily function in individuals with SMS.

1332/T

Background Strain and Natural Selection Improves Survival of HIBM Murine Model. *Y. Valles-Ayoub[1,2], Z. Khokher[1,2,4], A. Haghighatgoo[1,2], D. No[1,2,4], S. Esfandiarifard[1], C. Saechao[1], R. Carbajo[1,4], C. Creencia[3], S. Darvish[1,2], B. Darvish[2], D. Darvish[1,2].* 1) HIBM Research Group, Inc, Reseda, CA; 2) VA Greater Los Angeles (VA-GLA/UCLA), Los Angeles, CA; 3) Tissue Procurement Core Laboratory, Department of Pathology and Laboratory Medicine, UCLA School of Medicine, Los Angeles, CA; 4) Los Angeles Mission College, Sylmar, CA.

Recessive form of Hereditary Inclusion Body Myopathy (HIBM, IBM2, MIM:600737) is an adult onset muscle wasting disorders caused by hypomorphic GNE, the rate-limiting enzyme of sialic acid (Sia) biosynthesis. Unlike human patients, mice bearing the GneM712T/M712T genotype in C57BL/6 background strain suffer severe glomerular hematuria, incomplete podocyte development, and do not survive beyond the first few days of life. We crossed heterozygous mice (GneM712T/+) of B6 strain with FVB strain mice. In mixed inbred FVB;B6 background (N1), the homozygous mice show attenuated glomerular disease and survive longer (mean survival 23.48 ± 13.99 weeks, n=73). Within the first 2 generations, 26% of the homozygous mice survived passed the age of 40 weeks, and within the subsequent 3 generations the frequency of homozygous mice surviving pass age of 40 weeks has increased to 44%. Additionally, the homozygous mice (GneM712T/M712T) living past the age of 42 weeks begin to show muscle pathology. In summary, the background strain affects the disease phenotype, and natural selection may have an influence on long term maintenance of mouse models of human disease.

1333/T

Elucidating the Mechanism of Cardiac Teratogenicity in a Murine Model of Maternal PKU. *N.J. Seagraves[1,2], K.L. McBride[1,2].* 1) The Research Institute at Nationwide Childrens Hos, Columbus, OH; 2) The Ohio State University, Columbus, OH.

Phenylketonuria (PKU) is an inborn error of metabolism due to phenylalanine hydroxylase (PAH) deficiency which leads to elevated serum phenylalanine (Phe). Elevated Phe levels during pregnancy [Maternal PKU (MPKU)] cause heart and other defects. The mechanism of Phe teratogenicity upon the heart remains elusive. The BTBR Pahenu2 mouse provides a model to characterize the MPKU associated cardiovascular malformations (CVMs). We hypothesize there is a maternal serum Phe level and developmental period of exposure required to induce CVMs in offspring of mouse PKU females and the genetic background will influence the manifestations of CVMs. We established four exposure groups (control<360μM, low 360-600, mid 600-900, high>900) to determine the critical maternal Phe level. We screened 190 exposed BTBR fetuses and observed gross defects of the outflow tract (OFT) and vessel morphology including coarctation of the aorta. CVMs occurred in a dose-dependent manner as maternal serum Phe levels increase, from 5.6% of the controls to 20% in the high group (Nonparametric test for trends, p=0.03). We performed timed Phe exposure during gestation. Preliminary data indicates E8.5-E12.5 as the critical period. Early exposed fetuses have higher rates of transposition of the great arteries (TGA) while later exposure have truncus arteriosus (TA), double outlet right ventricle (DORV) and interrupted aortic arch (IAA). Further experiments are required to determine a more limited time period. We have generated congenic mice to investigate the effects of genetic background upon incidence and phenotypic variation of CVMs. We have not observed a difference in the incidence of CVMs among strains. We have observed phenotypic differences on each of the four Pahenu2 strains. The majority of affected fetuses on the BTBR background have TGA, while affected BL6 Pahenu2 have Dextrocardia with IAA, C3H/HeJ Pahenu2 have DOLV, and FVB/N Pahenu2 have TA. Defects of OFT and vessel morphology implicate the abnormalities involving cardiac neural crest cells (cNCCs). Using chick neural tube explants, to date we have not observed Phe exposure to affect migration or growth of the explant. Experiments to characterize cell signaling are ongoing. These results provide strong evidence that the murine model of MPKU is an excellent model to investigate the mechanism of Phe teratogenicity on heart development, which can aid in the understanding of the development of CVMs.

1334/T

***ESRRG* as a Candidate Gene for Human Congenital anomaly of the kidney and urinary tract (CAKUT).** *D.R. FitzPatrick[1], R. Berry[1], L. Harewood[1], J. Moss[1], M. Fisher[1], P. Branney[1], M. Whiteford[2], A. Howatson[3], N. Hastie[1], P. Hoenstein[1], D. Brownstein[4], L. Pei[5], R. Evans[5].* 1) Med Dev Gen Section, MRC HGU, Edinburgh, United Kingdom; 2) Department of Clinical Genetics, Yorkhill Hospital, Glasgow, United Kingdom; 3) Department of Paediatric Pathology, Yorkhill Hospital, Glasgow, United Kingdom; 4) Molecular Pathology, University of Edinburgh, Edinburgh, United Kingdom; 5) The Salk Institute, Howard Hughes Medical Institute, La Jolla, CA.

Congenital anomaly of the kidney and urinary tract (CAKUT) are common disorders of human development affecting the renal parechyma, renal pelvis, ureter, bladder and urethra that show evidence of shared genetic aetiology, although the molecular basis of this remains unknown in the majority of cases. We have previously reported a de novo, apparently balanced reciprocal translocation, t(1;2)(q41;p25.3) [Joss et al., 2003, Clin Genet, 63, 239-40], associated with non-syndromal bilateral renal agenesis. We performed breakpoint mapping using interphase FISH on nuclei extracted from archive tissue sections. Expression analysis in embryonic mouse kidneys of all the genes within 500Kb of both breakpoints implicated the gene encoding the nuclear steroid hormone receptor *ESRRG* as a strong candidate gene for CAKUT. We have found that Esrrg protein is detected throughout early ureteric ducts as cytoplasmic staining with nuclear localization seen only in the distal tip. In 14.5-16.5 dpc (days post conception) mouse embryos Esrrg localizes to the subset of ductal tissue within the kidney, liver and lung. The renal ductal expression becomes localized to renal papillus by 18.5 dpc. Perturbation of Esrrg function was then performed in embryonic mouse kidney culture: Pooled siRNA was used to inactivate Esrrg and a specific small-molecule agonist to induce abberent activation of Esrrg. Both result in severe abnormality of early branching events of the ureteric duct. Analysis of mouse embryos with a targeted inactivation of *Esrrg* on both alleles (*Esrrg*-/-) identified agenesis of the renal papillus with normal development of the cortex and remaining medulla. Taken together these results suggest that Esrrg is required for early branching events of the ureteric duct that occur prior to the onset of nephrogenesis. These finding confirm ESRRG as a strong candidate gene for CAKUT.

1335/T

Cranio-Encephalic Developmental Anomalies In A Mouse Model Of Fgfr3-Related Chondrodysplasia. *F. Di Rocco, E. Mugniery, N. Kaci, A. Jonquoy, C. Benoist, A. Munnich, L. Legeai-Mallet.* INSERM U781 Paris Descartes University, PARIS, France.

The fibroblast growth factors and their receptors regulate several cellular processes during embyonic and postnatal development. FGFR3 (Fibroblast Growth Factor Receptor 3) mutations are responsible for long bone anomalies such as achondroplasia or thanatophoric dysplasia (TD) but also craniofacial synostosis such as Muenke syndrome or Crouzon syndrome with acanthosis nigricans. Aim of the study is the analysis of FGFR3-related anomalies of cranial growth with a knock-in mouse model. Using the cre-lox system Fgfr3 mice express the mutation Y367C corresponding to the mutation Y373C responsible of TD type I in humans. The skull and the craniocervical junction of Fgfr3 mice (n:14) were compared to wild type (WT) controls (n:16). A macroscopic analysis was performed after alizarin blue and alcian red staining. It was completed by Xrays, Computed Tomography (CT) and Magnetic Resonance Imaging (MRI). Fgfr3 mutation induces an important deformation of the vault. All P21 fgfr3 mice presented a brachycephaly with a reduction of the anterioposterior lenght of the skull and brain and an increase of their height. They all had a defect in the ossification of the midline (all of mutant mice), going from an interfrontal defect to a complete absence of fusion of frontal and parietal bones. The nasal, the occipital bones and the atlas vertebra were reduced in size (79%,47%,48%, respectively, p<0.001) whereas the frontal and parietal bone sizes were unaffected. The interparietal bone size increased (116%, p=0.004). The shape of the skull was affected with a reduction of the angle between the frontal and nasal bones (139° vs 174° in WT, p=0.004). The mandible was prognatic in all mice. The foramen magnum was reduced in size, with a crowding of neural structures and a disappearance of cerebrospinal fluid signal at MRI. The cranial base was abnormal with an increased angle (140° vs 124° in WT, p=0.003) and a fusion of the clivus and sphenoid bone. In mutant newborns and E 16.5 embryos the interfrontal and interparietal distances were increased and the clivus abnormal. However its fusion with the sphenoid was absent in embryos and apparent at birth in most mice. Fgfr3 mutation disrupts the cranial base but also the skull vault. The anomalies progress during embryonic and postnatal development. The mouse cranial phenotype correlates with human FGFR3-related pathologies and highlights the primordial role of FGFR3 and endochondral ossification in normal craniofacial growth.

1336/F

A familial translocation as a model for decoding regulatory mechanisms associated with an inherited epimutation. *S. Minocherhomji[1], H. Goo Kim[4], C. Hansen[1], M. Bak[1], K. Henriksen F.[1], P. Guldberg[2], J. Michael Hertz[3], H. Hilger Ropers[4], Z. Tümer[1], N. Tommerup[1], V. Kalscheuer M.[4], A. Silahtaroglu[1]*. 1) Wilhelm Johannsen Centre for Functional Genome Research, ICMM, Faculty Of Health Sciences, University of Copenhagen. Blegdamsvej 3B, DK-2200, Denmark; 2) Institute of Cancer Biology, Danish Cancer Society, DK-2100 Copenhagen, Denmark; 3) Department of Clinical Genetics Aarhus University Hospital, DK-8000, Aarhus C, Denmark; 4) Max-Planck Institute for Molecular Genetics, Ihnestrasse 73, D-14195 Berlin, Germany.

Epigenetic modifications are first established at the time of gametogenesis in an allele specific manner and are maintained throughout development in differentiated cells. Imprinted regions, known to replicate asynchronously within the human genome, are marked by allele specific epigenetic modifications including DNA methylation and higher order chromatin structure. Inheritance of these epigenetic modifications, particularly the state of genomic DNA methylation has so far been disputed in the literature. Here, we report the altered state of particular epigenetic mechanisms affecting the disks large-associated protein 4 (*DLGAP4*) gene, induced by the balanced chromosomal translocation t(8:20)(p12;q11.23) downstream of exon 1, in individuals of a family having early onset non-progressive cerebellar ataxia. We observed the presence of an inherited transgenerational epimutation affecting the promoter region of the brain specific DGLAP4 isoform in individuals affected by the translocation. CpG dinucleotides present within and around transcription factor binding sites juxtaposed to the promoter CpG Island of the disrupted gene on the translocated allele of *DLGAP4*, become de novo hypermethylated compared to the retained normal hypomethylation state of the unaffected allele on chromosome 20. The *DLGAP4* locus affected by the translocation and involving chromosomes der8 and der20, becomes asynchronously replicating, whereas the normal allele of the gene on chromosome 20 remains early replicating. A perturbed landscape of histone modification marks consisting of both active and inactive marks is observed around the *DLGAP4* locus, individually corresponding to the unmethylated and methylated alleles. At the level of mRNA expression we show the long DLGAP4 transcript that could be disrupted in individuals with the translocation, to be specifically expressed in fetal and adult cerebellum, particularly in the Purkinje cell layer of both phenotypically normal mouse and human brains. An elevated mono-allelic expression level of the widely expressed short isoform of the DLGAP4 gene, downstream of the breakpoint, is seen to correlate with the translocation affected allele. This translocation, apart from associating *DLGAP4* as a candidate gene for early onset non-progressive cerebellar ataxia, serves as a model for understanding the effects of transacting regulatory mechanisms of epigenetic marks.

1337/F

Genome-wide methylation data analysis on the SOLiD™ System. *G. Marnellos[1], D. Krissinger[1], G. Meredith[1], M. Dudas[1], K. Clancy[2], C. Adams[1]*. 1) R&D/Epigenetics, Life Technologies, Carlsbad, CA; 2) R&D/Synthetic Biology, Life Technologies, Carlsbad, CA.

DNA methylation is an epigenetic modification crucial for organism development and normal gene regulation; aberrations in methylation are, among others, characteristic of many cancers in mammals. Next-generation sequencing technologies are enabling new methods for methylation profiling. Life Technologies has introduced a versatile MBD2 protein-based system (MethylMiner™) for the enrichment of methylated sequences from genomic DNA. This enrichment step along with the use of SOLiD™ System sequencing, allows for focused evaluation of genome-wide methylation patterns. This approach is an efficient and cost effective alternative to shotgun bisulfite sequencing of the entire genome to interrogate methylation marks, as only about 1% of the human genome is methylated and requires interrogation. Here we describe a comprehensive workflow for mapping and analyzing MBD2-enriched fractions of genomic DNA as well as bisulfite converted reads sequenced on the SOLiD™ System that employs freely-available public software tools and our own scripts and programs. The workflow enables characterization of methylation patterns at different levels of resolution, from broad genome region comparisons and profile differences between samples, to individual methyl C resolution. It provides the following functionality: • Mapping of unconverted and bisulfite-converted reads • Filtering of clonal reads • Mapping statistics: distribution of reads on chromosomes, coverage and read depth statistics, C and CpG counts in mapped readsPeak-finding in MBD2-enriched reads • Methylation analysis: methylation status of C residues in various sequence contexts, and bisulfite conversion efficiency • Level of enrichment in various genome regions (exons, introns, CpG islands, repeats, etc.) • Visualization of mapped reads and MBD2-enriched peaks with publicly available genome browsers (e.g. the UCSC or IGV browser) We have implemented this analysis pipeline analyze human data sets (IMR90 and MCF-7 cell lines), mainly MBD2-enriched fractions, bisulfite-converted and unconverted reads. Results showed good agreement with publicly available methylation data: peaks in MBD2-enriched reads have high coverage of genome regions with higher densities of published methyl-CpGs. This analysis workflow is a convenient and flexible solution for users which will allow the integration of methylation date with results from other SOLiD™ System applications (e.g. ChIP-seq and RNA-seq).

1338/F

Effect of heroin self-administration on epigenetic regulation of the BDNF gene in the nucleus accumbens of rats. *Q. Liu, F. Theberge, S. Fanous, B. Hope, Y. Shaham.* Behavior Neurosci Res, NIDA/NIH, Baltimore, MD.

CpG islands in gene promoter regions are usually hypomethylated and the levels of DNA methylation in the gene promoters are regulated by environment with long-term effects on gene expression. Changes of chromatin structure could also cause long-term gene expression changes that are influenced by histone modification. BDNF is known to be involved in the behavioral effects of abused drugs, and recent studies also suggest a role of DNA methylation and histone modifications. Therefore, we have begun an investigation of the effect of heroin self-administration (0.075 mg/kg/infusion; 10 days for 6 h/d; each infusion was paired with a discrete tone-light cue) or saline (control condition) self-administration on DNA methylation in BDNF promoter regions and histone modification in the nucleus accumbens of rats that were trained to self-administer. After ten days forced abstinence, half of the heroin self-administered rats were re-exposed to the previously heroin-paired cues in a 30 min extinction test (n=6) while the other half were not; saline-trained rats were not given the extinction test. We then measured DNA methylation in BDNF promoter I, IV and VI regions by bisufite sequencing and histone post-translational modifications of phospho-acetylation of H3, di-acetylation of H3, tri-methylation of H3, and di-acetylation of H4 by Western blot analysis. Preliminary results suggested that the pattern of DNA methylation of the promoter IV of BDNF gene at specific CpG sites was reduced in the heroin-trained rats exposed to the heroin cues during testing versus the heroin-trained rats that were not exposed to the cues. We did not observe any significant changes of histone modification in nucleus accumbens of the two groups. The functional significance of these DNA methylation changes is currently unknown.

1339/F

Brain weight is strongly correlated with DNA methylation across the extended IGF2-H19 imprinted region. *J. Mill, R. Pidsley, C. Troakes, E. Dempster.* SGDP Research Centre, Institute of Psychiatry, King's College London, London, United Kingdom.

Insulin-like growth factor 2 (IGF2) is a maternally imprinted gene that resides within a cluster of imprinted genes on chromosome 11p15.5, encoding a hormone involved in prenatal growth and development. Aberrant imprinting of the IGF2 gene is associated with congenital disorders such as Beckwith-Wiedermann Syndrome and Silver Russell Syndrome, which are characterized by growth abnormalities. More subtle epigenetic heterogeneity at IGF2 is also a feature of normal populations, and has been shown to be influenced by environmental factors including prenatal exposure to famine. Interestingly, prenatal exposure to famine is a risk factor for several psychiatric conditions including schizophrenia. In this study, we hypothesized that epigenetic variation across the differentially methylated regions (DMRs) regulating expression of IGF2, and its reciprocally-imprinted neighbor H19, may impact upon brain development, and particularly the cerebellum which is one of the largest structures in the brain and has a long developmental period. Using bisulfate-based fine-mapping approaches we quantitatively assessed inter-individual differences in DNA methylation across multiple DMRs at 11p15.5 in the cerebellum using two independent collections of post-mortem brain tissue carefully characterized for brain weight. We found that methylation across multiple DMRs in the 11p15.5 region was strongly correlated with total brain weight, with some evidence for sex-specific effects. Furthermore, a combined genetic-epigenetic analysis across the H19 imprinting control region (ICR) uncovered strong evidence for parent-of-origin-specific effects of SNPs on brain size. Our data have important implications for the field of psychiatric epigenetics, given the known association between brain weight and psychiatric disorders including schizophrenia and autism.

1340/F

Simple, automatable NGS library preparation method for global DNA methylation profiling in cancer research and diagnostics. *E. Kamberov, T. Kurihara, J. Langmore, V. Makarov, J. M'Mwirichia.* Rubicon Genomics, Inc., Ann Arbor, MI.

Next-generation DNA sequencing has revolutionized epigenetic research by enabling genome-wide sequencing and profiling of differentially methylated DNA regions associated with cancer and other diseases. Current protocols for preparing libraries for NGS DNA methylation analysis usually require microgram quantities of DNA and mechanical fragmentation, preparation of DNA ends, ligation of adaptors, gel size fractionation, library amplification, numerous purification steps, and isolation of methylated DNA by immunoprecipitation (MeDIP) or magnetic bead binding (MBD), which together require days of preparation time. We present a streamlined, automatable process (NGS-MethylPlex) for preparing libraries for methylation analysis using the Illumina Genome AnalyzerTM (GA) and results of genome-wide methylation profiling of human prostate cancer and benign cell lines using NGS-MethylPlex libraries. NGS-MethylPlex is substantially simpler, more sensitive, and faster than other sample preparation methods and requires only 50 ng of input DNA and 5 h total preparation time. The NGS-MethylPlex process is initiated by digesting input DNA with methylation-sensitive restriction enzymes, followed by attachment of universal sequences and subsequent PCR amplification. A second round of enzymatic treatment depletes non-GC rich sequences, and an additional amplification ensures enrichment of highly methylated DNA fragments. NGS-MethylPlex libraries were synthesized from 50 nanograms of genomic DNA isolated from LNCaP prostate cancer cells and PrEC benign prostate epithelial cells, and the libraries were sequenced using the Illumina Genome AnalyzerTM (GA). A Hidden Markov Model (HMM)-based algorithm detected enriched regions from mapped reads obtained in each sequencing run, and while both cell lines had a total of ~55,000 methylated regions each, LNCaP cells exhibited a seven-fold enrichment for promoter CpG island methylation compared to PrEC cells. Several methylated regions were validated by multiple independent approaches including bisulfite sequencing. Detailed promoter analysis revealed diverse methylation patterns around transcription start sites, including direct methylation of CpG islands, methylation of regions flanking CpG islands, and methylation of sites devoid of CpG islands. Methylated promoters correlated with gene repression, and there was an enrichment of novel regions methylated in LNCaP cells that were also methylated in prostate cancer tissues.

1341/F

Genetic and Epigenetic Alterations in Colorectal Cancer. *M.R. Schweiger[1], C. Grimm[1], L. Chavez[1], M. Kerick[1], S.T. Boerno[1], A. Fischer[1], K. Zatloukal[3], C. Roehr[1], B. Timmermann[2], A. Dahl[4], M. Isau[1], C. Barmeyer[5], R. Herwig[1], B.G. Herrmann[2], H. Lehrach[1].* 1) Vertebrate Genomics, Max Planck Institute, Berlin, Germany; 2) Max Planck Institute, Berlin, Germany; 3) Medical University Graz, Austria; 4) Technical University, Dresden, Germany; 5) Charité University Hospital, Berlin, Germany.

Changes in DNA methylation patterns of specific genes are commonly found during colorectal cancer progression. The recent development of next generation sequencing (NGS) technologies has brought about the possibility to investigate DNA methylation patterns in a genome-wide and unbiased manner. One of these technologies, the MeDIP technology, utilizes an antibody against methylated cytosine to immunoprecipitate and thereby enrich methylated DNA regions. We have adapted the MeDIP protocol to Illumina's Genome Analyzer as well as Applied Biosystem's SOLiD platform to identify precipitated DNA fragments in colon cancer cell lines as well as colon normal and tumor tissue samples. We will present our epigenetic studies in combination with whole exome sequencing results from the same patients. This combination of genetic and epigenetic experiments brings further access towards the identification of novel disease-causing genomic alterations in a genetically complex disease such as cancer.

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BRCA1 germline mutations and age-acquired methylation at stem cell target genes. *J. Zhuang[1], A. Teschendorff[2], M. Zikan[3], A. Jones[1], P. Pohlreich[4], D. Cibula[3], S. Peri[5], A. Bellacosa[6], I. Jacobs[1], M. Widschwendter[1].* 1) Gynecological Oncology Dept, UCL, London, UK; 2) Medical Genomics Group, UCL Cancer Institute, London, UK; 3) Oncogynecologic Center, Obstetrics and Gynecology Dept, Charles University Prague, Prague, Czech Republic; 4) Biochemistry and Experimental Oncology Dept, Charles University in Prague, Prague, Czech Republic; 5) Biostatistics Dept, Fox Chase Cancer Center, Philadelphia, PA; 6) Epigenetics and Progenitor Cells Program Dept, Fox Chase Cancer Center, Philadelphia, PA.

Women with BRCA1 germline mutations experience up to 80% risk of developing breast cancer. Age is the most important risk factor for cancer. Recently, CpGs become increasingly methylated with age are found to be heavily enriched for stem cell polycomb group target genes (PCGTs). PCGTs both reversibly repressed in stem cells and required for differentiation, and more likely to undergo cancer-specific promoter DNA hypermethylation than non-target. We demonstrated a signature comprising 69 age-hypermethylated CpGs mapping to PCGTs (Age-PCGTs) derived from peripheral blood cells distinguishes preneoplastic and neoplastic cells from their normal counterparts, whereas that of 156 age-hypermethylated CpGs not mapping to PCGTs (Age-NonPCGTs) is not. We hypothesised individuals with particularly high risk of developing a cancer are predisposed to premature hypermethylation at Age-PCGTs compared to non mutation carriers. PCGTs are discovered to be underexpressed in luminal progenitors compared with luminal cells (OR=2.76, P<1e-8) and the majority of Age-PCGTs showed underexpression in luminal progenitors (P=0.03), consistent with PCGTs being critically involved in the differentiation process of the breast epithelium which appears to be disrupted in BRCA1 mutation carriers. We also established if the Age-PCGT methylation signature is modified in peripheral blood DNA from women with BRCA1 mutation compared to controls. The results were validated in a set of 30 healthy age matched women and 30 breast cancer patients. The Age-PCGT methylation signature significantly discriminated BRCA1 carriers from controls irrespective of cancer status but not the Age-NonPCGT signature. We analyzed expression of Age-PCGTs in age-matched primary human mammary epithelial cells in women with and without BRCA1 mutation and observed an underexpression of Age-PCGTs but not of Age-NonPCGTs in BRCA1 mutation carriers. Also, Age-PCGTs were significantly underexpressed in a set of BRCA1 mutant basal cancers than age-matched non-BRCA1, suggesting the difference in expression might persist in tumour specimens. Finally, we illustrated genes that are hypermethylated and suppressed in tissue samples from BRCA1 mutation carriers to be generally enriched for PCGTs. This is the first report demonstrating an interaction between the germline genome and age leading to a specific alteration of the epigenome may eventually contribute to breast cancer.

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Subtelomeric Methylation in Patients with Dyskeratosis Congenita and their Unaffected Family Members. *S.M. Gadalla[1,2], H. Katki[3], F.M. Shebl[4], N. Giri[1], L. Mirabello[1], P.M. Lansdorp[5], B.P. Alter[1], S.A. Savage[1].* 1) Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, MD; 2) Cancer Prevention Fellowship Program, National Cancer Institute, Rockville, MD; 3) Biostatistics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Rockville, MD; 4) Infections and Immunoepidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Rockville, MD; 5) Terry Fox Laboratory, British Columbia Cancer Research Center, Vancouver, BC, Canada.

Dyskeratosis congenita (DC) is an inherited bone marrow failure and cancer susceptibility syndrome, caused by germline mutations in telomere biology genes. DC patients have very short telomeres (below 1st percentile for age). Recent data suggest that epigenetic regulation of telomeric chromatin impacts telomere length (TL). In mouse models and in-vitro studies, subtelomeric methylation was involved in TL regulation. We hypothesize that epigenetic factors may contribute to DC etiology and hence evaluated the role of subtelomeric methylation in DC. We identified 40 patients with DC (7 DKC1, 5 TERC, 4 TERT, 11 TINF2, 13 without mutations in known DC genes) and 51 unaffected mutation-free family members. All subjects were participants in the NCI's Inherited Bone Marrow Failure Syndrome cohort. D4Z4 subtelomeric repeat CpG methylation in leukocyte DNA was determined after bisulfite modification. Lymphocyte TL was measured by flow-FISH. Associations between DC, TL and % subtelomeric methylation were calculated using odds ratios(OR)and 95% confidence intervals(CI) from multivariable logistic regression models. Compared with unaffected family members, patients with DC were younger (median=14.4 vs. 45.6 years) and had very short TL (median=3.7 vs. 6.4 Kb). After adjusting for age and TL, our data suggested a positive association between subtelomeric hypermethylation and DC (OR=2.6, 95%CI=0.6-11.3, p=0.2). This association was statistically significant only in patients with a mutation in telomerase complex genes (DKC1, TERT, or TERC), or those without a mutation in the known genes (OR=1.8, p=0.04, and OR=30.9, p<0.0001, respectively), but not in patients with a TINF2 mutation (OR=1.6, p=0.2). We next evaluated the association between TL and subtelomeric methylation in cases and controls separately. Longer telomeres in DC patients were associated with higher levels of subtelomeric methylation; the reverse was true for controls (OR= 1.4 vs. 0.6, P-interaction=0.03). In conclusion, subtelomeric methylation may be associated with DC after accounting for age and TL, especially in patients without mutations in known DC genes. Absence of this association in patients with TINF2 mutation may indicate that methylation aberrations in patients with DC are telomerase dependent. Subtelomeric hypermethylation may protect against accelerated telomere shortening in DC. Our ongoing studies include mechanisms of epigenetic regulation of TL and its relationship to DC etiology.

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Whole exome, MeDIP-seq and RNA-seq analysis of monozygotic twins discordant for greater than 20 traits. *S.V. Ramagopalan, L. Handunnetthi, G.C. Ebers.* Dept Clinical Neurology, Oxford Univ, Oxford, United Kingdom.

Monozygotic twins have been widely studied to dissect the relative contributions of genes and the environment in human diseases. We report here a pair of female twins markedly different in appearance and discordant for more than 20 traits including, height, weight, personality, spinal stenosis, scoliosis, migraine, neuropathy, biliary cirrhosis, diabetes, hypertension, asthma, osteoporosis, hypertelorism and precocious puberty. These twins, were believed to be dizygotic but obstetric history of a common amniotic sac was validated by DNA testing. The extreme difference in phenotype observed here may be unparalleled. The discordance for many unrelated and unlinked conditions suggested diffuse epigenetic differences. Here we present the whole exome, messenger RNA transcriptome and DNA methylome sequences of peripheral blood mononuclear cells, CD4+ lymphocytes and buccal tissue from this twin pair. This twin pair implicates an epigenetic basis for the conditions for which they are discordant and twin-twin comparisons in them might be a rosetta stone for identifying key epigenetic differences for several common conditions. We believe our results will have more general implications for understanding the aetiology of these complex traits.

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A ChIP-Seq method for generating epigenome-wide profiles histone (H3K9) acetylation in post-mortem human brain tissue from clinical-pathologic epidemiologic studies. *C. Aubin[1], J. Schneider, MD, MS[2], G. Srivastava, PhD[3], A. Meissner, PhD[1,5], M. Szyf, PhD[6], D. Bennett, MD[2], P. De Jager, MD, PhD[1,3,4].* 1) Medical and Population Genetics, The Broad Institute of Harvard and MIT, Cambridge, MA; 2) Rush Alzheimers Disease Center, Rush University Medical Center, Chicago, IL; 3) Program in Neuro-Psychiatric Genomics, Department of Neurology, Brigham & Women's Hospital, Boston, MA; 4) Harvard Medical School, Boston, MA; 5) Department of Stem Cell and Regenerative Biology, Harvard University and Harvard Stem Cell Institute, Cambridge, MA; 6) Department of Pharmacology and Therapeutics, McGill University, Montreal, QC, Canada.

Cognitive decline is a complex function of experiential, genetic, and epigenetic factors that lead to the accumulation of neuropathologies or alter the brain's capacity to tolerate or compensate for the deleterious effects of neuropathologies. Over the past 15+ years, the Religious Orders Study and Rush Memory and Aging Projects have enrolled 2,500 persons who agreed to annual clinical evaluation and organ donation. More than 850 brains are already available. We are establishing a chromatin immunoprecipitation/ sequencing (ChIP-Seq) pipeline to generate epigenome-wide profiles of histone (H3K9) acetylation in fixed dorsolateral prefrontal cortex (DLPFC) tissue at the Broad Institute of Harvard and MIT. We identified the Millipore anti-H3K9Ac mAb (catalog # 06-942, lot: 31636) as a robust mAb for our ChIP experiment. Optimum sonication intervals have been shown to vary due to fixation interval in human cell lines. Therefore, we examined the effects of variations of fixation interval as well as post mortem interval (PMI, interval between death and autopsy) on the sonication interval best suited to fragment chromatin from this tissue for ChIP. Brain tissue (n=20) sampled from each quartile of fixation interval and PMI were sonicated for a range of times (6, 8, 10, and 12 minutes). A 10 minute sonication treatment was optimal to produce chromatin fragments with the desired proportion of target segments (length of 150-700 bp per Bioanalyzer assessment). At this time, we do not see a correlation between optimum sonication interval and either fixation interval or PMI; fixation times in excess of 200 hours and PMI in excess of 48 hours produced adequate material for library construction. Using 500mg of gray matter from the DLPFC tissue produced an average of 23.3ng of DNA fragments. Sequencing data from libraries derived from our ChIP is currently being produced. At the time of the conference, we will report on the extent of interindividual variation within the sequence data produced from the first 100 samples to be processed, and we will update our protocol on the optimal parameters with which to execute H3K9 acetylation profiling epigenome-wide in fixed brain tissue.

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Characterization of chromatin signatures using low cell number chromatin immunoprecipitation. *A.J. Notini[1], J.A. van den Bergen[1], D. Belluoccio[2], M. Burton[1], P.S. Western[1], A.H. Sinclair[1], S.J. White[1].* 1) Murdoch Childrens Research Institute, Royal Children's Hospital, Melbourne, Australia; 2) Agilent Technologies Pty Ltd, Melbourne, Australia.

A fundamental goal of modern biology is understanding how genes are regulated both temporally and spatially. It is becoming clear that this control is not only dependent on DNA sequence; rather epigenetic factors such as CpG methylation and histone modifications also play a major role. Diverse histone modifications are associated with different characteristics of transcriptional regulation, and techniques such as chromatin immunoprecipitation (ChIP) have started to reveal the complexity underlying this regulation. To date most genome-wide studies using this approach have used cultured cells as it is necessary to have millions of cells to obtain sufficient material for downstream applications. We have developed a method that can use as few as 100,000 cells per IP. Specifically, we have used this approach to study chromatin modifications in a purified population of somatic cells derived from embryonic mouse gonads. Following IP, the resulting material is amplified and hybridized onto custom arrays containing targeted genomic sequences for 28 genes known to be involved in gonad differentiation and development. We have used antibodies specific for histone modifications, including anti-H3K4me1, H3K4me2, H3K4me3, H3K4me27 and H3K36me3 to identify enhancers, active/inactive chromatin and transcript elongation. We will present chromatin profiles for somatic cells of the embryonic ovary and testis during different stages of development. In conclusion, we have developed low cell number ChIP to examine epigenetic changes during mouse gonad development. This approach can be applied to any biological question where the amount of starting material is limited.

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Analysis of DNA Methylation in Facioscapohumeral Muscular Dystrophy (FSHD). *C.H. Huichalaf[1,2], D. Gabellini[1,3].* 1) Regenerative Medicine, San Raffaele Scientific Institute, Milan, Milan, Italy; 2) International Ph.D Program in Cellular and Molecular Biology Università Vita Salute San Raffaele, Milan Italy; 3) Dulbecco Telethon Institute, Milan, Italy.

FSHD, the third most common muscular dystrophy, is an autosomal disorder with an incidence of 1:14.000. It is characterized by asymmetric muscle weakness and variable penetrance. FSHD is linked to deletion of the D4Z4 3.3 kb macrosatellite repeat, located in 4q35. In normal individuals 11 to 100 D4Z4 are found, while FSHD patients carry 1 to 10 units. While the human genome average GC content is 42%, each D4Z4 repeat unit contains 73% GC. Furthermore, in D4Z4 the CpG dinucleotide frequency is 10% compared to the average human genome frequency of 1%. As a result, in healthy subjects the region occupied by the D4Z4 repeats represents one of the more extended CpG islands of the human genome. This makes D4Z4 an excellent candidate for regulation mediated by DNA methylation, a common modification of mammalian DNA usually associated with gene repression. DNA methylation is executed by DNA methyltransferases (DNMTs) and usually occurs at cytosine residues of CpG dinucleotides. A few studies have been published regarding the methylation status of D4Z4 in FSHD. Unfortunately, all of them were based on the use of methylation-sensitive restriction enzymes and provided information on just two of the more than 300 CpGs present in the D4Z4 repeat. Our goal is to study DNA methylation across the entire D4Z4 repeat in healthy subjects and FSHD patients. We are doing this using a combination of bisulfite sequencing and methyl DNA precipitation assay. Our preliminary findings indicated that D4Z4 is highly methylated in healthy subjects, but this methylation is not uniformly distributed inside D4Z4. We have also found that this methylation does not correlate with the number of CpGs inside the region, and we have evidence for non-CpG methylation on the D4Z4 repeat. These studies are very relevant to develop therapeutic approaches aimed at controlling altered 4q35 gene expression in FSHD.

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Alterations in Maternal DNA Methylation are Associated with Congenital Heart Defects. *S. Chowdhury, M.A. Cleves, S.L. MacLeod, S.W. Erickson, W. Zhao, P. Hu, C.A. Hobbs.* Pediatrics Department, College of Medicine, University of Arkansas for Medical Sciences, Arkansas Children's Hospital Research Institute.

Background: Congenital heart defects are the most common structural birth defects. The etiology of the majority of CHDs are unknown, but are thought to result from an interaction of multiple genetic, epigenetic, and environmental and factors. Altered folate metabolism has been shown to be associated with CHDs, and is also implicated in altered DNA methylation patterns. Although evidence linking genetic and metabolic alterations in folate metabolism and CHDs exists, the relationship between maternal DNA methylation and CHDs remains relatively unexplored. We sought to investigate if alterations in maternal DNA methylation could be detected between mothers with CHD-affected pregnancies and mothers with unaffected pregnancies. Methods: The proposed study builds on the National Birth Defects and Prevention Study (NBDPS). Blood was collected from Arkansas NBDPS participants who delivered a singleton live birth with a non-syndromic CHD, and Arkansas NBDPS mothers who had a live birth without a major defect. Using a case-control study design, maternal DNA methylation was measured using three methods. In 180 cases and 187 controls, LINE-1 methylation was assayed via Methylight methodology, and gene-specific methylation was interrogated for over 14,000 genes in over 27,000 CpG sites by the Infinium® HumanMethylation27 BeadChip. Global DNA methylation was assessed in a subset of 56 cases and 52 controls by immunoassay. Lifestyle information was available for all subjects to perform covariate adjustments. Results: A 5% decrease in LINE-1 methylation resulted in a 15% increased risk of CHDs (OR=1.15; 95% CI: 1.03, 1.27; P=0.010). Multiple genes encompassing various cellular functions were found to be differentially methylated in cases when compared to controls. Within these genes, GDF3, EGFR, MAP4K5, and UGDH were significantly hypermethylated in cases, and have previously been identified as critical genes in heart development. Significant global DNA hypomethylation was observed in cases when compared to controls (p=0.045). Conclusions: Our findings indicate that maternal global DNA hypomethylation and gene-specific hypermethylation is associated with an increased risk of CHDs. Aberrations in maternal DNA methylation converge with previous evidence that indicate folate-dependent genetic and metabolic susceptibilities increase the risk of CHDs. These results suggest that further investigation into maternal epigenetic mechanisms as they affect CHD risk is warranted.

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Epigenomics of Vascular Cell Phenotypic Modulation in Atherosclerosis. *L. Mills[4], B. Wamhoff[1,2,3], B. Blackman[1,3], J. Connelly[1,2].* 1) CVRC; 2) Department of Medicine Cardiovascular Division; 3) Department of Biomedical Engerinnering; 4) Molecular Cell and Developmental Biology, University of Virginia, Charlottesville, VA.

Phenotypic modulation of both endothelial cells (EC) and smooth muscle cells (SMC) from quiescent to proliferative, inflamed, and migratory phenotypes occurs in vivo and leads to the progression of atherosclerosis. Regions of the vasculature that are subject to specific blood hemodynamic forces, including low time-average and oscillatory shear stresses, characterize regions of the artery predisposed to phenotypic modulation. We have previously defined a model system that recapitulates human hemodynamics in a culture system that mimics a vessel. This system was shown to recreate the phenotypes of ECs and SMCs found in atheroprone regions in vivo. We have confirmed that the same phenotypic changes occur in human aortic ECs and human aortic SMCs. When these two cell types are subjected to low time-averaged and oscillatory flow the expression of anti-inflammatory markers TEK, KLF2 and NOS3 decreases in ECs and the expression of the differentiation markers ACTA2, KLF4 and MYOCD decreases in SMCs. These results are the same expression changes that are found in diseased regions of arteries in vivo. Using this model we are testing the hypothesis that changes in DNA methylation status of genes in ECs and SMCs undergoing phenotypic modulation play a role in atherosclerosis initiation and progression. We have assayed DNA methylation using methylated DNA immunoprecipitation (Me-DIP) and quantitative real time PCR. We see enrichment of the highly methylated HIST1H2BA locus, in aortic smooth muscle cells and no enrichment of the promoter region of GAPDH. We are currently assessing methylation changes in smooth muscle cells on a genome wide scale using Solexa sequencing. Candidate regions will be identified, confirmed using bisulfite cloned sequencing and DNA from diseased and healthy human aortic tissues will be profiled to verify that changes seen in this system are also seen in tissue in which disease progresses.

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Evidence that early growth influences adiposity at age 9-13 years and is mediated by epigenetic regulation of gene expression. *A. Groom[1], D. Swan[2], S.M. Korada[3], H.J. Cordell[4], J.C. Mathers[1], M.S. Pearce[5], N.D. Embleton[3], C.L. Relton[1].* 1) Human Nutrition Research Centre, Institute for Ageing and Health, Newcastle University, Newcastle upon Tyne, Tyne and Wear, United Kingdom; 2) Bioinformatics Support Unit, Newcastle University, Newcastle upon Tyne, Tyne and Wear, United Kingdom; 3) Newcastle Neonatal Service, Royal Victoria Infirmary, Newcastle University, Newcastle upon Tyne, Tyne and Wear, United Kingdom; 4) Institute of Human Genetics, Newcastle University, Newcastle upon Tyne, Tyne and Wear, United Kingdom; 5) Institute of Health and Society, Newcastle University, Newcastle upon Tyne, Tyne and Wear, United Kingdom.

Early environmental events in postnatal life are postulated to cause aberrant epigenetic marking and precipitate altered expression of specific genes. This in turn may affect body composition and metabolic health in childhood.

Participants were drawn from a nutritional intervention study of preterm infants carried out in early postnatal life. Anthropometric, biochemical and nutrient markers in early life and at 9-13 years of age were collected. Blood and saliva samples were also collected at age 9-13 years for DNA and RNA analysis. Up-regulation of gene expression in 'slow growers' (n=12) versus 'rapid growers' (n=12) was assessed. 'Slow' and 'rapid' growth was determined by differences in z-scores for weight between term and term plus 12 weeks. Levels of DNA methylation at 7 CpG sites within *TACSTD2* were analysed by Pyrosequencing®.

245 loci were upregulated in females who were 'slow growers' versus females who were 'rapid growers' and 352 in males who were 'slow growers' versus males who were 'rapid growers'. Of these genes *TACSTD2* was selected for further investigation. *TACSTD2* was up-regulated 2.56-fold in males and 4.07-fold in females who were 'slow growers' compared to 'rapid growers' (p=0.0001). Methylation levels of *TACSTD2* (mean across 7CpGs) in DNA extracted from saliva and blood (n=52) were closely correlated (rho= 0.87, p=0.0001). However, methylation levels from saliva were considerably lower (mean % (SD) = 38.7 (15.4) compared to 66.9 (16.3)). *TACSTD2* methylation was lower in 'slow growers' and was also associated with an increase in total body mass at age 9-13 years (blood p=0.012, saliva p= 0.001). This latter association was driven by fat mass; change in fat mass per percentage change in DNA methylation; blood -146.4g (95% CI -242.4, -50.3) p=0.004; saliva -149.3 (95% CI -235.2, -63.3) p=0.001).

Preterm infants with a slower growth trajectory in early post natal life display considerable differences in gene expression levels and gene-specific DNA methylation levels at age 9-13 years when compared to infants who grew rapidly. These results show an association between early growth, epigenetic regulation of gene expression and body composition in childhood.

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A search for the silencing gene of mouse dactylaplasia. *H. Kano, T. Toda.* Physiology and Cell Biology, Kobe Univ., Kobe, Hyogo, Japan.

Dactylaplasia, characterized by missing central digital rays, is an inherited mouse limb malformation that is clearly dependent on a two-locus interaction between Dac and mdac (modifier of Dac). The dactylaplasia phenotype depends on the genotype at the mutated locus, Dac, and also requires homozygosity for a recessive allele at another unlinked locus, mdac. An LTR retrotransposon insertion was identified in the Dac locus on mouse chromosome 19. However, some mouse strains display normal phenotype despite carrying the insertional mutation. This is because of the presence of modifier allele, mdac, on mouse chromosome 13. The mdac is polymorphic (mdac/Mdac) among the inbred mice. The LTR retrotransposon insertion on the mdac background is unmethylated and the mice display the dactylaplasia phenotype. On the other hand, the insertion on the Mdac background is methylated and the mice display the normal phenotype. The aim of this study is to identify mdac/Mdac. Backcrossing test and haplotype block analysis refined the mdac locus to the ~1 Mb region. Among several genes, we focus on a gene, a member of zinc finger proteins. The gene has a SNP in its coding region, which causes an amino acid substitution between the mdac allele and the Mdac allele. Now, we are creating transgenic mice to confirm the role of the gene. The transgene was cloned from the Mdac allele and was microinjected into fertilized oocytes of the mdac background. By crossing the transgenic mouse with the dactylaplasia mouse, we expect that the LTR retrotransposon insertion would be methylated and the dactylaplasia phenotype would be suppressed by the presence of the transgene.

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Seq + PA: Combining Sequencing (Seq) and Individual Peak Analysis (PA) for Quantification of DNA Methylation and Minor Sequence Variation Detection. *E. Schreiber, A. Tobler, C. Davidson.* Applied Biosystems, Life Technologies, Foster City, CA. USA.

Fluorescent DNA sequence traces generated by Sanger sequencing on automated capillary electrophoresis (CE) instruments primarily reveal the composition and exact sequence of nucleotide bases. However, an additional layer of information may be present in the peak height of a given base reflecting a measure of abundance. Here we report a novel method, Seq+PA, that allows the separation of the four dye traces used in BigDye® Terminator 3.1 sequencing chemistry and inclusion of a 5th LIZ® dye-labeled size standard. This enables alignment by size of multiple samples and subsequent data analysis of each individual peak characteristics, such as size, height and area by GeneMapper® software. We have used the Seq+PA method for direct bisulfite PCR sequencing and were able to detect methylated cytosines in CpG pairs to a level of 5%. We have also applied the method for sensitive detection of somatic mutations. To that end, DNAs with a normal or mutant genotype at codon 12 in the KRAS gene were mixed in various ratios and processed for PCR and Seq+PA analysis. The mutant allele was noticeable at a 5% level. Taken together, the method described here has the potential to enable a more quantitative analysis of DNA sequence traces for sensitive detection of minor sequence variations. Disclaimer: For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.

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An adverse effect of ICSI on ovogenesis is suggested by concurrence in the same patient of trisomy 21 and Angelman syndrome. *M.G. Pomponi[1], L. Morciano[2], R. Pietrobono[1], F. Gurrieri[1], G. Neri[1,2].* 1) Medicina di Laboratorio, Genetica Medica, Roma, Roma; 2) Servizio di Genetica Medica, Ospedale “G: Panico”, Tricase.

Recent studies on the outcome of assisted reproductive technology (ART) have raised concern regarding the potential association between ART and chromosomal aneuploidy, imprinting anomalies, and congenital defects. It is still debated whether this association has a stochastic nature or is rather the result of a cause-effect relationship between infertility itself, induced superovulation and in vitro culturing of the embryo. We here report on a child, conceived with intracytoplasmic sperm injection (ICSI), who is affected with trisomy 21 and Angelman syndrome due to a sporadic imprinting alteration. Methods: we confirmed the diagnosis of Angelman syndrome by methylation sensitive Southern blot analysis and MLPA analysis using a commercially available Kit (SALSA MS-MLPA kit ME028-B1 Prader-Willi/Angelman (MRC-Holland). Genotyping of the propositus, his parents and maternal grandmother was performed with microsatellite markers from the PWS-AS critical region. Sequencing of the imprinting center was performed by standard procedures. Results: the molecular analysis showed that chromosomes 15 were of bi-parental origin in the propositus, therefore the altered methylation pattern was due to an imprinting defect. Such defect was de novo because no deletion or mutation was found in the imprinting center. In addition, haplotype analysis showed that the propositus inherited from his mother the grandmaternal haplotype for the 15q11-q13 region. We also confirmed that trisomy 21 was due to maternal non-disjunction, as expected. This is to our knowledge the second report of a double adverse effect of ART on the embryo, suggesting a vulnerability of the maternal genome to the ART-related treatment, the first being the one of a twin boy with Beckwith-Wiedemann syndrome and a 47, XXY caryotype (Yoon et al., 2005).

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Promoter methylation of *PDE11A* and *KITLG* modifies the risk of familial testicular cancer. *L. Mirabello, C.P. Kratz, S.A. Savage, M.H. Greene.* Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA.

Testicular germ cell tumors (TGCT) are the most frequent cancers among young men. No high-penetrance cancer susceptibility gene has been identified despite a high familial risk and likely inherited susceptibility. Recent genomic studies have identified several candidate loci associated with TGCT: *PDE11A*, *KITLG*, *SPRY4*, and *BAK1*. Mouse models suggest that transgenerational epigenetic interactions control susceptibility to TGCT in mice with a *Dnd1* mutation. These five genes are all involved in development of the normal testis and/or male infertility (a TGCT risk factor). Aberrant DNA methylation contributes to carcinogenesis, and may represent an alternative mechanism for TGCT susceptibility. We hypothesized that the promoter regions of these candidate genes (*PDE11A*, *KITLG*, *SPRY4*, *BAK1*, and *DND1*) may be regulated through epigenetic changes and investigated the association between promoter methylation and TGCT in multi-case familial testicular cancer family members. Pyrosequencing assays were designed to examine methylation at CpGs in the promoter CpG island of each gene in peripheral blood DNA from 153 TGCT probands and 116 healthy male relatives from 101 multiple-case families. Adjusted logistic regression models were used to investigate the association between promoter methylation of each gene and TGCT risk. Methylation levels for each promoter region, and for each individual CpG site, were categorized into tertiles, based on the distribution in the controls. A statistically significant upward trend was observed for *PDE11A* when comparing the middle and highest tertiles of methylation to the lowest [odds ratio (OR)=1.55, 95% confidence intervals (CI) 0.82-2.93, and 1.94, 95% CI 1.03-3.66], respectively; P_{trend}=0.042). A significant trend in the opposite direction was observed for *KITLG* when comparing the middle and highest tertiles to the lowest (OR=0.99, 95% CI 0.55-1.79, and 0.46, 95% CI 0.24-0.89, respectively; P_{trend} = 0.031). Our results suggest that TGCT susceptibility is associated with promoter methylation of novel candidate genes, and that ***PDE11A* is inactivated** and ***KITLG* activated** in familial TGCT cases compared with healthy male relatives. Both findings fit prior observations regarding how each gene/pathway modifies TGCT risk. This is the first study to investigate promoter methylation of these genes. Our data provide new insight into the complexity of TGCT genetics. Larger studies are warranted to further explore our preliminary findings.

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Safeguarding the human FMR1 promoter: the methylation barrier in its 5'-upstream region. *A. Naumann[1], S. Weber[1], A. Hoogeveen[2], E. Fanning[1,3], W. Doerfler[1,4].* 1) Institute for Virology, Erlangen University, Erlangen, Germany; 2) Department of Genetics, Erasmus Medical College Rotterdam, Nederland; 3) Department of Biological Sciences, Vanderbilt University, Nashville, TN 37235, USA; 4) Institute of Genetics, University of Cologne, D-50674 Cologne, Germany.

Recent studies of the methylation profile in the 5'-upstream region of the human FMR1 promoter revealed a distinct boundary between a highly methylated genome segment and the unmethylated, active promoter of the human FMR1 gene (Naumann et al., AJHG 85, 606-616, 2009). By using the bisulfite sequencing procedure, this boundary was located at a site about 700 nucleotides upstream of the (CGG)n repeat in the human FMR1 gene. The boundary is present in the genomes of all human cell types investigated as well as in the equivalent region in the mouse genome with only 46.7% homology. Nuclear proteins bind specifically to that DNA sequence. The region is thought to serve as a barrier against the spread of DNA methylation into the FMR1 promoter and thus to help maintaining its functionality. We have now investigated the methylation profile in that genome segment in different FRAXA premutation and mutation carriers. The barrier is lost in the genome of FRAXA males, but is preserved in premutation females and in high functioning males. In premutation females, the methylation profiles in that region are identical to those of normal females with one chromosome exhibiting the barrier as in healthy male chromosomes, the other allele being strongly methylated as can be expected. In a high functioning male genome with a (CGG)n repeat expansion (n = 330; Smeets et al. Human Mol. Genet. 4, 2103-2108, 1995), the methylation boundary is preserved and thus explains the normal activity of the FMR1 promoter. This finding further documents the importance of an intact methylation barrier in maintaining the function of the FMR1 promoter. In primary FRAXA fibroblasts, although not in peripheral mononuclear cells of FRAXA individuals, with loss of the methylation boundary, a genome segment well upstream of the methylation barrier has become hypomethylated. It is conceivable that this aberrant loss of methylation points to a structural instability in the boundary region in FRAXA genomes. We pursue the possibility that the chromatin structure at the methylation barrier is key to the functionality of the human FMR1 promoter. This research was supported by the Thyssen Foundation, Cologne, Germany (to W.D.), the Institute of Virology, Erlangen University Medical School, the Alexander von Humboldt Foundation, and by National Institutes of Health grant GM52948 (to E.F.).

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Are there changes in DNA methylation in response to chronic consumption and withdrawal of folic acid? *K.S. Crider[1], C. Bean[1], R.J. Berry[1], T.P. Yang[3], J.O. Brant[3], S.A. Rasmussen[1], L. Hao[4], Z. Li[4], D. Maneval[2], E. Quinlivan[2], L.B. Bailey[2].* 1) National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, Atlanta, GA; 2) Food Science and Human Nutrition Department, University of Florida Gainesville, FL; 3) Center for Epigenetics University of Florida College of Medicine Gainesville FL; 4) Peking University Health Science Center, Beijing, China.

We evaluated the effect of folic acid supplementation and withdrawal on DNA methylation at 1505 loci in 802 genes using bead array technology. Blood samples from 96 Chinese women (stratified by three methylenetetrahydrofolate reductase [*MTHFR*] genotypes) participating in a double-blind randomized trial of folic acid supplementation (100, 400, 4000 μg/day) were screened for changes in DNA methylation level and pattern at five time points: baseline, 1, 3 and 6 months supplementation and after a 3 month withdrawal of supplementation. Preliminary analysis showed overall mean baseline methylation patterns were highly correlated among the three MTHFR genotypes ($r^2 > 0.99$). At baseline > 99.4% of these loci had little variation in mean betas (ratio of methylated/unmethylated), with between -5 and +5 percent methylation difference between the three MTHFR genotypes. After supplementation the variation among individuals in locus-specific DNA methylation levels increased compared to the variation at baseline. The vast majority (>99.9%) of mean changes in methylation from baseline at each locus at any time point were between -20 and +20 percent. CpG sites that showed significant mean change ($p < 0.01$) from baseline to any time point were more likely to be part of non-CpG islands vs. CpG islands (OR 5.3 95% CI 3.9-7.1) and from loci on the X chromosome vs. autosomes (OR 3.3 95% CI 1.8-6.2). There were more loci with significant changes ($p<0.01$ and change in percent methylation of > +/-10) from baseline at any point during supplementation among those with either the variant TT (6.6%) or heterozygous CT (4.5%) genotype compared with the CC (2.5%) genotype. After withdrawal of folic acid there were limited numbers of loci with significant differences from baseline (CC 0.5%, CT 0.9% and TT 0.7%). No individual sites showed consistent long-term changes in DNA methylation in response to folic acid supplementation and withdrawal. However, specific CpG sites may be more susceptible to increased variability in methylation level in response to folic acid exposure; additional analysis and studies are ongoing. This work was supported by a collaborative CDC agreement and the GCRC Grant # MO1-RR00082.

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Genomewide search for imprinting and maternal effects in eczema. *J. Esparza Gordillo[1,2], K. Rohde[2], A. Bauerfeind[2], H. Schulz[2], N. Hübner[2], Y.A. Lee[1,2].* 1) Ped Pneumology/Immunology, Charite Univ Med, Berlin, Germany; 2) Max-Delbrück-Centrum (MDC) for Molecular Medicine, Berlin-Buch, Germany.

Eczema is a frequent inflammatory skin disease with complex etiology. Epidemiological studies have shown that children of eczema-affected mothers have a higher risk to develop the disease than children of eczema-affected fathers. This parent-of-origin (POO) effect may be due to genomic imprinting, feto-maternal interaction during pregnancy/lactation (maternal effect), or to maternal inheritance of the mitochondrial genome. However, few studies have aimed to identify such effects in eczema. We aimed to detect POO effects in eczema by analyzing 270 complete nuclear families with eczema, from which genome-wide high-density array SNP data was available (Esparza-Gordillo et al. 2009). Interestingly, in these families the frequency of eczema is much higher in the mothers than in the fathers (32% versus 18%, respectively), supporting the existence of POO effects. We have analyzed the genotype data according to the method of Weinberg (Weinberg, 1999). This method classifies families by mating types regarding the number of alleles carried by the father, mother and sibs and carries out a logistic regression over strata of the mating types containing information on imprinting and maternal effects. The independent imprinting and maternal effects can then be identified by appropriate likelihood ratio tests. Importantly, our scan tends to identify genomic regions containing predicted or experimentally confirmed imprinted genes, supporting the validity of our approach ($P < 0.05$). Additionally, we have used gene expression arrays to quantify the total mRNA levels in peripheral blood mononuclear cells (PBMCs) from a subset of these families. We are currently applying the method of Belonogova et al. (2009) in order to detect POO effects at the expression level. This new and powerful method enables the detection of expression differences among reciprocal heterozygotes by applying a regression model which includes a main allelic effect and an imprinting effect. Based on the genome-wide scan for POO in eczema, we will select 100 SNPs for follow-up in a set of 1000 independent complete nuclear families with eczema. We will prioritize hits which simultaneously show an imprinted pattern on disease risk and on gene expression or those located in imprinted regions (http://igc.otago.ac.nz/home.html). This is the first study to date performing a genome-wide POO association analysis in eczema and will increase our knowledge on this very interesting aspect of the disease etiology.

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Quantification of Methylation Levels by Next-generation Sequencing. *G. Wu[1], N. Yi[1], D. Absher[2], D. Zhi[1].* 1) Biostatistics, University of alabama at Birmingham, Birmingham, AL; 2) HudsonAlpha Institute for Biotechnology, Huntsville, AL.

Recently, next-generation sequencing-based technologies enable DNA methylation profiling at high resolutions and at low costs. Methyl-Seq and Reduced Representation Bisulfite Sequencing (RRBS) are two such technologies allowing for interrogating methylation levels at CpG sites throughout the entire human genome. With rapid reduction of sequencing cost, these technologies would enable epigenotyping a large cohort for associating phenotypes and epigenetic changes in a near future. Existing methylation quantification methods typically make binary calls of methylated versus unmethylated. However, many biological samples are consisting of heterogeneous mixtures of cells with variable methylation levels, for which the desirable quantification is often to estimate the percent of DNA molecules being methylated at each CpG site, which is called beta values in methylation microarrays. Meanwhile, the count-based sequencing analysis has its own properties: first, due to the random sampling nature, the sequencing coverage is not uniform and the estimate of beta values has heterogeneous variances. Moreover, the methylation levels at nearby sites or the same sites in different tissue sample libraries are correlated. Finally, quantification shall account for technical biases. Therefore, it is in urgent need to understand the statistical issues related to the quantification of methylation levels for these emerging technologies, with the goal of developing an accurate quantification method. In this paper, we discuss two methods for Methyl-Seq quantification. The first method, Maximum Likelihood estimate, is both conceptually intuitive and computationally simple. However, this estimate is truncated at extreme methylation levels and does not provide variance estimation. The second method, based on Bayesian hierarchical models, allows for variance estimation of methylation levels, and provides a flexible framework to adjust technical biases in the sequencing process. We compare previously proposed binary method, Maximum Likelihood method, and Bayesian methods using both simulation and real Methyl-Seq data. Our results show that the Bayesian methods outperform competing methods, displaying higher correlations with microarray beta value and larger areas under ROC curves. In addition, we apply these quantification methods to simulation data and show that, with sequencing depth above 300, Methyl-Seq offers a more consistent quantification than microarrays.

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An Information Resource to Support Epigenomics Research. *G. Schuler, R. Cohen, I. Fingerman, T. Hassan, S. Jiang, L. McDaniel, W. Ratzat, X. Zhang.* NCBI/NLM, NIH, Bethesda, MD.

Epigenomics is an emerging field of research aimed at understanding how—despite sharing a common genomic sequence—different cell types and lineages acquire distinct patterns of gene expression. The amount of data available from a few large-scale projects and many individual investigators has more than doubled in the past six months. We have developed an information resource on the NCBI website specifically aimed at highlighting epigenomics data (at www.ncbi.nlm.nih.gov/epigenomics). Epigenetic factors being examined include genomic DNA methylation, histone post-translational modifications, chromatin organization, and abundance of non-coding regulatory RNAs. Generally speaking, most experiments involve targeting specific regions of the genome using reagents like nucleases, methylation-sensitive restriction enzymes, and antibodies directed against methylcytosine or modified histones and then using either tiling arrays or massively-parallel sequencing for signal detection. Raw data from these experiments, together with extensive meta-data, are stored in the NCBI's GEO (Gene Expression Omnibus) and SRA (Sequence Read Archive) databases. The new Epigenomics resource provides a higher-level view, allowing users to search and browse the data based on biological attributes like cell type, differentiation stage, and health status, among many others. Data have been pre-mapped to genomic coordinates (to make "genome tracks"), so users are not required to be familiar with or manipulate the raw data. Tracks may be visualized in either the NCBI or UCSC genome viewers or may be downloaded to the user's computer for local analysis. Comparisons across tracks reveal loci that may play important biological roles and are candidates for further experimental study. At the time of this writing, the database includes 951 individual tracks derived from analysis of 260 biological samples (updates will be presented at the meeting).

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Exclusion of the GNAS locus in PHP-Ib patients with broad GNAS methylation changes provides evidence for an autosomal recessive form of the disorder. *E. Fernandez-Rebollo[1], G. Perez de Nanclares[2], B. Lecumberri[3], S. Turan[4], E. Anda[5], G. Perez-Nanclares[6], D. Feig[7], S. Nik-Zainal[8], M. Bastepe[1], H. Jüppner[1,9].* 1) Endocrine Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA; 2) Molecular Genetics Lab, Research Unit, Hospital de Txagorritxu, Vitoria-Gasteiz, Spain; 3) Endocrinology Service, Hospital La Paz, Madrid, Spain; 4) Department of Pediatric Endocrinology, Marmara University School of Medicine, Istanbul, Turkey; 5) Endocrinology Service, Hospital de Navarra, Navarra, Spain; 6) Endocrinology and Diabetes Research Group, Hospital de Cruces, Barakaldo, Spain; 7) Endocrinology and Metabolism, Mount Sinai Hospital and University of Toronto, Toronto, Ontario, CA; 8) Department of Medical Genetics, Addenbrooke's Hospital NHS Trust, Cambridge CB2 2QQ, United Kingdom; 9) Pediatric Nephrology Unit, Department of Pediatrics, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA.

Pseudohypoparathyroidism type Ib (PHP-Ib) is characterized by hypocalcemia and hyperphosphatemia due to PTH-resistance in the proximal renal tubules, which usually occurs in the absence of Albright's Hereditary Osteodystrophy. Most patients affected by autosomal dominant PHP-Ib (AD-PHP-Ib) carry maternally inherited microdeletions up-stream of GNAS (STX16del4-6 or STX16del2-4), which are associated with a loss of methylation restricted to GNAS exon A/B. Some AD-PHP-Ib patients carry microdeletions within GNAS (delNESP55/delAS3-4 or delAS3-4), which are associated with loss of all maternal methylation imprints. These latter epigenetic changes are often indistinguishable from those observed in patients affected by the sporadic form of PHP-Ib (sporPHP-Ib). We now investigated six females with PHP-Ib, who showed broad GNAS methylation changes and who were heterozygous for several microsatellite markers throughout the telomeric end of chromosome 20q, thus excluding a large deletion or partial paternal uniparental isodisomy (patUPD20) involving only small portions of the GNAS locus. The eleven children of these affected females are healthy and lack evidence for GNAS methylation changes. Furthermore, two of the sporadic PHP-Ib females had two and three children, respectively, who had inherited either the grandmaternal or the grandpaternal allele for the GNAS locus. These findings make it unlikely that the form of PHP-Ib that is present in our patients follows an autosomal dominant or an X-linked recessive trait. Instead, it appears plausible that the disorder in these cases is caused by a homozygous or compound heterozygous mutations in a gene involved in the establishment or maintenance of GNAS methylation.

1361/F

Homologous pairing of chromosome 15q11-q13 is associated with significant disruption of gene expression in human maternal chromosome 15 microcell transferred neurons. *S. Horike[1], K.N. Leung[2], D.H. Yasui[2], M. Oshimura[3], J.M. LaSalle[2], M. Meguro-Horike[4,5].* 1) Frontier Science Organization, Kanazawa University, Kanazawa, Ishikawa, Japan; 2) Medical Microbiology and Immunology, School of Medicine, University of California, Davis, USA; 3) Department of Biomedical Science, Institute of Regenerative Medicine and Biofunction, Tottori University, Yonago, Tottori, Japan; 4) Advanced Science Research Center, Kanazawa University, Kanazawa, Ishikawa, Japan; 5) JSPS Research Fellow.

Autism is a common neurodevelopmental disorder characterized by abnormalities in social, communicative, and behavioral functioning. Although the etiology of autism remains largely unknown, cytogenetic and genetic studies have suggested that autism may be influenced by genomic imprinting of 15q11-q13, through maternal copy number gains of 15q11-q13 occurring in 1-3% of autism cases. In this study, we focused on the homologous pairing of 15q11-q13 in human neuronal cells. Homologous pairing of 15q11-q13 was previously observed to be deficient in Rett Syndrome (RTT), Angelmann Syndrome (AS), and autism brain (Thatcher *et al*, 2005), and altered in maternal 15 q duplication (idic15) brain (Hogart *et al*, 2009). Our aim is to understand how the homologous pairing of 15q11-q13 is organized in the mammalian brain and associated with gene expression within the paired regions. Therefore, we investigated the impact of an extra human chromosome 15 on normal maternal to paternal 15q11-q13 interactions in a cell culture model. In this study, to model 15q11-q13 maternal duplication in a neuronal cell line, a paternal or maternal copy of human chromosome 15 was transferred into the human SH-SY5Y neuronal cells by microcell fusion. Then, FISH analysis was performed using probes to *SNRPN* and *GABRB3* to compare how the homologous alleles of 15q11-q13 are organized in human neuronal cells with a paternal or maternal copy of human chromosome 15. SH-SY5Y cells show an increase in the percentage of 15q11-q13 paired alleles following induced differentiation with 16 nM PMA. In contrast, homologous pairing of 15q11-q13 was disrupted in human neuronal cells with an extra maternal copy of human chromosome 15. Moreover, gene expression analysis of 15q11-q13 transcripts demonstrated significantly decreased expression of *SNRPN*, *GABRB3*, *CHRNA7* transcripts despite increased maternal dosage. Thus, our study revealed that gene expression can be altered in unexpected ways through epigenetic changes resulting from increased maternal 15q11-13 dosage, similar to what has been previously observed in a human brain sample with maternal 15q duplication and disrupted homologous pairing. Molecular investigation of gene expression in our autism model cells with an extra copy of 15q11-q13 provides insight into the potential complexities of other copy number variations in autism.

1362/F

Development of an ICON-probe based rapid diagnostic assay for imprinted diseases. *T. Kubota[1,2], S. Ishida[1], K. Miyake[1], T. Nakane[2], S. Saitoh[3], T. Hirasawa[1].* 1) Fac Med, Epigenetic Med, Univ Yamanashi, Chuo, Yamanashi, Japan; 2) Cntr for Genet Med, Univ Yamanashi Hospital, Chuo, Yamanashi, Japan; 3) Dept Pediatrics, Hokkaido Univ, Sapporo, Hokkaido, Japan.

Methylation-specific PCR (MSP) assay, which distinguishes the maternal allele from the paternal allele based on DNA methylation, is currently used to make a diagnosis of imprinted Prader-Willi (PWS) and Angelman (AS) syndromes. Recently, ICON (Interstrand Complex with Osmium for Nucleic acids) probe has been developed, which can crosslink to a methylated CpG in a unique sequence, and the osmium-DNA complexation inhibits subsequent PCR reaction. Taking advantage of use of this principle, we developed a new diagnostic assay for imprinted diseases.

We treated genomic DNA from three control individuals and two AS patients (in those DNA methylation pattern had been confirmed by MSP assay) with ICON probes designed either within the promoter region (ICON1) or within the downstream region of exon 1 (ICON2) of *SNRPN* gene. The treated samples were amplified using two primer pairs (SN-1, SN-2), which encompass the probe regions, respectively, and a control primer pair (LIS) within the *LIS1* gene region, using real-time PCR.

As a result, PCR amplification efficiency with ICON1 at the target region (SN-1) and that with ICON2 at the target region (SN-2) were lower in the controls (50% methylation) compared with AS patients (0% methylation) [ICON1: control 89±15%, AS 189±93%; ICON2: control 77±13%, AS 127±62%], whereas PCR amplification efficiency with ICON1 at the non-target regions (SN-2, LIS1) and those with ICON2 at the non-target regions (SN-1, LIS1) were not different between controls and AS patients.

These results suggest that crosslink of ICON probes to the *SNRPN* methylated allele inhibit PCR reaction in controls, indicating that ICON-based assay can distinguishes between the methylated allele and the unmethylated allele of *SNRPN*, which will be used for diagnosis of AS. Since this assay is faster than the conventional MSP assay because it does not include tedious bisulfite-treatment step, it potentially become one of the simplest method to screen CpG methylation in various research samples. We are currently improving the sensitivity of the assay in order to distinguish between controls (50% methylation) and PWS patients (100% methylation) at the *SNRPN* locus.

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Search for epimutations at differentially methylated regions in abnormal pregnancy. *K. Nakabayashi, Y. Yamaguchi, H. Torisu, N. Sugahara, M. Kusumi, K. Hata.* Dept Maternal-Fetal Biol, Natl. Res. Inst. for Child Health and Development, Setagaya, Tokyo, Japan.

Genomic imprinting, an epigenetic phenomenon whereby genes are differentially expressed according to their parental origin, is known to be crucial for placental development and fetal growth in mammals. Most imprinted loci contain a differentially methylated region (DMR) harboring allelic DNA methylation inherited from the male or the female gamete (germline DMR). Mutations/epimutations affecting DMRs cause rare imprinting disorders such as Silver-Russell syndrome (SRS) and Beckwith-Wiedemann syndrome (BWS). Whether imprinted genes also play critical roles in the more common abnormal pregnancy cases such as preeclampsia and intrauterine growth restriction (IUGR) is an important question yet to be fully evaluated. With the aim of addressing this question, we established quantitative COBRA (Combined Bisulfite Restriction Analysis) assay conditions for 30 DMRs from 22 loci that include the majority of known human imprinted DMRs. Using this COBRA assay system, we initially measured DNA methylation levels at these 30 DMRs in twenty blood samples from healthy individuals and twenty placental tissues from normal pregnancies, and determined the extent of tissue specificity and individual varieties of methylation levels. Subsequently, we have screened for epimutations at these DMRs in the placental tissues from approximately 100 IUGR cases. In two cases, among the DMRs examined, only H19- and IGF2-DMRs were found to be hypomethylated compared to the normal controls. Several other cases showed hyper- or hypo-methylation at the DMRs other than those in the H19/IGF2 locus, and are being further characterized. The genetic etiologies of IUGR are largely unknown and considered to be diverse. Our results suggest that placental epimutation at DMRs may account for the growth restriction phenotype of a subset of IUGR cases.

1364/F

Genetic characterization of the murine Angelman syndrome imprinting center. *E.Y. Smith[1], C.R. Futtner[2], S.J. Chamberlain[3], A.J. DuBose[1], R.A. Hallett[1], J.R. Resnick[1].* 1) Dept of Molecular Genetics & Microbiology, University of Florida, Gainesville, FL; 2) Duke University, Durham, NC; 3) Dept of Genetics and Developmental Biology, University of Connecticut, Farmington, CT.

Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are distinct neurological disorders resulting from improper gene expression from the imprinted domain on chromosome 15q11-q13, the PWS/AS locus. This locus is controlled by a bipartite imprinting center consisting of the PWS-IC and the AS-IC. Evidence suggests that the PWS-IC functions as a positive element to activate gene expression from the paternal allele. The AS-IC functions in the oocyte to inactivate the PWS-IC and thus silence the paternally expressed genes on the future maternal allele. The PWS-IC is located within a region of 4.3 kb just 5' to and including exon one of *SNRPN*. The AS-IC is contained within 0.88 kb, 35 kb upstream of *SNRPN*. Importantly, the AS-IC includes two of several *SNRPN* alternative upstream exons.

The PWS/AS locus is well conserved in the mouse but a murine AS-IC remains uncharacterized. As in humans, the mouse *Snrpn* locus includes several upstream exons postulated to function in silencing the maternal allele. We have taken a transgenic approach to study the potential regulatory role of these alternative exons. To do so, we utilized the bacterial artificial chromosome (BAC) 425D18, which contains *Snrpn* and approximately 120 kb of 5'sequence in which three alternative upstream exons reside. We first confirmed that this BAC transgene displayed proper imprinted expression in multiple transgenic lines thus demonstrating the presence of a functional AS-IC. Imprinting was further examined by analysis of the epigenetic status of the *Snrpn* differentially methylated region (DMR), which lies within the PWS-IC.

To determine whether the upstream exons on the 425D18 BAC confer silencing upon maternal transmission, we used recombineering techniques to create targeted deletions of these exons. Deletion of the three upstream exons resulted in robust *Snrpn* expression upon both maternal and paternal transmission of the transgene as well as a loss of the epigenetic imprint at the *Snrpn* DMR. These results indicate that the three upstream exons comprise the AS-IC on the 425D18 BAC. The discovery of the murine AS-IC in our transgenic model system has led to novel approaches for studying imprinting mechanisms at the PWS/AS locus.

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Monoallelic expression of NLRP7 and NLRP2: implications for autosomal recessive phenotypes resulting from mutations in imprinted maternal effect genes. *S. Wen[1, 2], M. McCarthy[1], X. Wang[1, 2], W. Liu[1, 2], C. Goodrich[3], I. Van den Veyver[1, 2].* 1) OB-GYN, Baylor College of Medicine, Houston, TX; 2) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 3) Rice University, Houston TX.

Objectives: Women with autosomal recessive mutations in NLRP7 in 19q13.42 have recurrent biparental hydatidiform moles with loss of DNA methylation at various paternally methylated imprinting control regions (ICRS). Women with autosomal recessive mutations in the neighboring and highly homologous NLRP2 gene have offspring with Beckwith-Wiedemann syndrome due to loss of imprinted DNA methylation at ICR2 in 11p15. A recent genome-wide screen suggested that NLRP2 is an imprinted gene itself. The goals of this study were to confirm mono-allelic expression and imprinting of NLRP2, determine if NLRP7 is monoallelically expressed and imprinted, and to describe models that explain how mutations in an imprinted gene can result in autosomal recessive inherited disorders. Methods: We designed primers for amplification of expressed single nucleotide polymorphisms (SNPs) in coding exons of NLRP2 and NLRP7 with a high frequency of heterozygosity. We then performed PCR amplification followed by direct sequencing of amplicons of genomic DNA from four human cell lines (HeLa, PA-1, BeWo, HEK293T) to find heterozygous SNPs. RNA was prepared and RT-PCR performed for all heterozygous SNPs. Results: Heterozygosity for several SNPs was confirmed in HeLa and PA-1 cell lines, but not in the BeWo and HEK293T cells. RT-PCR and sequence analysis of SNPs rs269950, rs269951, rs775883 was consistent with monoallelic expression of NLRP7 in the HeLa and PA-1 cell lines. Similar analysis of SNP rs1043680 and a novel coding SNP were identified, confirmed monoallelic expression of NLRP2 in the HeLa cell line, but not in the PA-1 cell line. Conclusion: Although our findings of mono-allelic expression do not yet confirm that NLRP2 and NLRP7 are imprinted genes, they are highly suggestive considering prior reported data on NLRP2. Experiments to determine the parental origin of the expressed alleles in human cell lines for which parental genotypes can be determined and in hybrid mouse crosses are ongoing. We propose a model that implicates (1)expression during the time of general erasure of imprinting marks in primordial germ cells and (2)persistence of the gene product in haploid gametes to explain the autosomal recessive inheritance, as opposed to the expected imprinted inheritance of disorders caused by mutations in imprinted maternal effect genes. Our data also raise the possibility that we have identified a novel imprinted gene cluster that contains at least two imprinted genes.

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Identification of novel germline DMRs by CpG island methylation profiling of Dnmt3L$^{mat-/-}$ embryos. *W. Yoshida, S. Sato, C. Tayama, M. Kusumi, K. Hata, K. Nakabayashi.* Dept. of Maternal-Fetal Biol., Natl. Res. Inst. for Child Health and Development, Setagaya, Tokyo, Japan.

Genomic imprinting, an epigenetic phenomenon whereby genes are differentially expressed according to their parental origin, is known to be crucial for placental development and fetal growth in mammals. Most imprinted loci contain a differentially methylated region (DMR) harboring allelic DNA methylation inherited from the male or the female gamete (germline DMR). Several germline DMRs have been shown to govern imprinted gene expression as a cis-acting imprinting-control region (ICR). *Dnmt3L*, a *Dnmt3* methyltransferase family member lacking a functional methyltransferase domain, is shown to be essential for the establishment of DNA methylation at DMRs during the maturation of germ cells. Mouse embryos produced from *Dnmt3L*$^{-/-}$ females arrest in development by embryonic day 10.5, and completely lack maternal methylation at germline DMRs. As an approach to identify novel DMRs, we obtained DNA methylation profiles for 16,000 CpG islands of these mutant and wild-type embryos (day 9.5) using the methylated-DNA immunoprecipitation (MeDIP) technique followed by array hybridization. Comparison of the mutant/wild-type profiles allowed us to detect decreased levels of methylation at 16 known maternally methylated DMRs as well as 17 novel candidates. Bisulfite sequencing analyses of oocyte and sperm for the top six candidates have revealed that three of them are germline DMRs that are methylated in oocyte but unmethylated in sperm, and that two of them are secondary DMRs, whose differential methylation status is established post-zygotically. One of the newly identified germline DMR was located approximately 180 kb away from the cluster of imprinted genes, *Xlr3b*, *Xlr4b*, and *Xlr4c* on the chromosome X. Since this is the first DMR mapped in the vicinity of these genes, it represents a primary candidate for the ICR of this imprinted chromosomal domain. To determine if the other newly identified DMRs are involved in the regulation of imprinted gene expression, allelic expression analysis is being conducted for the genes located close to the DMRs.

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Domain-wide tissue-specific differences in DNA methylation levels from Methyl-seq analyses. *D.I. Schroeder, D.H. Yasui, W.T. Powell, R. Woods, S.W. Chadwick, J.M. LaSalle.* Medical Microbiology and Immunology, UC Davis, Davis, CA.

In mammals, DNA methylation at CpG sites (mC) has traditionally been considered a repressive mark in the genome, as high DNA methylation in intergenic sequences and repetitive elements prevents spurious transcription while lack of DNA methylation in promoters and CpG islands promotes gene transcription. Surprisingly, though, recent genome-wide DNA methylation sequencing (Methyl-seq) analyses have shown that high CpG methylation is common in gene bodies where it positively correlates with transcription. Gene bodies contained in partially methylated domains (PMDs, genomic sequences with less than 70% average methylation over several hundred thousand bases) showed decreased expression compared to those contained in highly methylated domains (HMDs). We sought to better understand the role of PMDs in gene regulation, particularly in neurons, by comparing published Methyl-seq data from lung fibroblasts with new data from human neuroblastoma cells (SH-SY5Y). SH-SY5Y DNA was bisulfite converted, Solexa sequenced, and the reads mapped to the genome. Many PMDs were tissue-specific, with neuronal HMDs (N-HMDs, domains that were HMDs in neuronal cells but PMDs in lung cells) enriched for genes involved in neurogenesis, synaptogenesis, cell adhesion, and synaptic signaling. Lung HMDs (L-HMDs) were enriched for genes involved in lung development and immune response. Affymetrix gene expression data from the two cell lines confirmed that N-HMD genes are enriched for genes with neuronal-specific expression and L-HMD genes are enriched for lung fibroblast-specific expression. In addition, individual CpG sites outside of CpG islands were more consistently methylated in N-HMDs compared to those in PMDs. These data suggests that, at least in human cell lines, partially methylated domains encompass clusters of genes repressed in a tissue-specific manner, while highly maintained methylated CpG sites are found within and around a subset of actively expressed tissue-specific genes. Analyses of postmortem human tissues by these approaches and the examination of differential modifications of methylcytosine and hydroxymethylcytosine are in progress.

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Association between Parental Age and DNA Methylation Patterns in Newborn Umbilical Cord Blood. *R. Adkins[1], F. Tylavsky[2], J. Krushkal[2].* 1) Le Bonheur Child Med Ctr, Univ Tennessee-Hlth Sci Ctr, Memphis, TN; 2) Department of Preventive Medicine, Univ TN Health Sci Center, Memphis, TN.

In recent years, industrialized nations have witnessed a dramatic increase in the average age of women giving birth. While epidemiological and clinical genetic studies show an increased incidence of pregnancy complications, chromosomal abnormalities, and certain disorders such as cancer and neurocognitive diseases in the offspring of older parents, the molecular mechanisms of this increase are yet to be fully understood. Decreases in the levels of DNA methylation in adult tissues with age in a tissue-dependent manner have been well documented. However, much less is known about the influence of parental age on levels and patterns of DNA methylation in the offspring. We investigated the influence of parental age on neonatal genome-wide DNA methylation patterns. The patterns of methylation in umbilical cord blood were assayed at 27,578 CpG sites genome-wide in 168 newborns and related to numerous parental and newborn characteristics, including parental age. We observed a generally negative genome-wide correlation between parental age and leukocytic DNA methylation of autosomal probes in the newborn. Methylation of 144 CpG probes belonging to 142 genes was significantly correlated with maternal age at the genome-wide level. A correlation with paternal age was also present, but was weaker than that with maternal age. The products of many genes whose methylation levels were decreased in children of older mothers are involved in mesodermal development, neurological regulation, glucose/carbohydrate metabolism, nucleocytoplasmic transport, and transcriptional regulation. Many of the hypomethylated genes and probes were found to be involved in processes related to cancer development, and therefore changes in their methylation may be predisposing the newborn to increased cancer risk, in agreement with previous epidemiological observations. While age-related changes in DNA methylation in adults have been documented previously, we believe this is the first demonstration of an effect of parental age on DNA methylation patterns in the next generation at birth.

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Pediatric age-related DNA methylation changes. *R.S. Alisch, B. Barwick, B. Lynch, J.O. Mowrey, S.T. Warren.* Dept Human Gen, Emory Univ Sch Med, Atlanta, GA.

It is widely accepted that there are age specific DNA methylation (DNAm) changes and that they may contribute to human disease, including cancer. Previous studies have identified age-related differentially methylated loci (DML) in adults, suggesting that age-related DNAm changes manifest later in life, and epigenetic studies targeting pediatric cohorts might be less susceptible to this confounding factor. Here, we use a high-throughput approach to characterize the methylation status of 27,578 CpG loci in whole blood-extracted DNA from 281 children aged 4-17 years, and find DNAm changes associated with age. These pediatric DML significantly overlap with age-related DML identified in adults ($p<1x10-15$), suggesting that age-related methylation changes occur not only throughout adulthood but also earlier in life. These pediatric DML also significantly overlap with DML previously implicated in adult diseases such as systemic lupus erythematosus ($p<0.05$), indicating that many of these DML may be confounders and not associated with disease. Together, these findings corroborate previous studies by identifying loci with age-dependent methylation status in both pediatric and adult subjects and call for consideration of age as a covariate in methylation studies regardless of cohort age.

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Epigenetic Alterations In Radiation Induced Genomic Instability. *U. Aypar[1], W.F. Morgan[2], J.E. Baulch[1].* 1) Department of Radiation Oncology, University of Maryland School of Medicine, Baltimore, MD; 2) Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA.

Radiation-induced genomic instability (RIGI), results in an increased frequency of genetic alterations in the progeny of irradiated cells. RIGI is thought to be an early event in radiation-induced carcinogenesis, but it is unknown how this process is initiated. Epigenetic changes including aberrant DNA methylation and altered microRNA expression can be induced by radiation exposure and have been associated with some cancers. We are testing the hypothesis that irradiation results in epigenetic alterations that are perpetuated in clones with RIGI. The human-Chinese hamster ovary hybrid cell line GM10115 was irradiated using iron ions or x-rays and clones were obtained. Irradiation induced DNA damage in GM10115 cells and RIGI in clones (114, 118, CS9, LS12, 115, Fe5.0-8) was determined using micronucleus formation and fluorescence in situ hybridization analysis of chromosomal rearrangements. To study epigenetic changes, the post-irradiation status of genome-wide, repeat element, specific locus methylation, and microRNA profiles were evaluated. Arbitrary priming methylation sensitive PCR is being used to identify changes in methylation at random sites within the genome and combined bisulfite restriction analysis (COBRA) is being used to identify changes in methylation at LINE-1 and alu repeat elements. Methylation sensitive specific locus methylation assays and bisulfite sequencing is being used to detect aberrant promoter methylation for genes of interest. Preliminary data indicate that early effects of radiation exposure include aberrant genome-wide, repeat element methylation (LINE-1, alu) and alterations in some microRNAs. The genomically unstable clones also exhibited aberrant DNA methylation as well as altered microRNA profiles. Specifically, in unstable clones we observed global hypomethylation, hypermethylation of LINE-1 and hypomethylation of alu. Altered microRNA profiles were observed in unstable clones when compared to stable clones and the parental cell line. The array results are being validated with qRT-PCR and mRNA targets of the altered miRNAs will be determined based on mRNA arrays. These results suggest that the early effects of radiation exposure include alterations in epigenetic endpoints. Delayed effects include perpetuation of RIGI that appears to be associated with aberrant repeat element and global DNA methylation, and altered microRNA profiles.

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High-resolution DNA methylation profiles in monozygotic twins. *J.T. Bell[1,2], G.J. Brock[3], B. Zhang[3], C.L. Hyde[3], J.M. Harris[1], R. McEwen[4], B. Dougherty[3], S. Phillips[4], F.M.K. Williams[1], W. Jun[5], G. Burgess[4], S. John[3], T. Spector[1].* 1) Department of Twin Research and Genetic Epidemiology, Kings' College London, London, UK; 2) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK; 3) Pfizer Global Research & Development, Groton, CT, USA; 4) Pfizer Global Research and Development, Sandwich Laboratories, Sandwich, UK; 5) Beijing Genomics Institute (BGI), Chinese Academy of Sciences (CAS), Beijing, China.

DNA methylation is an important epigenetic mechanism involved in gene regulation and disease. Recently, studies of discordant monozygotic (MZ) twins have identified epigenetic changes as susceptibility factors in complex traits. We examined DNA methylation profiles in twenty-five MZ pairs of twins discordant for objective pain sensitivity, determined experimentally via heat-induced pain. DNA methylation was assayed in whole blood samples using a high-resolution MeDIP-sequencing (MeDIP-seq) approach. Following MeDIP, sequencing data were generated using the Illumina GAII platform, resulting in over 25 million paired-end reads per lane using 2 lanes per individual for a total of 100 lanes. Preliminary analyses using stringent alignment criteria indicate that MeDIP-seq fragment coverage was over approximately 819 Mb (26.6%) of an individual's genome, not accounting for repeats or CpG content. We examine the distribution of DNA methylation patterns genome-wide, comparing MeDIP-seq results to functional sequence annotations. To determine the effect of DNA methylation on pain sensitivity, we first assess power to detect differentially methylated regions (DMRs) that contribute to pain sensitivity differences. We then evaluate the role that epigenetic mechanisms play in sensitivity to pain by calculating DMRs using two complementary approaches testing for associations between epigenetic and phenotypic variation. Our findings have implications for epigenetic studies of phenotypically discordant MZ twins that may provide a powerful tool for identifying genomic regions that contribute to complex traits.

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High Density DNA Methylation BeadChip with Single CpG Site Resolution. *M. Bibikova, B. Barnes, C. Tsan, V. Ho, B. Klotzle, J. Le, D. Delano, R. McCauley, K. Gunderson, J.-B. Fan, R. Shen.* Dept Research & Dev, Illumina, Inc, San Diego, CA.

DNA methylation is one of the best known epigenetic modifications in human cells. Changes in DNA methylation patterns play a critical role in development, differentiation and diseases such as multiple sclerosis, diabetes, schizophrenia, aging, and multiple forms of cancer. We developed a new generation of genome-wide DNA methylation BeadChip which allows high-throughput methylation profiling of human genome. The new high density BeadChip will assay over 450K CpG sites. The innovative content includes coverage of 96% of RefSeq genes with multiple probes per gene, 95% of CpG islands from the UCSC database, CpG island shores and additional content selected from whole-genome bisulfite sequencing data. The well-characterized Infinium® Assay is used for analysis of CpG methylation using bisulfite-converted genomic DNA. In this assay, unmethylated cytosines (C) are converted to uracil (U) when treated with bisulfite, while methylated cytosines remain unchanged. The assay design employs single probe assays for CpG loci with up to two underlying CpG dinucleotides, and two-probe assays for CpG loci with multiple underlying CpG sites, with one probe querying the "unmethylated" allele and the other probe querying the "methylated" allele. The assays are designed under the assumption that methylation is regionally correlated (within 50 base pairs) and that all CpG sites underlying the probe are assumed to be "in phase" with the queried CpG site. For the BeadChip development, we used methylation standards created by de-methylating gDNA with Phi29 whole genome amplification, methylating the amplified DNA with SssI methylase and mixing the unmethylated and methylated DNA in a 1:1 ratio to create a 50% methylation state. 500 ng gDNA input is used for bisulfite conversion and is sufficient for two assays. Reproducible DNA methylation profiles were obtained between replicates (an average R^2 of 0.98). We applied this technology to DNA methylation analyses in normal DNA samples derived from multiple tissues and cancer cell lines of different tissue origin. Highly specific methylation signatures were obtained for each sample type. The ability to determine genome-wide methylation patterns will rapidly advance methylation research and ultimately lead to the development of powerful tools for diagnosis, prognosis, and treatment of human diseases.

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In-vitro embryo culture results in widespread alterations in preimplantation mouse blastocysts. *S. Brown[1], G. Brown[2], L. Brown[1].* 1) Dept OB/GYN, Univ Vermont, Burlington, VT; 2) Pathology Department, Memorial Hospital, Jasper, IN.

Super-ovulation and in-*vitro* embryo culture have been reported to result in altered genomic methylation, both at the blastocyst stage as well as in fully developed offspring. Most reports have focused on specific loci and most have reported that embryo manipulation is associated with hypomethylation. Given the public health importance of assisted reproductive technology (ART), it is important to increase our understanding of the alterations in genomic methylation caused by ART techniques. To this end, we have previously developed and validated a method for large-scale methylation analysis, based on methylation-sensitive DNA amplification followed by microarray assessment (ClinChem 2010). The method allows the simultaneous assessment of ~16,000 randomly chosen genomic loci.

In the present study, we have applied our method to the study of a series of preimplantation mouse blastocysts that were either flushed from the oviduct and cultured in-*vitro* beginning at 0.5 (E0.5) days post conception (experimental group) or were recovered from the uterus at E3.5 of normal gestation (control group). All embryos were normal in appearance and all had reached the blastocyst stage of development. Genomic DNA was prepared and subjected to methylation-sensitive amplification and then assessed by hybridization to a custom-designed microarray. Each hybridization was performed twice, with dye reversal, and resulting data were normalized by averaging the dye-reversed data. Non-methylated prokaryotic DNA was "spiked-in" to each experiment as a means of assessing data quality and normalization.

We found that genomic methylation was strikingly similar among control embryos, with pair-wise correlation coefficients of ~.95. Methylation in the experimental embryos was more variable, with pair-wise correlations of ~.65. Comparisons between experimental and control embryos showed that, in all cases, there was evidence of marked hypermethylation at the majority of loci in the in-vitro cultured embryos. The specific loci showing hypomethylation in the experimental embryos appeared to be non-random, suggesting that the impact of embryo culture on genomic methylation is variable and depends on genomic location. Future studies will be directed at understanding how in-*vitro* embryo culture results in these effects.

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DNA Methylation of the Oxytocin Receptor (OXTR) in Autism Spectrum Disorder (ASD). *D.T. Butcher[1], D. Grafodatskaya[1], R. Zhang[1], C. Zhou[1], S.W. Scherer[2], W. Roberts[3], E. Anagnostou[4], R. Weksberg[1].* 1) Genetics and Genome Biology, The Hospital for Sick Children, Toronto, Canada; 2) The Centre for Applied Genomics, The Hospital for Sick Children, Toronto, Canada; 3) Autism Research Unit, The The Hospital for Sick Children, Toronto, Canada; 4) Bloorview Research Institute, Toronto, Canada.

Epigenetics refers to alterations of genome function that are heritable through cell division but unrelated to changes in the primary DNA nucleotide sequence. Multiple epigenetic mechanisms that are essential for normal growth and development work in concert to regulate gene expression including, DNA methylation, non-coding RNAs, covalent modifications of histone proteins, and chromatin conformation. The study will focus on alterations in DNA methylation patterns. Autism spectrum disorders (ASD) are a group of childhood onset neurodevelopmental disorders characterized by problems in social interaction and communication as well as repetitive behaviours. The prevalence of ASD is estimated to be 1 in 110 with a male to female ratio of 4:1. Studies in children with autism have demonstrated lower levels of oxytocin (OXT) in the blood compared to normally developing, age-matched children. Previously in a small study increased DNA methylation of the oxytocin receptor (OXTR) has been demonstrated in lymphocytes of patients with autism. These changes were also demonstrated in post-mortem samples from the temporal cortex of autism patients. It has been suggested that dysfunction of the OXT pathway is associated with features of autism such as repetitive behaviour and impaired social cognition. Treatment of patients with ASD with OXT to ameliorate these behaviours has been proposed. We hypothesized that increased DNA methylation of the CpG island in the 5' UTR of oxytocin receptor (OXTR) could be a modulator of response to oxytocin (OXT) treatment. To determine the clinical significance of this DNA methylation variation, we undertook a methylation analysis of OXTR in a large OXT-naive cohort. Using quantitative sodium bisulfite pyrosequencing a number of CpG sites in the 5'-UTR CpG island of OXTR were analyzed which revealed a statistically significant increase in DNA methylation in the blood of males with ASD. We are currently correlating these DNA methylation patterns to a battery of clinical measures including, social cognition and repetitive behaviours, which have been collected for these patients. DNA methylation of OXTR could be an important modulator of response to oxytocin (OXT) treatment in which case the methylation pattern of OXTR could be a useful tool in determining the appropriateness of OXT treatment trials for individual patients.

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UVB radiation induces epigenetic modification in retinal pigment epithelial cells. *W. Chou[1,2], K. Chen[1,2], Y. Wang[1,2], S. Juo[1,2].* 1) Departments of Medical Research, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; 2) Department of Medical Genetics, Kaohsiung Medical University, Kaohsiung, Taiwan.

Purpose: Environmental factors are known to alter the fundamental epigenetic programming of the human genome. Ultraviolet (UV) radiation can damage ocular tissues and have phototoxic effects on the retina. The age-related macular degeneration (AMD) is caused by an increases in apoptotic cells in the RPE compared with normal retinas. SIRT1 has been found to function as a deacetylase for numerous protein targets involved in various cellular pathways, including stress responses and apoptosis. However, the role of SIRT1 in UV induces epigenetic modification remains unknown. The purpose of the study was to determine whether the UVB radiation induce alterations in human retinal pigment epithelium (RPE) cells by apoptosis and epigenetic regulation. Therefore, this study may gain more insight to AMD. Methods: ARPE-19 Cells (human retinal pigment epithelium cells) were exposed to different doses of UVB (0-75 mJ/cm2). Cells were incubated for 24 hours, and cell number was measured with trypan blue exclusion assay. Apoptosis and global DNA methylation were detected by flow cytometry. The mRNA expression of the SIRT1 gene (normalized to GAPDH gene) was detected by quantitative real-time PCR. Results: We found UVB radiation dose-dependently decreased cell viability and increased apoptosis in ARPE-19 cells. UVB radiation induced significant alterations in global methylation signatures of DNA in ARPE-19 cells. UVB down-regulated the SIRT1 gene expression in ARPE-19 cells. Furthermore, 5-aza-2'-deoxycytidine (AZA), an demethylating agent of hypermethylated DNA, could increase the mRNA expression of SIRT1 in ARPE-19 cells. But AZA only partly increase UVB-induced down-regulation of SIRT1 mRNA. Conclusions: We have demonstrated a regulation of DNA methylation under UVB exposure in the human RPE. Our results showed that epigenetic modification can regulate the SIRT1 gene expression of RPE cells. We are currently studying other genes that are also influenced by the epigenetic modification caused by UVB. Our studies suggest a new mechanism for UVB-induced AMD.

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Identification of early diagnostic markers for prostate cancer using the epigenome-wide screening of plasma circulating DNA. *R. Cortese[1], P. Boutros[2], A. Kwan[1], O. Bryzgunova[3], A. Bondar[3], M. Park[1], S. Jarmalaite[4], A. Laurinavicius[5], Z. Kaminsky[1], P. Laktionov[3], A. Petronis[1].* 1) The Krembil Family Epigenetics Laboratory, Centre of Addictions and Mental Health, Toronto, Ontario, Canada; 2) Informatics and Biocomputing Platform, Ontario Institute for Cancer Research. MaRS Centre, South Tower. 101 College Street, Suite 800. M5G0A3. Toronto, ON Canada; 3) Institute of Chemical Biology and Fundamental Medicine, SD RAS, Lavrentiev Ave. 8, Novosibirsk, 630090, Russia; 4) Department of Botany and Genetics. Faculty of Natural Sciences. Vilnius University, Ciurlionio 21. LT-03101 Vilnius. Lithuania; 5) National Center of Pathology, Baublio 5, Vilnius 2600, Lithuania.

Tumor-specific DNA methylation changes can be detected not only in malignant cells, but also in the cell-free circulating DNA (cirDNA) in blood and other bodily fluids of cancer patients. Aberrant DNA methylation identified in early stages of cancer can be used as a biomarker for early detection. We performed a large-scale microarray-based analysis of the cirDNA methylome of prostate cancer (CaP) and benign prostate hyperplasia (BPH) patients, as well as unaffected controls. To achieve this, we first developed a protocol for large-scale methylome analysis of cirDNA. Universal DNA adaptors were ligated to the ends of cirDNA fragments, followed by digestion with DNA methylation-sensitive and -specific restriction enzymes. cirDNA fragments that survived enzymatic hydrolysis were amplified by adaptor-mediated PCR and labeled with biotinylated nucleotides. This enriched differentially methylated DNA fraction was hybridized to commercially available microarrays containing 12,000 CpG islands. Following extensive quality control and data normalization, univariate multiple-testing adjusted statistical analyses were used to identify specific loci exhibiting differential methylation between cases and controls. As a proof of principle, we have identified DNA methylation profiles in cirDNA of 20 prostate cancer patients, 20 benign prostate hyperplasia patients, and 20 unaffected control individuals. We selected a profile of seven DNA regions showing significant disease-specific differences in cirDNA methylation. These novel candidates were verified using sodium bisulfite treatment-based mapping of methylated cytosines. First, the whole bisulfite-treated cirDNA was amplified using a random-primer based strategy. The genes and loci of interest were further amplified using specific primers that bind to the bisulfite-converted DNA, and analyzed by pyrosequencing. Tumor-specific DNA methylation markers in cirDNA can potentially serve as non-invasive biomarkers for early diagnostics of cancer, monitoring the efficacy of anti-cancer therapies, and tumor reoccurrence.

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Methylomic profiling in monozygotic twins discordant for major psychosis. *E.L. Dempster, R. Pidsley, L. Schalkwyk, T. Toulopoulou, M. Picchioni, E. Kravariti, R. Murray, J. Mill.* Institute of Psychiatry, King's College London, London, United Kingdom.

Studies of major psychosis (schizophrenia and bipolar disorder) have focused primarily on the interplay between genetic and environmental risk factors although recent research implicates a role for epigenetic processes in mediating susceptibility. Because monozygotic (MZ) twins share a common DNA sequence, the study of discordant twins represents an ideal design for investigating the contribution of epigenetic factors to disease etiology. We performed genome-wide methylomic profiling on peripheral blood DNA samples obtained from a unique set of MZ twin-pairs discordant for major psychosis (n=25 twin-pairs, 50 individuals) using the Illumina Infinium 27K Methylation array. Numerous loci demonstrated FDR-significant disease-associated DNA methylation different between twins for both schizophrenia and bipolar disorder, with a combined major psychosis group showing even strong epigenetic differences. Significant within twin-pair DNA methylation differences were independently quantified using the Sequenom EpiTYPER system and/or Pyrosequencing, and also assessed in post-mortem brain tissue from affected individuals and controls. Overall, our data provide further evidence to support a role for DNA methylation differences in the etiology of major psychosis.

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The level of DNA methylation may regulate the activity of CpG poor promoter of the *OTC* gene. *L. Dvorakova[1], J. Minks[2], M. Hnizdova Bouckova[1], H. Treslova[1], G. Storkanova[1], H. Vlaskova[1], L. Stolnaya[1], M. Jirsa[3], M. Hrebicek[1].* 1) Inst Inherit Metabol Disorders, Charles Univ, First F Med, Prague 2, Czech Republic; 2) Dept. of Medical Genetics, Faculty of Medicine, University of British Columbia, Vancouver B.C., Canada; 3) Laboratory of Experimental Hepatology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic.

Ornithine carbamoyltransferase (OTC) is a mitochondrial matrix enzyme, the deficiency of which causes the most common inherited defect of the urea cycle. The human *OTC* gene (Xp21.1) is expressed almost exclusively in the liver and its promoter lacks a CpG island. The *OTC* gene promoter and three dominant transcription start sites located at positions c.-169, c.-119 and c.-95 were recently described in Luksan et al., Hum Mutat, 31 (4), E1294 - E1303.

To find out if methylation contributes to tissue-specific expression of *OTC* gene we compared methylation status in autoptic samples (liver and blood) of four males, thus avoiding the confounding contribution of X-chromosome inactivation to the overall DNA methylation patterns. The methylation profile of 19 CpG dinucleotides in 1 kb region covering the promoter, 5' UTR, the first exon and a part of the first intron of the *OTC* gene was probed by bisulfite sequencing, when at least 10 cloned PCR products were analyzed. The results show that there are two distinct areas with respect to CpG methylation; 10 CpGs located 500-700 bp upstream of the *OTC* transcription start sites were heavily methylated (cca 90%) in both blood and liver. However, nine CpGs encompassing the transcription start sites (cca 230 bp upstream and 250 bp downstream from the start sites) were remarkably less methylated in the liver samples (~45%) than in blood (~90%). In conclusion, the level of methylation at CpGs surrounding transcription start site region inversely correlates with the known expression of *OTC* in the examined tissues, suggesting that despite the absence of CpG island, DNA demethylation is involved in regulation of *OTC* gene transcription. We speculate that aberrant DNA methylation patterns may contribute to phenotype variability of OTC deficiency or may explain the disease manifestation in patients in which the causative mutation was not found. Support: IGA MZCR NR/9364-3, VZ MSMCR 0021620806, VZMZCR64165.

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Novel Epigenetic Biomarkers of Type 2 Diabetes Susceptibility. *H.R. Elliott[1], M.S. Pearce[2], J.C. Mathers[1], M. Walker[3], C.L. Relton[1].* 1) Human Nutrition Research Centre, Institute for Ageing and Health, Newcastle University, Newcastle-upon-Tyne, Tyne and Wear, United Kingdom; 2) Institute for Health and Society, Newcastle University, Newcastle-upon-Tyne, Tyne and Wear, United Kingdom; 3) Human Nutrition Research Centre, Institute of Cellular Medicine, Newcastle University, Newcastle-upon-Tyne, Tyne and Wear, United Kingdom.

Background Genotype clearly plays a role in type 2 diabetes (T2D) susceptibility. However, the role of DNA methylation and its interaction with genotype is poorly understood. This study aims to identify whether variation in DNA methylation pattern is associated with risk of T2D or associated traits. A targeted approach was adopted; genes which harboured SNPs identified by genome wide association studies (GWAS) were analysed for variation in methylation patterns. In addition, we investigated if variation in DNA methylation pattern was associated with T2D-related traits

Methods Methylation analysis was conducted in two genes, *FTO* and *ADCY5*, using the Sequenom® EpiTYPER® platform, a high-throughput MALDI-TOF based system. DNA samples were derived from the *Relationship between Insulin Sensitivity and Cardiovascular Disease* (RISC) cohort; a group of healthy individuals aged between 30-60 years of age. DNA samples were collected at baseline and 3 year follow-up alongside detailed biological, physiological and lifestyle measures.

Results DNA methylation was analysed at 11 CpG sites in *ADCY5* (average n=304) and 10 CpG sites in *FTO* (average n=287). Spearman's tests for correlation between methylation and T2D related traits revealed a number of statistically significant observations including an inverse relationship between *ADCY5* methylation and fat free mass (rho -0.179;*p*=0.001) and an association with age (rho 0.148;*p*=0.007). In *FTO*, methylation correlated with BMI (rho 0.139;*p*=0.011) and fasting glucose (rho 0.125;*p*=0.022).

Conclusion These data provide evidence that the Sequenom® EpiTYPER® is a valuable high-throughput system for population-based studies to assess variation in methylation in DNA derived from leukocytes. Both *FTO* and *ADCY5* genes showed inter-individual variation in methylation and in turn were associated with T2D-related traits within the RISC cohort. These data suggest that, in addition to effects of genetic polymorphisms on T2D risk, epigenetic variants in the same genes may also contribute to disease risk.

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Cell-Specific Patterns of Methylation in the Human Placenta. *J. Ferreira[1], A. Grigoriu[2], S. Choufani[1], D. Baczyk[2], J. Kingdom[2], R. Weksberg[1,3].* 1) Gen & Genomics Program, Hosp Sick Children, Toronto, ON, Canada; 2) SLRI & Dept. Ob & Gyn., Mount Sinai Hospital, Toronto, ON, Canada; 3) Clinical and Metabolic Genetics, Hospital for Sick Children, Toronto, ON, Canada.

Epigenetic processes, such as DNA methylation, are known to regulate cell type specific gene expression in various organ systems. Alterations in these processes may be relevant to disease states such as severe IUGR or pre-eclampsia. DNA methylation variation among cell types within a tissue may complicate molecular investigations done at the tissue level. Therefore, our objective was to determine whether DNA methylation in the placenta is cell type specific. Placental samples from normal placentas obtained at 14 weeks (n=3) and 18-19 weeks (n=3) gestation were serially-digested using a trypsin-based protocol and Ficoll density column, followed by negative magnetic bead separation (CD45 and anti-fibroblast). Immunocytochemistry of plated fixed cells demonstrated 95% purity in C (cytokeratin-7) and 60-70% for fibroblasts (vimentin). Genomic DNA was extracted from whole placental villi, purified villous cytotrophoblasts (C) and mesenchymal core fibroblasts (F) using the QIAGEN MiniKit, bisulfite-modified and hybridized to the Illumina Methylation27 array that interrogates >27,000 highly-informative CpG sites covering >14,000 genes. Across the genome, the methylation pattern was similar amongst the different types of samples (r2= 0.90 - 0.95 [mean = 0.92]) and did not vary with gestational age (r2 = 0.97). Cluster analysis did not find a complete separation between the 3 components analyzed, but it showed two main clusters, one composed of placenta and C, the other one mainly of F. We identified 465 probes, corresponding to 386 genes, showing a statistically significant >20% difference in methylation between C and F. Interestingly, and as proof of principle, 6 of 8 probes mapping to the promoter regions of the beta chain of chorionic gonadotrophin genes were more methylated in F than C. Furthermore, probes that were more methylated in C were enriched (relative to the frequency in the array) for tumor suppressor gene related probes. Both of these findings suggest that epigenetic regulation of gene expression contributes to the functional specificity of cytotrophoblasts. Genome-wide specific methylation profiles of specific cell types in placenta will provide a useful resource for the interpretation of placenta methylation studies and will generate hypotheses regarding epigenetic regulation of cell specific expression patterns.

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Characterization of a new maternal differentially methylated region at chromosome 18q23. *D. Grafodatskaya[1], R. Rajendram[1], S. Choufani[1], Y. Lou[1], D. Niyazov[2], R. Weksberg[1,3].* 1) Gen & Genome Biol, Hosp Sick Children, Toronto, ON, Canada; 2) Department of Pediatrics, Ochsner Clinic Foundation, New Orleans, LA, USA; 3) Division of Clinical & Metabolic Genetics, The Hospital for Sick Children, Toronto, ON, Canada.

Terminal deletions of chromosome 18q are characterized by variable clinical phenotypes. Clinical features of 18q deletion syndrome include short stature, congenital aural atresia, delayed myelination and developmental delay. In addition 18q terminal deletions were reported in two patients with Beckwith-Wiedemann Syndrome-like macroglossia. The differences among phenotypes could be attributable to the size of the deleted region, as well as parent of origin effects if imprinted genes are located within the deletion. Here we report a patient with a 15 Mb de novo terminal deletion of chromosome 18q of paternal origin, typical BWS features and developmental delay. There were no identifiable molecular abnormalities in the chromosome 11p15.5 BWS critical region. We have generated DNA methylation profile of the chromosome 18q region using methylated DNA immunoprecipitation followed by Agilent CpG island array and have identified a new maternally differentially methylated region (DMR) located at the 18q23 between PARD6G and ADNP2 genes. Maternal pattern of DNA methylation was confirmed using methylation sensitive digestion with subsequent PCR amplification and sequencing in saliva samples of five trios informative for the SNPs within the DMR region. In addition we observed DNA methylation patterns consistent with a maternal parent of origin in blood sample of the patient carrying only maternal allele of 18q23 region and androgenetic hydatidiform mole, carrying two paternal genomes. These data suggest that the genes PARD6G and ADNP2 located adjacent to the newly identified DMR could be imprinted and potentially contribute to the different phenotypes of 18q terminal deletions.

1382/F

Genome-wide DNA methylation profiling in 40 breast cancer cell lines. *L. Han[1,2], S. Zheng[1,2], S. Sun[3], T. Huang[4], Z. Zhao[1,2,5].* 1) Biomedical informatics, Vanderbilt University, Nashville, TN; 2) Bioinformatics Resources Center, Vanderbilt University, Nashville, TN; 3) Case Comprehensive Cancer Center, Case Western Reserve University, Cleveland, OH; 4) Human Cancer Genetics Program, The Ohio State University, Columbus, OH; 5) Department of Cancer Biology, Vanderbilt University School of Medicine, Nashville, TN.

DNA methylation plays important roles in gene regulation and functions. Aberrant methylation, either hypomethylation or hypermethylation, has been reported to cause various diseases, especially cancers. Breast cancer ranked the fifth according to the number of cancer deaths in the world. To systematically characterize the epigenetic modification in breast cancer, we examined the genome-wide methylation profiling in 40 breast cancer cell lines. We identified a gene signature consisting of 345 differentially methylated genes, which could be used to discriminate estrogen receptor (ER)-negative and ER-positive breast cancer cell lines. This gene signature is promising for diagnosis and therapies of breast cancer. In the follow up functional analysis of this gene signature, three enriched networks could be highlighted. Interestingly, one of these networks contained estrogen receptor, implying its functional importance of ER-centric module. Finally, we examined the correlation between methylation and expression of these breast cancer cell lines. Very few genes showed significant correlation, suggesting that gene expression regulated by methylation is a complex biological process.

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Epigenetic Regulation of Osteoblast Differentiation and Genetic Variation. *T. Karaoli[1], A. Lira[2], W. Chen[2], V. Gilsanz[3], C.R. Farber[2], J.J. Connelly[1].* 1) Cardiovascular Research Center, University of Virginia, Charlottesville, VA; 2) Center for Public Health Genomics, University of Virginia School of Medicine, Charlottesville, VA; 3) Children's Hospital, Los Angeles, CA.

Osteoporosis results from an imbalance between bone resorption by osteoclasts and bone formation by osteoblasts. It is a major cause of morbidity in aged individuals and as such becomes important to molecularly define. Epigenetically regulated genes involved in bone formation could be used as drug targets for use in the modulation of osteoporosis. To identify such genes, we integrated gene co-expression network and epigenetic analyses. By applying Weighted Gene Co-expression Network Analyses to DNA microarray data generated from bone in 96 inbred mouse strains, we identified COL15A1 as a member of a co-expression network that plays an important role in osteoblast function. To epigenetically define COL15A1 in the process of bone development, we examined COL15A1 mRNA levels in differentiating primary mouse osteoblasts in vitro. We found that COL15A1 was significantly upregulated by day 8 of osteogenic differentiation (OD). Next, we inhibited DNA methylation in proliferating osteoblasts and found that loss of DNA methylation leads to an increase in COL15A1 mRNA levels. These levels are similar to the levels at day 8 during OD suggesting that the change in COL15A1 gene expression during OD may be solely controlled by loss of DNA methylation. Work is ongoing to assess COL15A1's role in OD and the epigenetic regulation that is governing the expression of this gene. In addition to beginning to define the function and regulation of COL15A1, we hypothesized that if the regulation of this gene leads to changes in bone phenotype then polymorphisms associated with bone density measurements in humans should exist. To determine if genetic variation in COL15A1 was associated with osteoporosis-related traits, we asked if SNPs spanning COL15A1 were associated with vertebral cancellous bone density (CBD) in 95 young Caucasian females. A total of three SNPs were associated with CBD at P<1.0 x 10-4. These variants spanned the COL15A1 promoter through intron 2. Studies are on going to determine if these polymorphisms affect the epigenetic or transcriptional regulation of COL15A1. The functional, epigenetic and genetic data we present suggest that COL15A1 may be important in bone formation and that a more rigorous validation of this gene in relation to osteoporosis is warranted. In addition, this study provides proof of principle for a genomewide study of epigenetically regulated loci involved in bone formation to identify manipulatable targets in bone disease.

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Quantification of the methylation at the GNAS locus identifies subtypes of sporadic pseudohypoparathyroidism type Ib. *S. MAUPETIT[1], C. BALLOT[1], V. MARIOT[1], C. REYNES[2], C. SILVE[1], A. LINLGART[1].* 1) INSERM U986, Hôpital Saint Vincent de Paul, 82 av Denfert Rochereau, 75014 PARIS, France; 2) INSERM U 973, MTi Université Paris Diderot, Rue Hélène Brion, 75013 Paris, PARIS, France.

BACKGROUND: Pseudohypoparathyroidism type Ib (PHP-Ib) is due to epigenetic changes at the imprinted GNAS locus including loss of methylation at the A/B differentially methylated region (DMR) and sometimes at the XL and AS DMRs and gain of methylation at the NESP DMR. Objective: To investigate if quantitative measurement of the methylation at the GNAS DMRs identifies subtypes of PHP-Ib. DESIGN AND METHODS: In 19 patients with PHP-Ib and 7 controls, methylation was characterized at the four GNAS DMRs through combined bisulfite restriction analysis and quantified through cytosine-specific real-time PCR in blood lymphocyte DNA. Results: A principal component analysis using the percent of methylation at 7 cytosines of the GNAS locus provided three clusters of subjects (controls n=7, autosomal dominant PHP-Ib with loss of methylation restricted to the A/B DMR n=3 and sporadic PHP-Ib with broad GNAS methylation changes n=16) that matched perfectly the combined bisulfite restriction analysis classification. Furthermore, we identified three sub-clusters of patients with sporadic PHP-Ib that displayed different patterns of methylation: incomplete changes at all DMRs compatible with somatic mosaicism (n=5), profound epigenetic changes at all DMRs (n=8), and unmodified methylation at XL in contrast with the other DMRs (n=3). Interestingly, PTH level at the time of diagnosis correlated with the percent of methylation at the A/B DMR. Conclusion: Quantitative assessment of the methylation in blood lymphocyte DNA is of clinical relevance, allows the diagnosis of PHP-Ib and suggests that, at least in some patients, the epigenetic defect is mosaic.

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Efficient whole-genome DNA methylation analysis of the Human Reference Genome (HuRef). *G. Meredith[1], M. Dudas[1], E. Levandowsky[2], T. Gilbert[2], D. Krissinger[1], G. Marnellos[1], V. Sheth[2], C. Adams[1], C. Lee[2], T. Harkins[3].* 1) R & D, Life Technologies Corp, Carlsbad, CA; 2) Beverly, MA; 3) Foster City, CA.

Aberrant DNA methylation is characteristic of many cancers and differences in methylation have been observed in a wide variety of genomic contexts; for example, both within "classic" promoter-associated CpG islands and also in distal, non-CpG island regions. Establishing a method to broadly and efficiently survey DNA methylation patterns genome-wide is the objective of the work presented. The method combines the power of methyl-CpG binding domain (MBD) proteins to sensitively and selectively bind methylated DNA sequences with the coverage, precision, and accuracy provided by high-throughput sequencing. Notably, MBD-affinity capture can also be used to sub-fractionate genomic DNA based on its average methyl-CpG content. To illustrate this method, Human Reference Genome (HuRef) DNA was enriched and salt-fractionated with a commercial MBD-based affinity reagent and high-throughput sequencing libraries, both bisulfite converted and unconverted, were prepared from each of the three fractions. The libraries were sequenced using error correcting codes and paired-end technology that yielded 75 bp read-lengths from one end and 30 bp read-lengths from the opposite end on a SOLiD 4 System. Peak analysis of the distribution of mapped unconverted reads permitted the discovery of thousands of locations of putative methylation in different genomic locus classes; the low salt fractions were depleted of CpG islands and enriched for exons while the highest salt fraction was enriched for CpG islands, exons, and promoter regions. Methylation at a large number of these positions was confirmed by bisulfite-sequencing of the same libraries. We conclude that such enrichment and fractionation, when coupled to high-throughput sequencing with or without bisulfite conversion, can be used to efficiently survey the majority of DNA methylation marks within samples of genomic DNA and to discover genomic loci of differential methylation. This method and reference dataset are intended to provide the community with a tool for large-scale methylation studies.

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DNA Methylation profiles distinguish Idiopathic Pulmonary Fibrosis from Normal Lung Tissue. *E.I. Rabinovich[1], Z. Yakhini[2], I. Steinfeld[2], K. Pandit[1], G. Yu[1], L.J. Chensny[1], N. Kaminski[1].* 1) PACCM, University of Pittsburgh, Pittsburgh, PA; 2) Technion-Israel Institute of Technology - Haifa/IL.

BACKGROUND: Idiopathic Pulmonary Fibrosis (IPF) is a pulmonary disease, characterized by extracellular matrix deposition, myofibroblast foci formation and alveolar epithelial cell hyperplasia, probably caused by interaction between susceptibility genes and diverse environmental factors. Little is known about the role of epigenetic changes in determining the lung phenotype in IPF. OBJECTIVES: To identify the global methylation patterns that characterize Idiopathic Pulmonary Fibrosis (IPF). METHODS: DNA was extracted from 12 IPF lungs,10 control lungs, and 10 lung adenocarcenomas using Qiagen DNeasy Blood & Tissue kit. Global methylation patterns were obtained using the Methylated DNA Immunoprecipitation (MeDIP) method and Agilent human CpG Islands Microarrays. Data analysis was performed using DNA Analytics 4.0.81 Software Validation was performed using EpiTYPER MassARRAY (Sequenom). RESULTS: Six hundred twenty five CpG islands were differentially methylated (false discovery rate<5%) between IPF and control lungs. EpiTYPER MassARRAY analysis validated a subset of these regions. The genes associated with the differentially methylated CpG islands are involved in apoptotis, Wnt, and ubiquitination pathways. Analysis of cancer samples revealed that IPF lungs represented an intermediate methylation profile between cancers and controls CONCLUSION: IPF lung exhibit significant changes in their methylation from controls. Interestingly, some of these changes are similar to those that are seen in Cancer.

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Evaluating DNA methylation and gene expression variability and identifying its underlying causes in the human term placenta. *W. Robinson[1], L. Avila[1], D. Diego-Álvarez[1], R. Yuen[1], M. Peñaherrera[1], D. McFadden[2], P. von Dadelszen[3].* 1) Dept Med Gen, Univ British Columbia, Vancouver, BC, Canada; 2) Dept Pathology, Univ British Columbia, Vancouver, BC, Canada; 3) Dept ObGyn, Univ British Columbia, Vancouver, BC, Canada.

Background: Abnormal placental growth and function may be influenced by genetic errors (e.g. trisomy) as well as epigenetic changes, such as those involving altered DNA methylation. However, before an association between DNA methylation and placental pathology can be investigated, normal intraplacental variation and the effects of sample location, local cell composition, gestational age, as well as mode of delivery and sample processing needs to be understood. Methods: To further our understanding of normal variability in DNA methylation, detailed sampling was obtained from 14 normal term placenta. Sites exhibiting variable methylation (KISS1, PTPN6, CASP8, APC, AR, and LINE1) were quantified by pyrosequencing. Expression of genes specific to a subset of placental cells (CDH1, CDH11, ID2, PLAC1 and KISS1) were evaluated by real-time PCR. Multiple sites from 5 placentae were also evaluated using the Illumina Beadarray technology for both expression and methylation. Results: Despite considerable intra-placental variability, significant inter-placental differences in gene expression and methylation were present. Most variability was not correlated with site location or depth. However, processing time (0-24 hours) had a dramatic effect on mRNA level for some genes including KISS1; In contrast, DNA methylation for the sites examined, including KISS1, remained relatively constant over this same time period. Observed correlations in expression and methylation from unrelated genes are suggestive that much methylation and expression variability largely reflects cell-composition differences between samples rather than true differences in gene expression at the cellular level. Sites that are differentially methylated in different placental cell types can be used to infer cell composition associated with abnormal placentation, while those methylation differences that are not influenced by cell type may be more informative for understanding the cellular/physiological changes between normal and abnormal placentae.

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Methylation of *HSPA4* in umbilical cord blood DNA from Children of Mothers with Mood Disorders. *J.W. Schroeder[1,2], V. Kilaru[2], K.N. Conneely[2], D.J. Newport[1], Z.N. Stowe[1], J.F. Cubells[2], A.K. Smith[1].* 1) Department of Psychiatry and Behavioral Sciences, Emory University, Atlanta, GA; 2) Department of Human Genetics, Emory University, Atlanta, GA.

Maternal illness can alter the intrauterine environment that constitutes the developmental context for the fetus. Mood (MD) and anxiety disorders (AD) in women are common during pregnancy and can result in long-lasting, stress-related outcomes in offspring. We hypothesize that DNA methylation may mediate some of these effects. In this study, we examined methylation patterns in DNA extracted from umbilical cord blood of 41 neonates whose mothers sought evaluation for psychiatric disorders during pregnancy, comparing the offspring of women with DSM-IV MD (N=28) and AD (N=8) as determined by SCID interview, to those with no Axis-1 diagnosis (ND, N= 5). Genomic DNA was interrogated across 27,578 CpG sites using the Illumina HumanMethylation27 BeadChip yielding a β value, which estimates the proportion of cytosine methylation at each CpG locus. For each CpG, we assessed the ability of the β value to predict maternal psychiatric diagnosis using a linear mixed model that included random effects to adjust for potential chip effects. The β values for each locus were adjusted with a conservative Bonferroni correction (α=1.81E-6) to minimize type I errors. We found several CpG loci that were nominally significant ($p<0.05$) in comparisons of MD vs. ND (N=1355; 5.98E-7<p<0.05) and AD vs. ND (N=1835; 2.65E-5£p<0.05). One locus, cg13778073, showed decreased methylation meeting criteria for experiment-wide significance (t=6.48; p=5.98E-7) in MD vs. ND samples; this locus resides near the transcriptional start site of *HSPA4*, which encodes heat shock protein A4 (HSPA4). In a *post hoc* examination, the methylation patterns in the offspring of women with major depressive disorder vs. ND (t=5.04; p=7.35E-5) and bipolar disorder vs. ND (t=4.47; p=2.91E-3) were consistent with the combined MD vs. ND test. HSPA4 is a chaperone protein involved in folding and regulation of the glucocorticoid receptor, which transduces signals mediated by cortisol, the major output hormone of the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis is often dysregulated in patients with psychiatric disorders. Our results suggest that maternal psychiatric diagnosis may alter maternal or fetal HPA axis function leading to detectable differences in methylation of genes related to cortisol function. Validation of the result is underway in addition to an extended evaluation of the methylation status of CpGs in the surrounding region in umbilical cord blood and placental tissue from these neonates.

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Differential DNA Methylation in Posttraumatic Stress Disorder and Related Phenotypes. *A.K. Smith[1], K.N. Conneely[2], V. Kilaru[1], K.B. Mercer[2], T.E. Weiss[1], B. Bradley-Davino[1], Y.L. Tang[2], C.F. Gillespie[2], J.F. Cubells[1,2], K.J. Ressler[2].* 1) Psychiatry & Behavioral Sci, Emory University SOM, Atlanta, GA; 2) Human Genetics, Emory University SOM, Atlanta, GA.

Human and animal studies suggest that DNA methylation mediates persistent changes in gene function following chronic stress, and a recent study reports that genes are differentially methylated in subjects with Posttraumatic Stress Disorder (PTSD). We evaluated 110 African American subjects matched by age and sex, and stratified into four roughly equal categories: with and without PTSD diagnosis (Clinician-Administered Posttraumatic Stress Disorder Scale), and with and without a history of childhood trauma (Childhood Trauma Questionnaire). Total Life Stress (TLS) was assessed in all subjects using the Stressful Events Questionnaire. We evaluated DNA from the peripheral blood of each subject using the HumanMethylation27 BeadChip and analyzed both global methylation (average β value across 27,578 CpGs) and methylation at individual CpG sites for association with PTSD, childhood trauma, and TLS using a linear mixed model that included random effects to adjust for potential chip effects. We noted an increase in global methylation in subjects with a current PTSD diagnosis compared to controls (P=.0035). Also, 2156 CpG sites were nominally associated with current PTSD (4.90E-6<P<.05, 7.8% of sites), 1446 CpG's with child abuse (2.17E-5<P<.05, 5.2%), and 5089 with TLS (1.16E-7<P<.05, 18.5%). CpG sites in 2 genes (*KRTHA5* and *NPFFR2*) associated with TLS met criteria for experiment-wide significance following Bonferroni adjustment (P<1.81E-6). KRTHA5 is activated in response to p65/RelA, and NPFFR2 is activated in response to inflammatory pain. Given these results and reports of immune system dysregulation in subjects with trauma history, we measured plasma levels of proinflammatory cytokines (IL6, IL2 and TNFa) using a multiplex ELISA and examined the association between these immune response markers with PTSD, child abuse, and TLS. While subjects with child abuse have higher TNFa (P=.038), we observed no other associations with child abuse or PTSD. However, IL6 (P=.019), IL2 (P=.039) and TNFa (P=.0044) concentrations increased with increasing TLS scores. Our results suggest repetitive psychosocial stress may alter global or gene-specific DNA methylation patterns potentially associated with peripheral immune dysregulation. Analyses are currently being performed to validate these observations and to evaluate further the role of DNA methylation in the development of stress-related disorders.

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Genetic variants associated with altered DNA methylation in schizophrenia. *K.R. van Eijk[1], M.P. Boks[2], F. Colas[3], E. Strengman[1], E. Janson[1], R.S. Kahn[2], S. Horvath[4], R.A. Ophoff[1,2,3,4].* 1) Medical genetics, University Medical Center Utrecht, The Netherlands; 2) Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, The Netherlands; 3) Center for Neurobehavioral Genetics, Neuropsychiatric Institute, University of California, Los Angeles, USA; 4) Department of Human Genetics and Biostatistics, University of California, Los Angeles, USA.

There is compelling evidence that epigenetic modifications play a role in schizophrenia susceptibility. Using a genome wide approach we investigated DNA methylation in whole blood and the extent to which differences between patients and controls are related to genotype. In a sample of 179 medicated patients and 172 controls, we obtained DNA methylation levels using Illumina HumanMethylation27 BeadChip and genotype data using Illumina 550k array. We used a linear model to analyze DNA methylation differences at 27,578 CpG sites. Differences smaller than detection sensitivity of the arrays were discarded. For the CpG sites associated with schizophrenia, we subsequently calculated association of DNA methylation levels with genotype (mQTLs) using a linear model with age as covariate and gender and disease status as factors. Analyses were performed in PLINK and R. After FDR correction for multiple testing we found 7,346 CpG sites that showed significant differences between cases and controls. Of these, 591 CpG sites showed a significant association with genotype. For 291 CpG sites the association to disease status was entirely due to genotype effect. Our results show the presence of vast differences in DNA methylation levels in whole blood of schizophrenia patients of which a small fraction is entirely regulated by genetic variants. The genes linked to these CpG sites are therefore prime candidate genes. We also identified 300 CpG sites associated with schizophrenia where genetic variation is associated with DNA methylation differences over and above the association with schizophrenia. Although further study is required to gain a better understanding of the biological relevance of these findings, these analyses can facilitate our understanding of diseases susceptibility.

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Bisphenol A Decreases the Methylation Status and Expression of Estrogen Receptor Alpha in C57BL/6 Mouse Embryo Brains. *J.T. Wolstenholme[1], K.H. Cox[1], E.F. Rissman[1], J.J. Connelly[2].* 1) Biochem & Molecular Genetics, University of Virginia, Charlottesville, VA; 2) Cardiovascular Medicine and Cardiovascular Research Center, University of Virginia School of Medicine, Charlottesville, VA.

Bisphenol A (BPA) is an environmental endocrine disruptive compound found in plastics; it accumulates in the blood of embryos, infants, children and adults. Exposure to such endocrine-active compounds alters normal hormonal function, affects neural organization and ultimately may modify behavior and lead to neurological disorders. Given that humans born after 1950 have been exposed to increasingly high levels of BPA from the time they were conceived, we have undertaken a behavioral and epigenetic study in the mouse to further characterize the affects of BPA exposure. BPA has two modes of action: as an endocrine-active compound and as a DNA hypomethylator. We hypothesize that in utero BPA exposure causes persistent, epigenetically trackable alterations in the brain and these changes influence social behaviors in mice and potentially in humans. Recently, we have shown that *in utero* exposure to BPA at human-relevant doses alters typical social interactions in C57BL/6 mice. Here, we report the ability of BPA to affect methylation and expression of estrogen receptor alpha (*Esr1*) in embryonic mouse brains. Female C57BL/6J mice were placed on phytoestrogen-reduced chow with or without 5mg/kg BPA, yielding equivalent blood levels as reported in humans. A week later, females were mated, checked for plugs and brains from gestating embryos were collected at embryonic day 18.5. To identify epigenetic mechanisms underlying these altered behaviors, the methylation status of several candidate genes with or without BPA exposure was determined. Methylated DNA was immunoprecipitated and subjected to quantitative PCR. We find that both a conserved promoter region shown to change with licking and grooming in the rat and a region within the CpG island in the second exon of *Esr1* is hypomethylated in BPA exposed embryos compared to controls. Interestingly, *Esr1* mRNA levels are decreased following BPA exposure but maintain sexually dimorphic expression. In stem cells, DNA methylation in the body of genes has recently been show to increase gene expression, suggesting that the methylation changes we see in the second exon of Esr1 may drive the change in gene expression. Studies are underway to identify genome-wide BPA-induced alterations in the DNA methylome in order to identify candidate epigenetically regulated genes that may play a role in human behavioral disorders. This work is supported by NIH grants R01MH08711 and Autism Speaks grant #4892 (EFR).

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OxLDL up-regulates miR-29b leading to epigenetic modifications of MMP-2/MMP-9 gene: a novel mechanism for cardiovascular disease. *K.C. Chen[1], C.Y. Hu[3], Y.S. Wang[1], W.C. Chang[2], S.H. Juo[1,2].* 1) Department of Medical Research, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; 2) Department of Medical Genetics, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; 3) Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan.

Background: MicroRNAs (miRNAs) are small non-coding RNAs and can control gene expression by binding to their target genes for degradation and/or translational repression. Epigenetic mechanisms are defined as heritable changes in gene expression that do not involve coding sequence modifications. Both miRNA expressions and epigenetic regulations play an important role in maintaining physiological functions and are also related to disease development. However, few studies report that miRNAs-mediated epigenetic regulations involve in atherosclerosis. **Purpose:** We investigated a miRNA-regulated epigenetic mechanism for MMP-2/MMP-9 genes using human aortic smooth muscle cell (HASMC) treated with oxidized low density lipoprotein (oxLDL). **Methods:** Primary HASMC were grown in medium 231 and co-cultured with 40 μg/ml oxLDL for 48 h. Cell migration was measured by wound healing and transwell migration assays. The expression levels of MMP-2, MMP-9, DNA methyltransferase (DNMT) 1, DNMT3a, DNMT3b and miR-29b were respectively estimated by either quantitative real-time-PCR (Applied Biosystems.) or western blot analysis. DNA methylation levels of MMPs were measured by methyl-specific PCR. 400 ng DNMT3b shRNA or 50 nM antagomiR-29 (Applied Biosystems.) was transfected into HASMC with lipofectamine 2000 (Invitrogen) to knockdown DNMT3b or miR-29b, respectively. **Results:** oxLDL significantly increased HASMC migration through MMP-2/MMP-9 up-regulation. DNA methylation levels of MMP-2/MMP-9 were also decreased in oxLDL-treated HASMC. Either mRNA or protein level of DNMT3b showed a dose-dependent down-regulation in oxLDL-mediated HASMC. Knockdown DNMT3b expression by DNMT3b shRNA enhanced oxLDL-induced DNA demethylation levels of MMP-2/MMP-9. The expression of miR-29b, directly targeting to DNMT3b, was up-regulation by oxLDL treatment in a dose-dependent manner. OxLDL-mediated MMP-2/MMP-9 up-regulation and DNMT3b down-regulation were both attenuated after knockdown miR-29b expression by antagomiR-29b. Knockdown miR-29b expression also abolished oxLDL-mediated DNA demethylation of MMP-2/MMP-9. **Conclusions:** We find that oxLDL can up-regulate miR-29b expression resulting in DNMT3b down-regulation in HASMC. oxLDL-mediated miR-29b expression epigenetically regulated MMP-2/MMP-9 genes involved in HASMC migration. These results show that miRNAs-mediated epigenetic regulations may be a novel mechanism in atherosclerosis.

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microRNA's role and genetics in alcoholism. *A. Pietrzykowski[1], O. Anees[1], Y. Wang[1], N. Boulghassoul-Pietrzykowska[1,2].* 1) Department of Animal Sciences, Rutgers University, New Brunswick, NJ; 2) Capital Health, Internal Medicine Residency Program, Trenton, NJ.

Alcoholism is a worldwide disease with a strong genetic component. However the exact genetic and molecular underpinnings of this debilitating disease are yet to be determined. We have recently discovered that exposure of neurons to alcohol leads to the development of tolerance to its effects by upregulating expression of one of microRNA species, called miR-9, in these cells. microRNAs belong to a family of short, non-coding RNAs and are essential regulators of gene expression. They don't encode proteins but regulate mRNA stability by RNA:RNA interactions. Typically, one microRNA can regulate expression of hundreds of targets. miR-9 is abundant in leukocytes regulating their key developmental stages and functions. Malfunction of leukocytes in alcoholism can contribute to immunosupression and cancer associated with alcohol abuse. We observed that in humans, two out of three miR-9 genes are located within loci of high susceptibility to alcoholism. Interestingly, a promoter of each miR-9 gene has a completely different structure suggesting differential expression of each miR-9 gene. Together, our findings describe new mechanisms of alcohol actions and could provide novel explanation of polygenic nature of alcoholism.

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Characterization of newly designed Sirtuin 1 FRET and fluorogenic substrates. *V. Rakhmanova, N. Nguyen, W. Cruz Jr, B. Sun, A. Hong.* AnaSpec, Inc., Fremont, CA.

Sirtuins comprise a unique class of nicotinamide adenine dinucleotide (NAD+)-dependent histone deacetylases (class III HDACs). Sirtuin 1 (SIRT1), the human homolog of yeast Sir2 (Silent Information Regulator 2), has been implicated in various cellular processes, including genomic stability, DNA repair, p53-mediated apoptosis and adipogenesis. It represents a treatment target of age-related diseases and type II diabetes. Recently, there has been much interest in characterizing small molecules that affect sirtuin activity. Resveratrol, an anti-aging compound found in red wine, has been reported to act as a SIRT1 activator, and this property has been proposed to account for its anti-aging effects. However it was determined that activation of sirtuins by resveratrol was observed mostly for an AMC (7-amino-4-methylcoumarin) coupled fluorogenic substrate generating blue fluorophore. To facilitate sirtuin drug discovery, we developed two new substrates, fluorogenic Green SIRT1 and 520 FRET SIRT1 substrates. The former substrate, derived from p53 sequence, releases a green fluorophore after incubation with SIRT1-containing samples and developer. This fluorophore can be detected with excitation/emission= 490/520 nm. The peptide for the FRET substrate was derived from the human FOXO3 sequence surrounding the deacetylation site of SIRT1. This peptide is coupled to a quencher, QXL™ 520, and a fluorophore, 5-carboxyfluorescein (5-FAM). Upon reaction of the FRET substrate with sirtuin and developer, an increase of fluorescence can be monitored at excitation/emission=490/520 nm. Both substrates are highly sensitive and detect activity at nanogram level of SIRT1. The substrates were validated for use in inhibitor screening assay with a previously described SIRT1 inhibitor. The long wavelength fluorescence of the green dyes employed in these two substrates is less interfered by the autofluorescence of cell components and test compounds. Resveratrol tested in the 520 FRET SIRT1 based assay showed no effects on enzyme activity. Interestingly, when the fluorogenic Green SIRT1 substrate was used in the assay, we were able to detect an increase of SIRT1 activity by resveratrol only at low concentrations of NAD+ and not at high concentrations. The assay with AMC based commercial substrate however, showed increase of sirtuin activity after incubation with resveratrol at a wide range of NAD+ concentrations.

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γH2AX in pluripotent stem cells: more than a DNA damage response signal? *C. Giachino[1], Y. Sanchez-Ripoll[2], V. Minieri[1], J. Foster[2], B. Kumpfmueller[2], V. Turinetto[1], P. Porcedda[1], L. Orlando[1,2], M. Welham[2].* 1) Department of Clinical and Biological Sciences, University of Turin, Orbassano, Italy; 2) Department of Pharmacy and Pharmacology, University of Bath, Bath,UK.

Histone H2AX phosphorylation (γH2AX) is the earliest indicator of DNA double strand break (DSB) and the main signal that triggers DSB repair. Recently, it has been shown that mouse embryonic stem cells (mESCs) have very high basal levels of γH2AX, even when they have not been exposed to genotoxic agents. We have confirmed this finding and have also observed high basal levels of γH2AX in mouse induced pluripotent stem cells and mouse blastocyst inner cell masses. In contrast, hESCs exhibited low basal levels of γH2AX, despite high sensitivity to genotoxic agents and a rapid repair response. To investigate whether γH2AX is linked to DNA repair signalling or plays an alternative role in pluripotent mouse cells, levels of γH2AX were examined following (i) treatment with genotoxic agents; (ii) mESC differentiation, where a decrease correlated with differentiation or (iii) inhibition of de-acetylation, methylation or key signalling pathways. The results of these analyses will be presented and potential roles discussed.

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X-chromosome wide DNA methylation analysis of human males and females in multiple tissues. *A. Cotton[1,2], J. Affleck[1,2], I. Wilson[3,4], M. Peñaherrera[1,5], D. McFadden[5,6], M. Kobor[1,5], W. Lam[3,4], W. Robinson[1,5], C. Brown[1,2].* 1) Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada; 2) Molecular Epigenetics Group, Life Sciences Institute, Vancouver, BC, Canada; 3) British Columbia Cancer Research Centre, Vancouver, BC, Canada; 4) Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada; 5) Child & Family Research Institute, Vancouver, BC, Canada; 6) Department of Pathology, University of British Columbia, Vancouver, BC, Canada.

DNA methylation is one epigenetic change that occurs during X-chromosome inactivation and distinguishes the inactive X (Xi) from the active X (Xa) in 46, XX females. It is well established that CpG island promoters on the Xi are hypermethylated compared to the Xa. Using the Illumina Infinium array we examined 1081 probes in 597 promoters on the X in human male (n=19) and female (n=24) tissues (n=5). Little variability in methylation was observed between muscle, spinal cord, brain and kidney, while placenta showed hypomethylation consistent with previous reports. 65% of the probes are associated with CpG islands, of which over 10% are unmethylated in non-placental tissue in both males and females. As the majority of genes known to escape inactivation are unmethylated, the 16 novel genes that fail to show promoter methylation are candidates to escape X-chromosome inactivation. Furthermore, 12% of island probes showed a tissue-specific methylation pattern, suggesting that for some genes escape from inactivation may vary between tissues. Methylation at CpG island promoters is thought to be an important epigenetic lock in maintaining gene silencing; however, further roles for methylation are not well-characterized. To examine more than promoters we used MeDIP on male and female blood followed by hybridization to a NimbleGen 2.1M array containing the X. We confirmed an X-chromosome-wide decrease in hypomethylated CpG island promoters in female versus male blood. Both male and female non-genic and intronic regions show moderate to high methylation whereas exonic regions show a bimodal pattern of methylation with the majority being highly methylated. Non-promoter high density islands are similarly hypomethylated in both males and females, whereas non-promoter intermediate density islands are moderately methylated in both sexes. Elucidation of the X methylation pattern in chromosomally normal tissues allows us to compare how chromosomally abnormal tissues are methylated. We are analyzing deviations from the normal XaXi status of 46, XX females that occur in triploids and sex-chromosome aneuploids. Contrasting methylation patterns between the Xa and the Xi highlights regions where differences in methylation may in turn have an effect on gene silencing. Determining the regions where methylation correlates with gene silencing is not only of importance in understanding X-chromosome inactivation but may also inform studies of other epigenetic regulatory pathways.

1397/T

Informed consent for biobank research: a mixed-methods study in cancer patients. *J. Mancini[1, 2], I. Pellegrini[2], C. Chabannon[3], F. Viret[3], N. Vey[3], C. Julian-Reynier[2].* 1) LERTIM, Aix-Marseille Université, Marseille, France; 2) Inserm, UMR 912, Marseille; 3) Institut Paoli-Calmettes, Marseille.

Biobanks have become strategic resources for biomedical research. Cancer patients' information and consent are key issues but might be suboptimal. We investigated the quality of patients' informed consent when they are solicited to grant access to their own biological specimen for research projects. A mixed-methods design was used to study the experiences of cancer patients who had a tumour or blood sample cryopreserved in a French Regional Cancer Centre (Paoli-Calmettes Institute). Nineteen patients (aged 28-82) treated for colorectal cancer or leukaemia were in-depth interviewed using grounded theory methodology and 574 patients (aged 20-89, response rate=77.0%) treated for colorectal, breast cancer or a haematological malignancy answered to a self-administered questionnaire on average 16.5 months after their cancer diagnosis. In the quantitative survey, 213 patients (37.1%) declared they had given consent. The others mainly did not, or couldn't remember, they had received the consent form; only two patients (0.9%) refused explicitly to consent. There was a poor concordance between declared and actual consent (Kappa=0.23) traducing a poor recall of the consent process. The majority of patients (60.8%) stated that a signed consent should be mandatory for biobank research, 90.9% judged satisfactory to participate in this decision, and only 6.6% would let the physician deciding alone in this context. Only a third (37.0%) of patients that had received the consent form, understood that the consent included an authorisation to access to medical files. Qualitative survey pointed out feelings of personal valorisation for the patients through their participation to the research and highlighted different profiles of participating to the research. Some patients recalled precisely practical details and their answers while others could not even remember if they had been solicited. In order to maintain an opt-in procedure valuable for patients, quality of consent and communication should be improved.

1398/T

Cardiogenetics: Developing a Model for Successful Implementation of Personalized Genetic Medicine. *D.A. McDermott, L.L. Cohen, S.M. Dolan, R.W. Marion.* Montefiore-Einstein Center for CardioGenetics, Departments of Pediatrics, Divisions of Cardiology and Genetics; Medicine, Division of Cardiology; and Obstetrics and Gynecology and Women's Health, Division of Reproductive Genetics, Albert Einstein College.

Recent advances in molecular medicine and pharmacogenomics have led to the belief that the era of personalized medicine (PM) is upon us. Although the ability to identify asymptomatic individuals' genetic susceptibility to disease is possible, new developments in cardiogenetics raise additional challenges not only with the capability of identifying mutations causing lethal arrhythmias and tailoring evidence-based medical management, but also resolving ethical, legal, and social issues (ELSI) that arise in the clinical setting. The Montefiore-Einstein Center for Cardiogenetics (MECCG) represents the leading edge of this PM revolution. Located in the Bronx, NY, the MECCG is a multidisciplinary clinic composed of medical genetics and genetic counseling, pediatric and adult cardiology, psychology and social work, and pathology that focuses primarily on individuals and families with a history of, or risk for, sudden cardiac death. Genetic testing of numerous individuals evaluated by the clinic has identified mutations in genes known to be causative for cardiac channelopathies such as the Long QT syndromes (LQTS) and Brugada syndrome. The information gleaned from genetic testing, in conjunction with personal and family history is used to guide personalized recommendations for these individuals as it pertains to medical management and preventive lifestyle factors. We will present clinical vignettes that illustrate some of the ELSI issues that will be equally pertinent in other PM clinical settings. We are currently planning focus group meetings for individuals followed at the Center, to better evaluate the translation of complex genetic information, appropriate timing of such information, and modes of family communication. Information from these focus groups will be critical in allowing further personalization of recommendations for current and future patients, and will serve as a model for other such multidisciplinary programs. Preliminary thematic findings from these focus groups will hopefully improve the infrastructure for PM-guided health care.

1399/T

Copy Number Variation at Genomic Regions Containing Short Tandem Repeats Used in Forensic Genetic Identity Analysis. *R.E. Pyatt[1,2], A.K. Bailes[1], D. Ell[1], L. Erdman[1], A. McKinney[1], C. Weber[1], D. Lamb-Thrush[1,3], C. Astbury[1,2], S. Reshmi[1,2], J.M. Gastier-Foster[1,2,3].* 1) Dept. of Laboratory Medicine, Nationwide Children's Hospital, Columbus, OH; 2) Dept. of Pathology, Ohio State University, Columbus, OH; 3) Dept. of Pediatrics, Ohio State University, Columbus, OH.

Copy number variation (CNV) typically refers to genomic segments that are 1 kilobase in size or larger and can vary from the normal, two copy state. Approximately 5-12% of the human genome is believed to exhibit CNV in normal individuals. Microarray-comparative genomic hybridization (array-CGH) is now routinely used clinically for the assessment of copy number changes and chromosomal imbalances for constitutional disorders. Over the last five years in our laboratory, routine array-CGH analysis using custom BAC and oligonucleotide platforms has identified 7 cases of CNV at genomic regions containing short tandem repeat (STR) loci used in genetic identity analysis. The examination of CNV at these loci was limited to alterations of 1 kb in size or smaller. These STRs are the core loci for CODIS (COmbined DNA Index System), the United States national database of convicted offender profiles. This set of STRs was originally selected based on their high levels of heterogeneity, minimal PCR amplification artifacts, lack of association to pathogenic alterations, and rare microvariant alleles which can complicate profile interpretation. We identified CNV including deletions of regions containing D13S317 and vWA, and duplications of regions containing TH01 and Amelogenin. Both deletions (1) and duplications (2) of the region containing TPOX were also noted. Duplication CNV ranged in size from 175 kb to 995 kb and deletion CNV from 247 kb to 505 kb. A review of The Database of Genomic Variants revealed that duplication CNV containing TPOX and deletion CNV containing D21S11 have been previously reported. In instances where individuals are heterozygous for STRs within duplicated CNV, amplification of these regions could result in peak height imbalances for the heterozygous allelic profiles. As empirical interpretation of DNA mixtures in forensic casework is based on heterozygous STRs demonstrating similar allele peak heights from a single individual, allelic imbalances due to duplication CNV could incorrectly suggest a mixture rather than a single DNA source. These results show that regions containing STRs used in genetic identity analysis appear to be susceptible to variation in copy number which can have direct and important implications in mixture interpretation as a part of forensic casework. Consequently, the Database of Genomic Variants may currently under-represent CNV at these loci.

1400/T

Genetic Burden in a Rural Population of Andhra Pradesh, India. *A. Jyothy, K. Venkata Karunakar, B. Pullareddy, M. Srinivas, M. Sujatha.* Cell and Molecular Biology, Institute of Genetics and Hospital for Genetic Diseases, Osmania University, Hyderabad,Andhra Pradesh, India.

Genetic disorders are the most burdensome of all human afflictions. Every year, thousands of families are affected by the birth of an abnormal child. About 0.5% of new borns have a chromosomal abnormality with moderate to severe phenotypic effects. Another 0.5-1% suffer the consequences of single gene defects (dominant, recessive or X-linked) and about 2% have a malformation that may be due in part to heritable factors. Altogether about 4% of new borns have a serious defect that is recognized at birth or within their first year. Facilities to detect genetic disorders are available for urban population but not in rural areas where majority of Indian people live. Genetic screening is the systematic search of populations for persons with latent, early, or a symptomatic disease. It is highly useful for medical intervention and research, for reproductive information, for enumeration, monitoring and surveillance, and for registries of genetic disease and disability. The present study was carried out to identify genetic disorders in a rural population and provide management and counseling. A total of 14,929 households including a total population of 73,551 individuals were screened by door to door survey in Mahboob Nagar district of Andhra Pradesh, India. Reproductive performance of the women showed that the percentage of still births was high followed by abortions and premature births. The prevalence of genetic disorders was assessed and Genetic counseling was offered to all these cases for proper management and prevention of these disorders.

1401/T

Considerations in consenting participants to whole-genome sequencing research protocols. *J.C. Sapp, F.M. Facio, L.G. Biesecker.* National Human Genome Research Institute, NIH, Bethesda, MD.

Novel sequencing technologies call for new approaches to informed consent. Controversy exists regarding the return of variant results from large-scale sequencing protocols, and a major challenge is that there is a paucity of data regarding how to inform research participants about the possible availability of variant data in a way that is both meaningful and appropriate. We describe here our protocol for informed consent for two large-scale sequencing studies at our Institute. Our approach to the consent discussion and process in each protocol is distinct and dependent upon the aims of the study and the target cohort. The ClinSeq™ study has enrolled over 800 participants and a primary research aim is to investigate the return of different categories of genetic variant results to adult participants, the majority of whom are healthy. In contrast, our rare disease sequencing protocol aims to elucidate the molecular etiology of rare phenotypes in a largely pediatric population (likely to be cognitively impaired) by sequencing affected probands and both parents. As participants in both protocols may choose to learn individual genotype results, informed consent of participants in both studies centers on this issue. Our approaches converge with respect to the variety of possible results, limitations of sequencing technology and challenges in interpretation, options for withdrawal given that some data will be deposited in publically-available databases, and override of requests not to receive results in instances where results are of medical urgency. The studies' distinct scientific aims and participant characteristics mandate some differences in approach as well. Because the rare disease protocol ascertains families, sequencing data are often generated for minors and cognitively-impaired participants and a greater emphasis is placed on the implications that results could have on family relationships; this is not a focus of the ClinSeq™ study. As well, the consent discussion differs with respect to the threshold used for the return of results, which is much broader in the ClinSeq™ study. Informed consent of participants to these two studies has proved challenging, complex, and nuanced. We conclude that approaches to the return of results and informed consent of participants in protocols involving whole-genome/exome sequencing should include careful consideration of specific research aims and participant characteristics.

1402/T

New technology, new challenges: Barriers to informed decision-making for first trimester aneuploidy screening. *R.M. Farrell, P.K. Agatisa.* Bioethics and OB/GYN, Cleveland Clinic , Cleveland , OH.

Purpose: First trimester aneuploidy screening was developed to identify fetal risk for aneuploidy several weeks earlier than conventional second trimester modalities. This study investigated key aspects of the decision-making process for this new form of prenatal genetic screening for the purpose of identifying barriers to informed decision-making and improving informed consent. Methods: Pregnant patients (N=93) were recruited from outpatient OB/GYN clinics in Northeastern Ohio. Participants completed a self-administered, multiple-choice questionnaire assessing knowledge and decision-making factors for first trimester aneuploidy screening, demographics, gestational age, and reproductive history. Data analysis was performed using R 2.9.1. Results: Participants demonstrated a low baseline understanding of the key concepts associated with first trimester aneuploidy screening. Specific knowledge gaps pertained to Down syndrome with 40% of participants unfamiliar with intellectual disabilities and 64% unfamiliar with health issues associated with Down syndrome. Participants were unfamiliar with the indications for first trimester aneuploidy screening, with 42% unable to correctly identify current recommendations for universal screening of all pregnant women regardless of maternal age and 62.3% unfamiliar or uncertain about testing procedures. Only 31.9% were able to correctly identify personal risk from the screening test result in contrast to 60.7% who were incorrect or uncertain about interpretation of an abnormal test result. Participants demonstrated knowledge gaps about follow-up testing for an abnormal result, with only 35.1% aware of the role of second trimester sequential or integrated protocols. In terms of immediate follow-up diagnostic testing, 67.8% were familiar with chorionic villus sampling but only 29.4% demonstrated understanding of its procedure-related risks. Conclusions: While first trimester aneuploidy screening provides fetal genetic information at an earlier time in the pregnancy, it also introduces novel challenges for informed decision-making. Our data demonstrate significant challenges for the process of patient education and informed consent for this new test. These findings have important implications for prenatal genetic counseling, particularly in light of the rapid pace of clinic translation of new prenatal genetic testing options.

1403/T

Genomic Risk Profiling: Physician attitudes and use in personal and clinical care. *S.B. Haga[1], M.M. Carrig[2], J.M. O'Daniel[1], L.A Orlando[3], L.A. Killeya-Jones[1], A. Cho[1,3].* 1) Inst Genome Sci & Policy, Duke Univ, Durham, NC; 2) Department of Psychology and Neuroscience, Duke University; 3) Department of Medicine, Duke University.

Genomic risk profiling involves the analysis of single nucleotide polymorphisms (SNPs) linked to a range of diseases and traits through statistical associations. There is considerable controversy as to how, and even whether, to incorporate these tests into routine medical care. Since little is known about their clinical impact, physician attitudes, or physician uptake, we surveyed a national group of primary care clinicians who are currently offering testing as part of their practice. The group, MDVIP, established a partnership with Navigenics, Inc in 2008 to provide testing at no cost to network physicians and a discounted cost to their patients, if physicians completed four educational modules. One third of respondents ordered a test for themselves and 42% for a patient. The odds of having ordered personal testing were 10.51X higher for those who felt well-informed about genomic risk testing ($p<0.0001$, 95% CI [3.79-29.15]). Of those who did not order a test for themselves, 53% were concerned about life and long-term/disability insurance discrimination and 49% about health insurance discrimination. Respondents were almost evenly divided about their intentions to order testing for their patients in the next 6 months (34% did vs. 39% did not). Ordering a test for their patients was 2.82X higher among respondents who had ordered testing for themselves ($p=0.028$, 95% CI[1.12-7.12]). The odds of ordering testing in the near future for patients were 2.9X lower for internists than family medicine practitioners ($p=0.040$). Of those who had ordered testing for patients, the following concerns were associated with a lower relative frequency of intention to order testing in the future: insurance coverage ($p=0.014$), uncertain clinical utility ($p=0.034$), unfamiliarity with testing ($p=0.016$), and concern about ability to communicate genomic risks ($p=0.040$). Among respondents who had never ordered a genomic risk test for a patient, there was a significant association between the uncertain clinical utility of testing when considering testing personally and for a patient ($p<0.0001$). This is the first study to assess initial uptake, interest, and concerns of genomic risk testing among primary care physicians. Our findings demonstrate that, with educational and interpretive support, primary care physicians are willing to include this type of information in a patient's risk assessment, even as concerns about uncertain clinical utility remain.

1404/T

Connecting geneticists with the public: changing our perspectives. *J.M. O'Daniel[1, 2], S.B. Haga[1], M.K. Rosanbalm[3], G.M. Tindall[1], L. Boles[4], T.M. Livingston[4].* 1) Duke Institute for Genome Sciences & Policy, Duke University, Durham, NC; 2) Illumina, Inc, San Diego, CA; 3) Center for Child and Family Policy, Social Sciences Research Institute, Duke University, Durham, NC; 4) Museum of Life & Science, Durham, NC.

Given the rapid pace of genetic and genomic research and technology development, it is increasingly important for the public to gain an understanding of genetic concepts and associated ethical and policy issues so as to enable informed deliberation of how it may affect their lives. Likewise, it is essential for genetic researchers to be aware of public perceptions and potential concerns about their work. Researchers, however, may be hesitant to take on the part of engaging the public due to their own perceptions of public knowledge and attitudes as well as concern for their ability to communicate with diverse, lay audiences about the importance and relevance of their work. To address these issues, we developed and piloted the Genome Diner discussion activity as a means to facilitate the engagement of genetic/genomic researchers with members of the local community. For the two year pilot, the community groups were composed of middle school students and their parents. In total 40 researchers, 76 students and 83 parents/guardians participated in one Genome Diner session. Program impact was assessed via pre/post surveys for each participant group. The most significant changes were demonstrated by researchers' responses. Researchers' assessment of public adult understanding of genetics concepts and whether the public believed research affected them increased significantly following participation ($p < .001$, $p < .01$ respectively). Positive changes were also measured in regards to researchers' perceptions about how the public viewed both genetic research and researchers including whether researchers liked interacting with subjects ($p < .01$) and whether participating in research was safe ($p < .01$). Post-Diner, researchers were also more convinced of the impact of researcher-community interaction on the conduct and focus of their research ($p < .01$). Lastly, researchers noted that the program increased their knowledge of strategies for communicating science ($p<.001$) and building relationships with diverse peoples ($p<.01$). In conclusion, through an interactive discussion with students and parents, researchers gained valuable insight of public perspectives about genome science research and technology. By building trust and essential relationships between the two groups, the engagement format of the Genome Diner program presents a novel method to better inform both the public and the researchers whose work may depend on their opinions and participation.

1405/T

Five clusters were assumed in the structure of the public attitude toward the genome research by the latent class analysis in Japan. *Z. Yamagata[1], T. Maedea[2], K. Muto[3], A. Tamakoshi[4], A. Nagai[1], I. Ishiyama[5].* 1) Dept of Health Sciences, Univ Yamanashi, chuo, Japan; 2) The Institute of Statistical Mathematics, Tokyo, Japan; 3) The Institute of Medical Science, The University of Tokyo, Tokyo; 4) Aichi Medical University, Aichi, Japan; 5) Teikyo-Gakuen Junior College, Yamanashi, Japan.

[Aim] The aim of this study was to clarify the structure of the public attitude toward the genome research by the latent class analysis. [Methods] The nationwide surveys about the attitude toward the genome research were conducted in 2005, 2008 and 2009 in Japan. The participants were comprised of 4,000 people (age, 20-69), selected from the Japanese general population by using the two-step stratified random sampling method. [Results] Five clusters were assumed as an explanation model of six variables related to the knowledge of genome and attitudes toward genomic research promotion about three themes; basic genome research, genome research related to agriculture and medicine at the survey in 2005. They were able to be named “Group of aggressive promotion” (40.8%), “Group of passive support” (20.2%), “Group not making judgment” (18.5), “Group making prudent judgment” (16.5%), and “Group not interested in genome”. The results in 2008 and 2009 were almost the same as that in 2005. It is possible to forecast to which cluster to belong according to respondent’s attribute, and we can forecast the reaction to other questions by using a cluster oppositely. For examples, “Group of aggressive promotion” is the layer of a high academic background, and is positive to donate their blood for the genome research, “Group making prudent judgment” is high academic background persons as same as “Group of aggressive promotion”, and is interesting in the science and technology, but is negative to the blood donation for the research.

1406/T

Psychological and Behavioral Impacts of Direct-to-Consumer Personalized Genomic Risk Testing. *C.S. Bloss[1], E. Silver[2], L. Ornowski[1,3], M. Cargill[2], V. Vanier[2], N.J. Schork[1,4], E.J. Topol[1,3,4].* 1) Scripps Genomic Medicine, Scripps Translational Science Institute, and Scripps Health, La Jolla, CA; 2) Navigenics, Foster City, CA; 3) Scripps Clinic, La Jolla, CA; 4) Dept of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA.

Direct-to-Consumer (DTC) personalized genomic tests are controversial for many reasons, one being a lack of information regarding the impact of this type of testing on consumers. We examined the psychological and behavioral impacts of genomic testing with the Navigenics Health Compass (23 conditions assessed), a currently marketed genomic test, in a sample of convenience recruited between October 2008 and September 2009. A cohort of 3639 individuals was recruited from health and technology companies. Participants purchased the Health Compass at a discounted rate. We measured dietary fat, exercise behavior, and anxiety symptoms at baseline and an average of 5 months post-testing (follow-up), as well as test-related distress and intention to complete health screening behaviors with greater frequency at follow-up. A total of 2059 individuals completed follow-up. Primary analyses showed no significant differences in covariate-adjusted measures of fat intake ($p = .90$), exercise behavior ($p = .70$), or anxiety symptoms ($p = .77$) between baseline and follow-up. The number of health screening behaviors participants intended to complete with greater frequency post-testing was significantly increased from zero (Mean = 2, $p < .001$). Secondary analyses incorporating genetic risk estimates revealed that intention to complete screening behaviors with increased frequency was positively correlated with average estimated lifetime risk (ELTR) across the conditions assessed ($r_s = .048$, $p = .030$). A similarly positive correlation was also observed for test-related distress ($r_s = .18$, $p < .001$), however, over 90% of the sample had scores indicating no distress. DTC genomic testing did not result in significant short-term psychological risks, and data suggest that testing may be associated with increased compliance with recommended health screening practices. In the sample as a whole, no significant improvements in diet or exercise behaviors were observed.

1407/T

Reprogenetics and Cross Border Reproductive Care: What’s So Unique With Reprogenetics? *V. Couture, C. Bouffard.* Division of Genetics, Department of Pediatrics, Université de Sherbrooke, Sherbrooke, Quebec, Canada.

Introduction: It is only in recent years that procreative tourism, also known as crossborder reproductive care, has attracted the interest of the scientific community because of the depth of the ethical, legal and social issues it raises. Despite this sudden concern, little research has been done on the particular situation of reprogrenetics. Moreover there is still confusion regarding the many ethical, legal and social issues associated with this crossborder reprogenetics. A large part of the work published does not always distinguish between procreative tourism to overcome infertility and the one based on the diagnosis of genetic or hereditary diseases and chromosomal abnormalities. Based on the literature on reproductive tourism, we developed an analytical frame of the ethical, legal and social issues that are specific to reprogenetics tourism. Methods: Qualitative analysis of the literature on reproductive tourism (n=151) from the major database provider in clinical sciences and social sciences (OVID, Journals@Ovid Full Text; EBSCO, 14 databases including MEDLINE, ERIC and CINAHL).Results: Four levels of analysis have emerged from the literature. The first level has allowed a mapping of reprogenetic tourism integrating: a) the diversity of legal frameworks between different states b) the location of centers of excellence and c) the users’ itineraries. The second level involves the mobility of practitioners and b) and of biological material. Third, the scarcity of reprogenetic services compared to those dedicated to fertility. This scarcity is based on: a) its high cost and b) the level of expertise required for its implementation. The fourth level reflects the interaction and the unique relationship established between the user and the caregiving personnel. It integrates: a) the linguistic and symbolic issues of cross-cultural counseling, b) the responsibility and the accountability of practitioners in monitoring and in referencing) and c) the psychological stress specific to users of reprogenetic services. Conclusion: Although there are many interrelationships between the medical, reproductive and reprogenetic tourisms, each has a configuration of its own. Reprogenetics has always been the object of major ethical and moral considerations. A better understanding of the actual state of the practice is a “fundamental condition” to the development of norms and guidelines that are adapted to the social changes and are equitable and people-centered.

1408/T

Enrolling Children with Mitochondrial Disease in a Research Biobank: The Challenge of Meaningful Assent. *L. Eisenberg[1], A. VanDenBoom[2], W. Highsmith[1, 2], D. Oglesbee[1, 2].* 1) Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN; 2) Medical Genetics, Mayo Clinic, Rochester, MN.

Title 45 of the US Code of Federal Regulations, §46.408 states that every IRB overseeing research with minors “shall determine that adequate provisions are made for soliciting the assent of children.” Assent is defined as a child’s affirmative agreement to participate in research, which is a complement to traditional informed consent granted by the child’s guardian. Federal regulations grant IRBs leeway in defining what constitutes “adequate provisions,” as the statute only states that age, maturity, and psychological state should be assessed when reviewing a child’s capability of assenting. It is unclear if merely soliciting assent is sufficient, or if assent must be obtained in order to proceed with enrollment. As part of the development of a biobank studying mitochondrial diseases, we examined the practice of obtaining assent through an analysis of previously published literature. We then formulated contemporary guidelines for obtaining assent, and utilized our strategy during biobank recruitment over the last six months. Our analysis showed that there are few common practices regarding assent. Minimum ages for assent ranged from seven to fifteen years old, depending upon the researcher’s conclusion about the age at which a child is able to appreciate the risks and benefits of research participation. There was also varying credence given to the impact of a child’s cognitive delays or symptoms limiting cognitive function on the assent process. The relatively rarity of mitochondrial disease makes it necessary for us to obtain consent for our biobank through the mail, a complexity largely neglected in assent research. Furthermore, few studies deemed a child’s refusal to assent as grounds to exclude the child from participation. Some minors with mitochondrial disease will never develop the capacity to give meaningful assent. Participation is unlikely to directly benefit the enrolled child, and this leads to reluctance to utilize children as research subjects. However, without research on children, it is difficult to advance understanding of diseases that are terminal in childhood. Therefore, it is important to standardize guidelines for assent and enhance the potential of research studies to directly benefit children. Thus far, none of our assented pediatric participants have refused to participate. Our experience suggests that by actively seeking assent, it is possible to meet high ethical standards while not significantly decreasing participation.

1409/T

Use of GWAS markers from HapMap 3 can be used to create a SNP-based individual information panel with high information content and very little population bias, which could provide an improvement over the current STR-based CODIS 13 forensic DNA panel. *J. Fagerness[1, 2, 3], R. Siburian[1, 2, 3], S. Purcell[1, 2, 3], B.M. Neale[3], C. O'Dushlaine[3], A. Kirby[3], M.A. Rivas[3], J.W. Smoller[1, 2, 3], M.J. Daly[3], D.L. Pauls[1, 2, 3].* 1) Dept Psychiatry, Massachusetts Gen Hosp, Boston, MA; 2) Psychiatric and Neurodevelopmental Genetics Unit, Massachusetts Gen Hosp, Boston, MA; 3) Center for Human Genetic Research, Massachusetts Gen Hosp, Boston, MA.

The FBI was given formal authority to establish a National DNA Index System (NDIS) in 1994 and has been using a 13 short tandem repeat (STR) marker panel as a way to forensically test individual identity for the purposes of law enforcement. In 2005 the DNA Fingerprint Act was passed and the Combined DNA Index System (CODIS) database has quickly grown to over seven million profiles. We wanted to assess the information content across a number of populations in order to determine how well the 13 CODIS markers uniquely identify any given individual from any given population. Using popSTR (http://spsmart.cesga.es/popstr.php?dataSet=strs_local) we found that the information content in the 13 CODIS markers can vary by more than 10-fold from one population to the next. Given the dramatic improvements in genotyping technology, we identified 85 autosomal SNPs that are maximally and equally informative (>40% minor allele frequency (MAF)) in all 11 populations of HapMap3 and are represented on all 22 chromosomes at greater than 10Mb apart from one another, leaving negligible linkage disequilibrium (LD) between any SNP-pair. Further, these 85 SNPs confer a random match probability (RMP) of greater than one in $1x10^{25}$ for any given individual to any other individual. This is significantly better than the 13 CODIS marker's RMP of between one in $1x10^{9}$ to $1x10^{10}$. In order to verify that our 85 SNP panel was useful in more than just the 11 populations represented in HapMap 3 we used data from the Human Genetic Diversity Panel (HGDP) and popSTR. These two datasets contain similar populations allowing us to examine the information content of the 13 CODIS markers and compare them to the 44 of 85 SNPs that are present in the HGDP SNP dataset. The 44 SNPs vary in information content less than 1.5-fold and have an RMP of one in $1x10^{13}$. The power of equal information content becomes apparent when looking at the combinatorix algorithm for "cold-hit" cases. For example, not all of the CODIS markers contain equal information and certain populations have very little information in some of the 13 CODIS markers. Therefore, hundreds to thousands of "positive" matches can seemingly occur on any 9 out of the 13 CODIS markers, with certain populations having orders of magnitude greater chance for seeing these matches. Using this new technology and a genome-wide approach to human genetic variation should allow for stronger statistical proof of guilt or innocence in the justice system.

1410/T

Conducting high-throughput genomic research in the setting of a healthcare cooperative: recommendations from a consensus development panel. *S.M. Fullerton[1], S.B. Trinidad[1], D.C. Grossman[2], G.P. Jarvik[3], E.B. Larson[2], W. Burke[1,2,3].* 1) Dept Bioethics & Humanities, Univ Washington Sch Med, Seattle, WA; 2) Group Health Research Institute, Group Health Cooperative, Seattle, WA; 3) Medical Genetics, Univ Washington Sch Med, Seattle, WA.

As part of the electronic MEdical Record and GEnomics (eMERGE) Network, the Group Health Research Institute hosted a seven-month long consensus development process aimed at discussing genome-wide association studies (GWAS) and related whole-genome research and the ethical, legal, and social implications of pursuing such studies with Group Health members. Consensus development approaches are used for many purposes, including practice guideline development and healthcare priority-setting, to ensure the incorporation of different stakeholder views. Structured deliberative procedures can elicit and combine different types of evidence; promote accountability and transparency; and produce guidance that is acceptable to both experts and the public. Our consensus development panel, which was convened to develop research policy recommendations for Group Health leaders, comprised 13 members chosen to represent key stakeholders, including general Group Health members, researchers, healthcare providers, and legal and administrative representatives. The panel met in person seven times to deliberate on issues it identified as key priorities, including informed consent, return of research findings to participants, and sharing research data with researchers outside of Group Health. Informed by the results of several local empirical investigations and Group Health's commitment to trustworthy research practice, the panel arrived at 11 key recommendations, outlining the ways in which research risks should be described to prospective participants, the advisability of re-consenting subjects for research uses materially outside the scope of original consents, the need to offer aggregate - and where appropriate, individual - research findings to participants, and advocating for the transparent description of anticipated data sharing as part of the informed consent process. The panel also urged the Institute to avoid recourse to genetic exceptionalism in research review, while acknowledging public perceptions that genetic research can be more risky than other forms of health research. These recommendations formed the basis of a final report presented to the Group Health Cooperative Board of Trustees in December 2009.

1411/T

Deriving Individual Genotypic Barcodes from Gene Expression Data. *K. Hao[1], E.E. Schadt[1,2].* 1) Research Genetics, Rosetta Inpharmatics, Seattle, WA; 2) Department of Medical Genetics, University of Washington, Seattle, WA.

Backgrounds. mRNA profiling study simultaneously captures the expression pattern of many genes. The expression levels are often under genetic control, characterized as expressional quantitative trait loci (eQTL). A growing amount of mRNA expression data has been deposited into public databases, which may expose the identity of participants. Methods. Employing published eQTLs as the prior, we developed a Bayesian approach to predict genotype based on mRNA expression data. The predicted genotype on many eSNPs forms an inferred genotype vector for each individual, which can reveal individual's identity. We evaluated this novel approach on three large human cohorts. Results. We find strong eQTLs are highly consistent among studies on the same tissue and across different tissues (e.g. liver and adipose). Such high consistency allows us to use published eQTLs and predict eSNP genotypes of an independent mRNA expression dataset. On empirical data, our method was shown to offer very high accuracy. In within-tissue scenario (eQTLs and testing data are on the same tissue type), we resolved 98% testing subjects' identity at p£1E-5 confidence level. In cross tissue scenario, we resolved 92% subjects' identify at p£1E-5. Conclusions. Individual's identity can be accurately resolved using mRNA profiling data. We discuss a number of implications. For example, this novel method provides quality control against DNA-RNA pair mis-annotation in eQTL studies. More importantly, deriving genotypic information from mRNA expression datasets of public domain may unmask participant's identity.

1412/T

Ethical implications emerging from research into the genetic and genomic aspects of autism spectrum disorders: A qualitative study of parental opinions in Japan. *J. Higashijima[1], K. Takahashi[2], M. Oi[1], K. Kato[2].* 1) Kanazawa University, Kanazawa City, Ishikawa, Japan; 2) Kyoto University, Kyoto City, Kyoto, Japan.

The tragic history of eugenic social policy is a dreadful example of past social reactions to genetic research into the human mind and behavior. This history demonstrates the necessity of considering the social and ethical implications of each research project in the research field. Today, genetic and genomic research related to autism spectrum disorders (ASD) has grown to become one of most prominent research areas concerning the human mind and behavior. As such, consideration of the social and ethical implications of this research is essential for both the scientific community and society at large (Journal of Genetic Counseling, 15, 41-50; American Journal of Medical Genetics, Part C, 142C, 52-57). As part of this process, an examination of the voices of related parties in each cultural context is essential. The aim of the current research is to clarify and examine Japanese parental attitudes towards, and knowledge regarding, genetic and genomic ASD research. The data was collected through semi-structured interviews with parents of at least one child diagnosed with ASD. Approval for this study was obtained from the Medical Ethics Committee of Kyoto University, Japan. The core questions were divided into four parts: (i) Demographics; (ii) Parental knowledge of gene mutation; (iii) Parental knowledge of ASD research; and (iv) Parental attitudes toward ASD research, focusing especially on genetics. The 50 interviews conducted for this study revealed several controversial issues for researchers, medical personnel, and genetic counselors to discuss. In general, our results showed that Japanese parents had little scientifically valid knowledge of genetic and genomic ASD research, even though they expressed serious interests and concerns about the matter. Parents were highly ambivalent about super early diagnosis and prediagnosis, as a result of the close relationship between these subjects and abortion, parental mental health, the Japanese social support system, and social attitudes to ASD. Clarification of the existence of any genetic differences between people with and without ASD was also regarded as controversial. In this presentation, we will discuss the issues mentioned above in detail.

1413/T

Individual Return of Research Results (IRRS) and Incidental Findings (IFs) in Stem Cell Research: Exploring the Ethical & Legal Implications. *R. Isasi, B.M. Knoppers.* Centre of Genomics & Policy, McGill University, Montreal, PQ, Canada.

Recognizing that incidental findings (IFs) are intrinsic to the general research endeavor, an emerging debate centers on whether providing feedback to research participants (IRRS) is an ethical duty of researchers. Even if the definitional issues are solved, the development of what would be the best mechanisms to manage IRRS and the roles and responsibilities of all stakeholders e.g. (researchers, participants, licensing/oversight bodies, ethics review committees, biobanks, etc) is still in its infancy. The emergence of an ethical duty to provide IRRS not only argues for a re-evaluation of such roles and responsibilities, but also for the reappraisal of the traditional informed consent process, which usually maintains that no individual results will be returned. In the specific context of stem cell research, the scientific and ELSI implications concerning IIRS and the discovery and management of IFs are rarely addressed. Notable exceptions are recommendations adopted by the EGE of the European Commission and guidelines adopted by the US National Academies of Science. In the abovementioned policies, the potential discovery and management of clinically significant information are considered essential elements of the informed consent process. Furthermore, these policies call on stem cell banks to adopt protocols governing the delivery IRRS back to donors. Given the peculiarities of stem cell research, both the general research and clinical contexts could serve only as ethicalguidance concerning the establishment of appropriate mechanisms addressing the IRRS and IFs. A prospective and nuanced analysis must address questions such as: Do stem cell researchers have a duty to return research results to research participants ? Do different sources of stem cell lines warrant a different policy approach? Who, when, how and to whom should research results and IFs be communicated? Finally, will policies pertaining to IFs strength or weaken donors' rights (autonomy, privacy and confidentiality, etc.)? This presentation will provide a prospective analysis of the scientific, socio-ethical and legal issues arising from the disclosure of IRRS and the identification of IFs in the context of stem cell research (e.g. embryonic and iPs cell lines). Drawing from examples arising from the general research context, we will further explore policy approaches and mechanisms so as to address the identification, management and disclosure of IRRS and IFs.

1414/T

The eMERGE Consortium: An NIH-supported model for integrating bioethics into leading-edge genomic research. *B. Koenig[1], S. Fullerton[2], A. Lemke[3], C. McCarty[4], E. Wright Clayton[5].* 1) Mayo Clinic 200 First St. SW Rochester MN, 55906; 2) University of Washington 1959 NE Pacific Avenue Seattle, WA 98195; 3) Northwestern University 676 N. St. Clair Street, Suite 1260 Chicago, IL 60611; 4) Marshfield Clinic Research Foundation 1000 North Oak Avenue - MLR Marshfield, WI 54449; 5) Vanderbilt University 2525 West End Ave., Suite 400 Nashville, TN 37203.

Genomic research technologies are evolving rapidly, generating unprecedented ethical challenges. Ethical, legal, and social issues (ELSI) include: requirements for sharing of genotype and phenotype data collected by NIH-funded researchers; recognition that new models of biobank governance are required when data are shared widely; increasing evidence that research participants expect to receive research results; and the increasing integration of sensitive electronic medical record (EMR) information into genomic research. Furthermore, simple "de-identification" of data is an inadequate strategy to protect human subjects and may not ensure trust in the research enterprise. The eMERGE network is a "proof-of-principle" consortium created by NIH to develop, disseminate, and apply innovative approaches to research that links DNA biorepositories with phenotypes obtained directly from the EMR. While funding several genome-wide association studies and associated bioinformatics research, the eMERGE network simultaneously funded bioethics researchers at 5 institutions (Group Health/UW, Marshfield, Mayo, Northwestern, Vanderbilt). Empirical bioethics research and analysis is fully integrated into ongoing genomic research. The Consent and Community Consultation (CCC) workgroup is tasked with identifying challenging ethical issues and developing guidance for researchers. The CCC is divided into sub-groups targeting the following areas: the identifiability of DNA and EMR data, return of results, IRB oversight, engagement with communities, data sharing, and informed consent. The consortium's goal is to produce recommendations and best practices for researchers and policy makers not only within eMERGE, but also for the research community at large. Outcomes of empirical bioethics research, comparisons of diverse approaches to community engagement, and development of model informed consent language have directly informed eMERGE research protocols, oversight, and the development of new research infrastructure. The eMERGE network represents a novel approach to conducting bioethics research in the context of a large multi-center research project. Through the eMERGE experience, we illustrate a novel approach to bioethics research, demonstrating how empirical studies inform normative deliberation, which in turn informs the creation of recommendations and best practices developed in direct collaboration with colleagues in bioinformatics, basic science, and clinical genomics.

1415/T

The General Public's Understanding and Perception of Direct-to-Consumer Genetic Test Results. *J.W. Leighton[1], K. Valverde[1], B. Bernhardt[2].* 1) Arcadia University Genetic Counseling Program, Glenside, PA; 2) Penn Center for the Integration of Genetic Healthcare Technologies, Division of Medical Genetics, Hospital of the University of Pennsylvania, Philadelphia, PA.

Direct-to-consumer (DTC) genetic testing allows consumers to discover their risk for developing common complex disorders without involving a medical professional. Consumers may not understand test results, leading to negative consequences including unnecessary concern, false reassurance, or unwarranted changes in screening behaviors. To investigate consumers' perceptions and understanding of DTC test results, an online survey was posted on facebook.com that included questions relating to 4 sample test results for risk of developing colorectal cancer, heart disease, and skin cancer. Genetic counselors (GCs) were used as a comparison group and completed the same survey through the NSGC listserv. 145 individuals from the general public (GP) and 171 GCs completed the survey. A significant difference ($p<.05$) was found between the GP and GCs understanding of genetic test results in 3 of 4 scenarios. When results were presented in terms of relative risks, GP respondents were significantly more likely to misinterpret risks than were GCs. When presented with a scenario providing results indicating a slight reduction in absolute risk, GP respondents were significantly more likely to believe their risk was "much lower." With respect to medical management, the GP thought results in all four scenarios would be significantly more helpful than did GCs. Although the majority of GP respondents rated the results either very easy or easy to understand, they often were unable to correctly interpret the results. For example, in the scenario involving a relative risk of 1.45 for developing colon cancer, 71 of the GP respondents felt that results were easy to understand, but only 42 (59%) of them correctly interpreted the results. These findings imply that the GP has the potential to misinterpret DTC results without appropriate assistance. Further research is needed exploring optimal methods of providing DTC test results and ways to minimize the risk of negative consequences for consumers.

1416/T

Developing Global Genetics. *K. Leppig[1], M. Laurino[2], D. Sternen[3], J. Thompson[3], G. Snudden[4].* 1) Gen Services, Group Hlth Cooperative, Seattle, WA; 2) Public Health Genetic Department, University of Washington, Seattle, WA; 3) Genetic Medicine, Seattle Children's Hospital, Seattle, WA; 4) BlueGnome Limited, Cambridge, UK.

The development of global genetics in emerging countries has been addressed in recent ASHG and ACMG annual meetings. While health care in emerging countries has initially focused on areas of nutrition and infectious disease, there has been an increased recognition that genetic services can improve healthcare for both individuals and a population. A number of clinical geneticist and genetic counselors who have worked in emerging countries have been approached for assistance in developing clinical care and education programs in emerging countries. While individual efforts are essential, the development of a global genetics requires a coordinated effort between medical professionals in emerging and developed countries. Ongoing projects that serve as examples include a partnership to develop clinical genetics programs at Hue College of Medicine and Pharmacy, Vietnam and the development of the first genetic counseling program at University of Manila, the Philippines. Challenges identified thus far include difficulties working with diverse cultural, language and social differences. With limited trained clinical geneticist and genetic counselors, there is a need to develop robust training programs within the emerging countries partnering with established genetic programs. The advances in technology are essential for developing cost-effective genetic testing for genomic and metabolic disorders. The successful development of global genetics will depend on the coordination of training genetic professionals,application of technologic advances in genetic testing, the development of the necessary infrastructure, and a commitment from those in the medical genetics community.

1417/T

Attitudes and beliefs of Hispanics in South Florida regarding participation in genomic research. *R.J. Martinez, M.J. Gavier, S.E. Hahn, J.L. Robinson, B.T. Lemelman, M.A. Pericak-Vance, M.L. Cuccaro.* John P Hussman Inst Human Genomics, Univ Miami, Miami, FL.

The success of genomic research relies on participation from diverse populations. Representation of minorities in genomic studies is paramount to ensure potential ethnic differences are considered in disease patterns. Hispanic populations are underrepresented in genomic research. The urgency to include Hispanic populations in genomic research has led to development of targeted, culturally sensitive, community-based enrollment strategies. Nonetheless, limited data exist on the attitudes, beliefs, and perceptions of the Hispanic community concerning participation in genomic research. The goal of this research was to identify barriers to participation in genomic research among Hispanics using a focus group methodology. Focus group participants were recruited from South Florida in Miami-Dade County, which has a large rapidly growing Hispanic population (62% Hispanics, U.S. Census Bureau). Focus groups were organized by ethnicity (Colombia, Cuba, Dominican Republic, Honduras, Peru, and Puerto Rico). The groups ranged in size from 7-10 participants (average 9 per group). Participants were 38% male and 62% female. Average age of participants was 46 years(sd=13.5). All focus group sessions were conducted and analyzed in Spanish by multicultural facilitators using a standard format. The primary qualitative data were derived from analysis of common ideas, categories, and themes (e.g., access to health/ genetic studies information, knowledge/ understanding of genomic research, perceptions/attitudes regarding genomic research, cultural barriers/motivators to participation in genomic research, and effective communication strategies). These data were compared across the different Hispanic groups and will be used to develop specific recruitment strategies for each group. Our initial results revealed several prominent concerns such as access to trusted sources of medical/ genetic information for their respective communities and the perception that genomic research was not beneficial to their community. We will present passages for each of the themes by groups. Focus groups are valuable tools for exploring the attitudes and beliefs of community members. To increase participation of Hispanic groups in genomics studies it is crucial that we use focus groups to identify specific barriers. The knowledge generated from such focus group research can help investigators tailor recruitment procedures and improve participation rates among Hispanic individuals.

1418/T

Attitudes toward genetic testing in Japan: National surveys in 2005, 2008 and 2009. *K. Muto[1], A. Nagai[2], A. Tamakoshi[3], T. Maeda[4], I. Ishiyama[5], Z. Yamagata[1].* 1) Dept Pub Policy, IMS, Univ Tokyo, Tokyo, Japan; 2) Dept. of Health Sciences, School of Medicine, Univ. of Yamanashi, Chuo, Japan,; 3) Aichi Medical University, Aichi, Japan; 4) The Institute of Statistical Mathematics, Tokyo, Japan; 5) Teikyo-Gakuen Junior College, Yamanashi, Japan.

[Aim] The aim of this study is to explore attitudes toward genome research applied to medicine among the general population of Japan, focusing on willingness to undergo genetic tests for disease susceptibility and pharmacogenetic tests. [Methods] Postal questionnaire surveys were conducted in 2005, 2008 and 2009 in random samples of the general public in Japan. The questionnaire included items on genetic knowledge, attitudes toward the application of genomic studies to medicine and crop science, basic genomics research, and technology. We analyzed the data with a focus on the attitudes toward the application of genomic studies to medicine. [Results] There were 2171 participants in 2005 (response rate, 54%), 1613 in 2008 (response rate, 54%) and 2005 in 2009 (response rete, 50.1%). In both surveys, most respondents had positive attitudes toward these genetic tests. The logistic regression analysis showed that significant predictors of the willingness to undergo both genetic tests were to perceive more benefit and having positive impression and interest in the research. Considering that the use of genetic testing is likely to increase in the near future, we propose that more information about genome and communication between scientists and the public should be provided in Japan.

1419/T

The HumGen Spanish Portal, a tool for Public Health Genomics in Latin America: Improving, legal and policy frameworks, based on knowledge transfer networks. *P.F. Oliva - Sanchez[1,2,3], J. Zaga - Galante[2], R. Isasi[3], A. Carnevale[1], B. Knoppers[3].* 1) ELSI Dept, National Institute of Genomic Medicine, México City, Distrito Federal, Mexico; 2) School of Health Sciences, Dept. Medicine, Anahuac University, Mexico; 3) HumGen International Project,Centre of Genomics and Policy at McGill University (CGP), Canada.

Public health genomics (GSP) is a multidisciplinary field that concerns responsible and effective translation of knowledge and risk-based genomic technologies. Through the genomic variation in populations and gene - environment interactions, it designs and develops applications for improving health and prevention, based on genomic knowledge. One of the challenges in translational genomic medicine, in addition to its analytical and clinical validation, are the ethical, legal and social issues (ELSI) surrounding this genomic applications. In response, since January 2008, the National Institute of Genomic Medicine in collaboration with the Centre of Genomics and Policy at McGill University (CGP) in Canada, have been successfully developing the Spanish HumGen Portal. This is a researchable database, where normative texts related to human genetics ELSI issues are compiled, combining documents with national, regional and international influence. The selection of these laws is done through a process of rigorous qualitative research. The portal is aimed at the general public, but with an emphasis on decision-makers with the capacity to formulate policies on matters related ELSI genetics and human genomics. The developing countries are facing a problem, the applied genomics research to public health have a significant advance, but the legal information is not developed in tandem with scientific advances. HumGen is a valuable tool transfer knowledge, accessible and free, strengthening the content of these strategies through the analysis of Comparative Law. In the portal there are 64 laws and policies, of which 54% are regional normative texts of the Latin American countries, 24% are national laws and policies and 22% are decrees of international organizations. The topics included in the international and regional documents are: ethical principles in human genetics research, data protection, genetic diversity and patents. National laws focus on cloning, human reproduction and stem cells. Latin American countries are not taking a future perspective, because they do not regulate and legislate the technology derived from research in human genetics. The promotion of this portal in legislative and professional forums through knowledge networks, is one of principal strategies that will help to the development of regulatory frameworks in the Spanish speaking countries appropriate to the current scientific progress.

1420/T

Patients' and researchers views of the etiology of addiction: the interplay of social and genetic factors. *J.E. Ostergreen[1], J.B. McCormick[1,2], M. Dingel[3], B. Partiridge[1], M.E. Robinson[1], B.A. Koenig[1,2].* 1) Health Sciences Research, Mayo Clinic and College of Medicine, Rochester, MN; 2) Medicine, Mayo Clinic and College of Medicine, Rochester, MN; 3) University of Minnesota-Rochester, Rochester, MN.

Research on the biology of addiction has implicated a number of neurotransmitter systems in both the reinforcement and dependence-inducing effects of various substances including nicotine and alcohol. One of the more widely studied is the dopamine system, which is thought to be involved in brain reward functions. Mounting evidence from neuroscience, bio-behavioral, and pharmacological studies suggests that genetic factors are involved in vulnerability to substance addictions including alcohol and nicotine. Evidence for the genetic basis of smoking was first suggested by family and twin studies, and more recently, genetic association studies examining the role of specific candidate gene variants. The science is moving quickly and producing exciting findings with potential clinical application. To ascertain how biological understanding of addiction may be influencing prevention and treatment measures, we did a NIH funded study aimed at examining the complex interplay of the social and scientific "causes" of addiction. We have interviewed patients undergoing treatment for either alcohol or nicotine dependence. Our informants expressed varied interpretations of the "causal" elements of their addiction. For some, addiction is all genetic; for others environmental factors are more important. A few expressed anxiety that an "addiction gene" would mean loss of free will and control; others saw hope that a genetic link would mean less stigma and more social acceptance of the "addiction disease." To complement these data we investigated researchers' concepts of addiction as a biological question and a public health problem. Analysis reveals that some scientists view addiction as a condition with both genetic and environmental components. They acknowledge the complexity of not just GxE interactions but also gene/gene interactions in determining the underlying biological basis of addiction. Some also noted that defining an addiction phenotype is a limitation in study design and analysis. Our researcher participants refer to addiction as a disease and a behavior, and view pharmacological and behavioral therapies as important options. Determining biological underpinnings of addiction uses a reductionistic approach and can influence how the research findings are translated from bench to clinic. Ascertaining what researchers' and patients' view as the etiology of addiction is critical to informing public health policies targeted at treatment and prevention.

1421/T

Privacy concerns regarding large prospective genetic research. *J. Scott, D. Kaufman, S. Devaney, J. Murphy Bollinger.* Gen & Pub Policy Ctr, Johns Hopkins Univ, Washington, DC.

Objective: Large prospective studies that use DNA annotated with medical, lifestyle and environmental data are important research tools. However, the breadth of information collected and the potential to widely share these data with researchers raises privacy concerns among potential participants. To explore public concerns about privacy and access when considering participating in such studies, we conducted 10 focus groups with 89 people across the US. **Methods**: Between October 2009 and January 2010, 8 focus groups were conducted in-person in DC, Philadelphia, and Denver and 2 groups were conducted online. Participants represented a range of demographics including race, gender, age, ethnicity, socioeconomic status, and social networking behavior. We described a potential US cohort study that would protect participants' information by (1) removing all personal identifiers and replacing them with a code; and (2) only sharing coded data with those researchers whose applications have been approved by the NIH. We asked participants about privacy issues and opinions on data access and sharing. Participants were also asked whether law enforcement should have access to the database to solve a crime or to identify victims of a mass tragedy. We asked participants their opinions about the implications of certificates of confidentiality (COCs) and GINA on privacy. **Results**: Most participants said that coding as described would provide adequate privacy protection, and most supported sharing data with qualified researchers. However, majorities in all focus groups did not want insurance companies or employers to have access to the research database for fear of discrimination. Almost all focus group participants vehemently opposed law enforcement accessing the database for fear it would deter people from participating. While a few individuals were willing to let law enforcement access the database in the event of a mass tragedy, most held firmly to their belief that law enforcement should not have access. Participants were unaware of the recent passage of GINA and most were not reassured by the protections it offered. There was unanimous support for researchers obtaining COCs. **Conclusions**: Privacy concerns are important to potential research participants. The concerns center on how the study information in the database could be used against them, especially by insurers and employers. Protections afforded under GINA did little to reassure participants.

1422/T

Analysis of Exome Sequence Data for Potentially Returnable Research Results. *H.K. Tabor, NHLBI's Exome Sequencing Program—and Lung Project and ELSI Project Teams.* Ctr Pediatric Bioethics, Seattle Children's Hosp, Dept of Pediatrics, University of Washington, Seattle, WA.

Exome sequencing is rapidly becoming an important tool for the identification of genes involved in rare Mendelian traits and perhaps common complex traits as well. As the popularity of this approach increases, researchers are facing new ethical challenges regarding the return of clinically important results to research participants. Because the coding regions of nearly all genes are targeted by exome sequencing, variants of *possible* clinical significance are likely to be found in nearly every participant. Whether such research findings should be returned to participants, how this is best done, and what might be the impact has been a topic of vigorous debate within genetics and bioethics. To date, this debate has been informed primarily by research findings in linkage or GWAS studies, where the identification of clinically significant findings is predicted to be a rare event. There are virtually no data about the range of possibly returnable findings that might be identified with exome sequencing. We analyzed exome data from 65 persons with cystic fibrosis (CF) sequenced as part of a study to identify genetic modifiers of CF. We cataloged known and novel variants in CFTR and selected genes involved in Mendelian disorders, risk for common diseases, and pharmacological traits. CFTR was included to compare exome results to those from previously obtained clinical testing. Exome sequencing identified several novel variants in CFTR that were not identified by clinical testing and missed one variant that had been found by clinical testing. In 70% of participants, we found variants of potential clinical importance in at least one gene other than CFTR. Twenty percent of participants had variants of potential clinical importance in two or more genes. These results suggest that exome sequencing will identify a potentially large number of clinically important and possibly returnable findings, including novel changes and changes of unknown significance. However, exome sequencing will not identify all pathogenic variants in coding regions. Accordingly, exome sequencing presents new and unique challenges for investigators who consider return of results. These findings have implications for the development of policies and procedures for return of results for experimental approaches utilizing either exome or whole genome sequencing. No decisions have been made yet about whether to return findings for this cohort.

1423/T

Attitudes on DNA Ancestry Tests. *J. Wagner.* Dept Anthropology, Penn State Univ, University Park, PA.

The DNA ancestry testing industry currently has 39 companies selling ~200 products and >500,000 customers. Yet the industry remains young and uncertain. No obvious industry-wide standards have been established regarding methods or communication of results. Where does one begin to evaluate the potential ethical, legal, and societal consequences of an industry that lacks a clearly defined product, clearly delineated market, or uniform commercial vision? The type and degree of the potential implications, however, must be dependent upon the attitudes on DNA ancestry tests held by those aware of the tests. Those attitudes are undoubtedly variable and changing, yet data on those attitudes are surprisingly scant. This anthropological study explored attitudes on DNA ancestry tests, including what motivates individuals to purchase the tests, how they value or interpret the scientific capabilities, and for what purposes they believe the results should be used. Questionnaires, designed and administered using Survey Monkey, were administered to general participants (N=176) recruited in State College, PA and to targeted participants (N=76) recruited through Facebook DNA ancestry-related groups. General results suggest individuals view the relationship between DNA ancestry and race in a more nuanced way than is frequently acknowledged. Test-takers are more knowledgeable about genetics generally. The main motivations for testing are education, entertainment, and curiosity. Results of this study do not support the oft-cited reasons for individuals avoiding DNA ancestry tests, namely fear of results, desire not to know, and privacy concerns. While individuals support use of DNA ancestry tests in research and support personal rights to access such information, individuals generally oppose most potential uses and are notably reluctant to permit such tests in litigation or law enforcement settings. Targeted results suggest individuals are motivated to get tested for genealogical purposes and are not motivated by legal purposes. Those who had not purchased tests avoided thembecause of cost and opportunity, not because of fear of results, preference not to know, and privacy concerns. These Facebook individuals were purchasing tests from multiple companies and reporting some changes in identity and behavior in light of the DNA ancestry testing, Results suggest the discussion about negative psychological effects of DNA ancestry testing may be overstated in the literature.

1424/T

Determining the effect of bacterial infection of human remains on the quality and quantity amplifying of aged bone DNA. *a. ghasemi[1,2], m. Tavallaei[3], m. Naderi[3], s. Habibi[3], m.m. Aslani[4], a.t hajizade Sharif[5], n. Mahdieh[2], m. Sarafi Farzad[2], m. Kargar[1], f. Kafilzadeh[1], a. Shirkavand[6,2], z. Zafari[7,2], s. Zeinali[8,2].* 1) 1Azad University of Jahrom, Fars, Iran; 2) 2Kawsar Human Genetics Research Center, Tehran, Iran; 3) 3Human Genetic Research Center, Baqyatallah Medical Sciences University, Tehran, Iran; 4) 4Microbiology Research Center, Pasteur Institute of Iran, Tehran, Iran; 5) 5Academic Center of Education, culture & Research, Yazd, Iran; 6) . 6Biology Group, Faculty of Sciences, Razi University, Kermanshah; 7) 7Khatam University, Tehran, Iran; 8) 8Biotechnology Research Center. 9 Pasteur Institute of Iran, Tehran, Iran.

One of the most important applications of DNA profiling is identification of human remains. These tests are applied for identification of human remains from natural disasters, wars, archaeological remains, in forensic laboratories. For this purpose, bone and teeth are the major source for genomic DNA. When a sample is aged, many problems may occur with the genomic DNA, such as DNA degradation and DNA contamination and presence of PCR inhibitors. These problems may lead to difficulties in performing PCR and act as inhibitory factors for PCR based techniques. To overcome these problems, we have checked the existence of bacterial DNA (as a contaminant) in DNA extracted from Iranian human remains from the 8 year Iran-Iraq war. We checked these DNA using ABI Identifier kit.We have tested probable effects of bacterial DNA on amplifying aged bone DNA .In spite of high volume of extracted DNA, we could not amplify aged bone DNA .We extracted DNAs from blood, aged bone (20-30 yrs ago) and bacteria. Artificial condition as a positive control for contaminated DNA was created by adding bacterial DNA to human blood DNA Using different aged bone and bacterial DNA dilutions along with PCR based methods; we tried to test their positive, negative or inhibitory effects on each other. Quantification of these effects is carrying out by real-time PCR. We also created artificial conditions as a positive control for contaminated DNA. For this, we simply added bacterial DNA to human bone DNA, In order to assess and check the inhibitory effects. Our experiments showed that bacterial DNA does not act as an inhibitory factor. Therefore, presence of bacterial DNA does not have any negative effect in amplifying DNA. Indeed it may act as a molecular marker to test human DNA for its ability to be amplified by PCR prior to using expensive kits.

1425/T

Role of DTC Relationship Testing Laboratories in Human Trafficking Prevention. *S.H. Katsanis.* Genome Ethics, Law & Policy, Institute for Genome Sciences & Policy, Duke University, Durham, NC.

Genetic identification technologies can deter human trafficking by confirming claims of biological relationship in immigration and adoption procedures. Over the last decade in Guatemala, reports of adoption fraud led the US Embassy to request DNA tests to confirm the relationship of a relinquished child and biological mother in adoption procedures. However, reports revealed that DNA testing also was conducted through fraudulent means, primarily through US-based direct-to-consumer (DTC) relationship testing (RT) laboratories. While the sources of fraud are under continuing investigation with Guatemalan authorities, the responsibility of the RT laboratories in maintaining chain-of-custody remains unclear. RT laboratories process over 400,000 cases per year including samples required for international adoptions and immigration. RT laboratories are subject only to professional oversight and guidelines. Of the 39 US laboratories offering RT, all are AABB certified, 11/39 are CLIA certified, and 14/39 are accredited for forensic testing. 32/39 companies market for immigration testing, 17/39 market testing for adoption, and 13/39 also offer ancestry testing. Authorities in Guatemala and other countries are working with US-based RT laboratories to develop cross-border DNA protocols to deter human trafficking. The lack of US federal oversight for RT laboratories warrants an evaluation of RT laboratory policies, particularly the retention of biological samples. Retaining a specimen makes it available for additional analysis using new technologies and for verification of a profile after a match. Yet, samples retained without legal authority have few privacy protections from the potential misuse or release of genetic material. Samples may be retained as source specimen (e.g., whole blood, saliva), stain cards, purified DNA, or amplified products. The policies for retaining samples collected by RT laboratories vary depending on 1) type of sample retained; 2) intended use of sample; 3) whether the sample was voluntary or required; 4) whether the laboratory is private or public; and 5) whether the laboratory follows CLIA or forensic oversight standards. Implementation of international DNA databases of missing persons to deter human trafficking could potentiate profound societal benefit. It is vital at this early stage to establish policies to guide laboratory operations and protect the individuals at risk of misuse of their samples and resulting profiles.

1426/T

A Survey of Attitudes Toward Behavioral Genetic Testing. *A. Wise[1], J. Bosson-Heenan[1], J. Gruen[1,2].* 1) Yale University, New Haven, CT; 2) JS Genetics, New Haven, CT.

Though many genetic studies today seek to identify genetic markers associated with behavioral conditions, there is a dearth of research regarding the public's attitude toward using these research results for genetic testing. Is there a demand for genetic testing for behavioral conditions? What are the publics' concerns with such testing? Does interest depend upon how genetic the public perceives the condition to be? Our research study aims to examine attitudes toward genetic testing for behavioral conditions, such as attention deficit hyperactivity disorder (ADHD), dyslexia (a reading learning disability), and addiction (alcoholism, nicotine dependence, or other drug addictions). Subjects were recruited off the street and at local fairs, markets, and clinics in New Haven, CT to obtain the most representative and unbiased population possible. Participation in the study involved completing a brief 16-question survey that required approximately 5 minutes of the subject's time. Preliminary results of the survey indicate that ~80% of respondents believe that genetic testing should be made available for ADHD, dyslexia, and addiction. When queried if they personally would consider genetic testing for themselves or their children, the positive response rate remained the same when the result of the test was personalized treatment/therapy or an individualized education plan, dropping to ~75% when the testing result was a risk of developing the condition. A further drop in approval was seen when asked if prenatal testing should be made available (~60% yes). In general, individuals' responses did not appear to depend on how genetic or environmental they felt the conditions to be (percent of positive responses were similar across conditions, while overall addiction was felt to be less genetic than ADHD or dyslexia). When asked about their general concerns, participants voiced fears about subjects such as insurance coverage, accuracy of the result, abortions, stigmatization of children, and benefit of the knowledge versus risk of the test. Potential benefits focused on individualized approaches, early detection and preparation, possible cures or prevention, and general knowledge gain.

1427/T

Prevalence of genetic disorders in the northwest of Iran. *T. Mizani[1], S. Dastgiri[1], M.J. Bonyadi[2].* 1) Department of Community Medicine, Tabriz University of Medical Sciences; 2) Department of Medical Genetics, Tabriz University of Medical Sciences.

Background and aim: genetic disorders are responsible for a major proportion of mortality, morbidity, and handicap across the world varying by racial, ethnicity and cultural differences. The aim of this study was to estimate the prevalence of genetic disorders in the northwest of Iran. Methods & Materials: in this descriptive/cross-sectional study, 2968 cases (53% males and 47% females) with confirmed genetic disorders or carriers of the traits diagnosed by molecular methods were identified between 2005 and 2009. Prevalence rate, descriptive statistics and 95 percent confidence intervals were used for data analysis. Results: the study subjects included patients (n=1312), carriers (n=1474), suspected cases (n=157) and unknown cases (n=25). The most prevalent (for five years) disorders were identified as Familial Mediterranean Fever (20.6/100000, CI95%: 19.1-22.07), Inherited Deafness (11.4/100000, CI95%: 10.6-12.2), Spinal-Muscular Atrophy (11.1/100000, CI95%: 10.1-12.1), Cystic Fibrosis (7.9/100000, CI95%:7.1-8.2), Duchenne Muscular Dystrophy (7.8/100000, CI95%:6.2-8.7) and Down Syndrome (5.1/100000, CI95%:4.5-5.4). The overall prevalence of genetic disorders was higher in males compared to females (M/F = 1.1). Conclusion: estimating the true prevalence of the genetic disorders may help in the planning of health care and screening programs. The prevalence of these diseases in the region indicate the necessity to establish a population-based center for genetic disorders in the area. More population-based investigations are however needed to develop effective preventive strategies to control genetic disorders in the region.

1428/T

The NINDS Human Genetic Resource Center: A Resource for the Discovery of Genetic Risk Factors for Neurological Disorders. *K. Gwinn[1], E.R. Londin[2], M. Self[2], A. Ansbach[2], K. Reeves[2], R. Zhang[1], M.A. Keller[2].* 1) National Institute for Neurological Disorders and Stroke, Bethesda, MD; 2) Coriell Institute for Medical Research, Camden, NJ.

Neurological disorders affect an estimated 50 million Americans each year. Though there are rare familial forms of neurodegenerative diseases where the causative gene has been identified, most individuals diagnosed with neurodegenerative disorders such as Parkinsonism and Amyotrophic Lateral Sclerosis (ALS) have no identifiable genetic cause of disease. In most cases, these diseases are multifactorial, involving the interaction of many genes with environmental factors. Genome-wide association studies (GWAS) have identified genetic loci associated with neurodegenerative disorders. These studies require large numbers of cases and controls that can be facilitated by collaborative efforts to collect and manage biological samples and corresponding data. The National Institute of Neurologic Diseases and Stroke (NINDS) Human Genetic Resource Center (NINDS Repository) is a public resource established to provide a centralized and open resource of biological samples (DNA and cell lines) and corresponding phenotypic data collected. Many NINDS-funded researchers involved in neurodegenerative disease research have integrated into their study protocols the submission of biologic samples and data to the Repository. Since its inception in 2002, more than 30,000 individuals have been banked, with more than 16,000 samples from individuals with motor neuron diseases (2,221), epilepsy (742), Parkinsonism (3,719), cerebrovascular diseases (5,092), Tourette syndrome (194), neurologically-normal controls (3,761) and unaffected primary blood relatives (918) have been made publicly available through the Coriell Cell Repositories' web catalog (ccr.coriell.org). To aid high-throughput gene discovery, the Repository offers disease-focused panels of genomic DNA samples in 96-well plate formats as well as custom plate design services. Additionally, genotype data from over 4,000 NINDS Repository samples are available on dbGaP. More than 20,000 samples have been distributed to researchers by the repository since inception. Analyses of NINDS Repository samples have been published in more than 200 peer-reviewed scientific articles. Furthermore, the Repository has begun to establish and bank fibroblast cell cultures from individuals with neurodegenerative disorders for purposes of cellular reprogramming to induced pluripotent stem cells. The NINDS Repository facilitates genetic discoveries aimed at unraveling the genetic and cellular mechanisms that lead to neurological disorders.

1429/T

Clinical and ethical perspectives of a case of false positive in a prenatal diagnosis (PND) of Turner syndrome (TS). *A. BROUSSIN DUCOS, K. KRABCHI, R. DROUIN, C. BOUFFARD.* Genetics Service, Department of Pediatrics, Faculty of Medicine and Health Sciences, University of Sherbrooke (Quebec, Canada).

Following a prenatal diagnosis of trisomy 21 indicating a high risk, a pregnant woman chose to submit to an amniocentesis which revealed a fetal TS. Following a meeting in genetics where the parents were informed of genotype and phenotype of TS, they decided to continue the pregnancy. Subsequent morphological ultrasound showed that the fetus was male, which led to the realization of a PCR analysis. This establishes the existence of the SRY gene and the presence of a segment of chromosome Y. In postnatal, an analysis by microarray confirmed the loss of the Yq of the proximal portion of Yp and translocation of the remaining segment to the distal extremity of the 11q. Methodology: Qualitative approach, case study (a couple), analysis of 22 articles relating to the PND of TS. Results: Clinically, this case shows the need for further genetic testing to establish with certainty a PND of TS. Ethically, it raises the importance of providing patients with comprehensive information and the urgency to develop additional knowledge as regards to the problems caused by certain clinical and social practices in relation to a PND of TS. As an example, the fact that a decision to perform an abortion can be taken on the basis of incomplete information and an inaccurate diagnosis raises serious ethical questions, reinforced by the number of abortions, reported in the literature in relations to PND of TS. That the number of abortions is also higher for TS than for Klinefelter syndrome generates other questions regarding parental representations of TS and, more broadly, the socio-cultural representations of female and / or male infertility. Conclusion: Nevertheless, in the specific context of the clinical case investigated, we should retain that the implementation of complementary genetic investigation and the assurance of a genetic counsel would decrease the ethical, clinical and socio cultural problems inherent to an inaccurate PND of TS.

1430/T

Bioethics and socioethics: An urgent need for probative data to guide and regulate medical and social uses of reprogenetics. *C. Bouffard.* Dept Ped, Fac Med Hlth Sci, Univ Sherbrooke, Sherbrooke, PQ, Canada.

OBJECTIVES: With reprogenetic practice no longer confined to the research setting or futuristic scenarios, its medical and social uses are multiplying without showing signs of slowing down. In a worldwide market economy that fosters reproductive tourism and direct-to-consumer genetic testing, the deployment of reprogenetic practice is raising unprecedented ethical, legal, professional-code, and social issues. The greatest challenge will arise from the fact that the people who offer and access reprogenetic services will soon be exercising more power over reprogenetic practice than the policy makers and experts whose mandate is to regulate it. To meet this challenge, it will first be necessary to re-examine the foundations of the decision-making processes employed by the policy makers who establish the ethical and legal norms that govern reprogenetic practice and set up the health policies that determine the conditions of service delivery. METHODS: Ethnographic study conducted in France and under way in Quebec and the rest of Canada: Analysis of scientific, legal, and ethical literature; participant observation in clinics and laboratories for preimplantation genetic diagnosis (PGD), a paradigmatic reprogenetic practice (990 hours); 79 semi-structured interviews. RESULTS: When PGD had not yet been transferred to the clinical setting, it was understandable that the decision-making regarding the development, governance, and institutionalization of PGD and reprogenetic practices in general should be based on the speculations of policy makers and experts. Today, appropriate inquiry must be based on: 1) probative data about national and international conditions for the development and delivery of reprogenetic services; 2) the motivations of these services' users and providers; and 3) the impact of State intervention in citizens' reproductive lives. CONCLUSION: Given the issues raised by reprogenetics, if we wish our health policies and our legal, professional-code, and ethical norms to have their hoped-for efficacy and usefulness, it is important to re-evaluate the modes of operation that underlie our decision making; the more so since political, medical, and community policy makers will have a foundational impact not just on the conditions for service delivery but also on the future of new medical paradigms based on the use of the human embryo.

1431/T

Signatures of natural selection in the first pilot experiment of the 1000 Genomes Project. *R.D. Hernandez[1], J.L. Kelley[2], S.C. Melton[2], A. Auton[3], G. McVean[3,4], G. Sella[5], M. Przeworski[2], 1000 Genomes Project.* 1) University of California, San Francisco, Department of Bioengineering and Therapeutic Sciences, San Francisco, CA, 94158; 2) University of Chicago, Department of Human Genetics, Chicago, IL, 60637; 3) University of Oxford, Wellcome Trust Centre for Human Genetics, Oxford, OX3 7BN, United Kingdom; 4) University of Oxford, Department of Statistics, Oxford, OX1 3TG, United Kingdom; 5) The Hebrew University, Jerusalem, Department of Evolution, Systematics and Ecology, Jerusalem, JR 91904, Israel.

Nearly 200 genomes from four populations have been resequenced at low coverage for the first pilot experiment of the 1000 Genomes Project. These data avoid many of the ascertainment biases that have plagued previous large-scale human data sets, allowing long standing evolutionary questions to be resolved. We used these data to characterize genomic signatures of natural selection using patterns of genetic diversity. We show that neutral diversity increases with genetic distance from coding regions, suggesting that selection on coding regions distorts patterns of diversity in their vicinity. In particular, we report a clear footprint of natural selection on diversity patterns around human-specific amino acid substitutions. In addition, we identified novel regions of the genome with extreme differences in allele frequencies between population samples. While all three findings reflect the action of natural selection, it remains unclear to what extent they are explained by adaptive evolution or purifying selection, with recent reports offering conflicting conclusions in this regard. To disentangle the relative contributions of the two evolutionary forces, we ran extensive simulations of the human genome, incorporating information about functional annotations, fine-scale genetic maps, and realistic demographic models of all four populations.

1432/T

The power of HLA for the discrimination of human populations. *W. Klitz[1], L. Gragert[2], M. Maiers[2].* 1) Pub Hlth, Univ California, Berkeley, CA; 2) National Marrow Donor Program, Minneapolis, MN.

PURPOSE: While only a fraction of human genetic variation is distributed between populations, of that variation the HLA complex appears to be the most powerful system in this regard for the discrimination of one human group from another. Here we utilized the high resolution HLA typing and exceptionally large and ethnically specified population sizes of the National Marrow Donor Program adult donor database to portray the genetic relationships among a number of Asian population groups. METHODS: We analyzed estimated HLA A-B-DRB1 haplotype frequencies in eight Asian-American populations, including (abbreviation and samples size in thousands), South Asia (SA, 211), Philippines (F, 57), Hawaii (H, 12), Vietnam (V, 27), other South East Asia (SEA, 29), China (C, 108), Korea (K, 97), and Japan (J, 30). RESULTS: The haplotype diversity in each population was very large: the ordered haplotype frequency list reached 50% of the sample ranging from 72 in the Filipino to 295 in the Other Southeast Asian group. Principal components analysis (PCA), neighbor joining (NJ) trees and a clustering package (cluto) were used to reveal relationships among the populations. PCA indicated that the South Asian and Other Southeast Asian groups fall at the center of the Asian populations. Three pairs of populations diverge in different directions, including a Pacific island group (F and H), an East Asia group (C and V) and a derived East Asian group (K and J). The NJ analysis found SA and SEA at the root with a single clade heading to all of the other populations. The cluto analyses revealed the HLA similarity of these same pairs, along with the specific most frequently associated haplotypes for each population. CONCLUSIONS: These results mirror the conclusion of a recent genome wide analysis of these same groups pointing to a founding population source for East Asian peoples from Southeast Asia. More generally this study points to the power of a single genetic system, the HLA complex, to define population differentiation in our species.

1433/T

DIVERGENOME: a bioinformatics platform to assist population genetics and genetic epidemiology studies. *W.C.S. Magalhaes[1], M.R Rodrigues[1], A. Sene[2], B. Araujo[2], M. Machado[1], A.A. Faria-Campos[2], G.B. Soares-Souza[1], M.L. Iannini[1], E. Tarazona-Santos[1].* 1) Departament of General Biology, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil; 2) Department of Computer Sciences, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.

The huge amount of data that is emerging from human genetics and genomics initiatives requires appropriate bioinformatics tools that are suitable also for research groups of different sizes. Such tools should allow the storage of public and private genotypic and phenotypic data together with efficient and secure management, perform database integration, and facilitate recovery and visualization. Studies in different fields of genetics that require this capabilities are not limited to GWAS, but include: (a) Evolutionary inferences that use state-of-the-art methods to infer past demographic parameters of populations, such as statistical phylogeography approaches; (b) Inferences about the action of natural selection; (c) Epidemiological studies designed to capture rare polymorphisms responsible for complex traits; and (d) Screenings for mutations in families or small populations with high-incidence of specific genetic diseases. To address the requirements above, we are developing DIVERGENOME, a bioinformatics platform focused on academic use that includes two integrated components: (1) DIVERGENOMEdb a relational database; and (2) DIVERGENOMEtools. The former component DIVERGENOMEdb is suitable for storing different types of genotypic data (SNPs, re-sequencing, STR, CNVs) and phenotypic variables. Here users can store both public and private data, according to their interest. A set of resources for database integration are under development to combine information from different sources, such as metabolic pathways. DIVERGENOMEtools is a set of scripts (modules) that can be combined in a pipeline to handle a variety of popular file formats from population genetics and statistical software, so that users can convert the output file of specific software into the appropriate input file format of another. DIVERGENOMEtools can handle file formats for the following population genetics software: PHASE, FastPHASE, DNAsp, Haploview, STRUCTURE, SWEEP, and genetic epidemiology software such as GLU and PLINK. DIVERGENOME includes a set of quality control steps in its different components to ensure data integrity and is built with a modular architecture envisioning the easy integration of other data types, which opens up prospects for future improvements. A first version of DIVERGENOMEtools was developed focusing on facilitating re-sequencing studies, and is publicly available since May 2010 as a web-based re-sequencing pipeline at www.cebio.org/pipelineldgh.

1434/T

A Novel Statistic for Testing Selection on Pair of Genes. *G. Peng, M. Xiong.* Dept of Biostatistics, University of Texas School of Public Health , Housotn, TX.

It is well known that gene function in a concert, rather than in isolation. Complex phenotype variations are caused by dynamic interaction among many genes and many environmental exposures through regulation and metabolism. Adaptations are often influenced by multiple interacted genes. However, in the conventional population genetics, most researches have primarily focused on natural selection acting on a single locus. Little attention has been paid to determining how the natural selection acts on multiple interacted genes in response to environmental perturbation. Recently, we performed genome-wide scan for natural selection in human population using 1000 genome pilot 1 data. We identified 14 pathways enriched with candidate selection genes including pathways, involved in the development of neural system, metabolism of carbohydrates and phosphates, cell communication and signal transduction. We found that all six pathways enriched by high genes in the recent paper by Amato et al. were also confirmed in our studies. A remarkable feature in our finding is that several pathways showed enrichment of selective signals in at least three populations. This strongly demonstrated that natural selection may jointly act on multiple genes. To reveal how multiple genes that are often organized into interacted networks are evolved to adapt dramatic changes in environment, lifestyle and culture, we used genome-continuum models and stochastic calculus to develop a unified framework for genome-wide scans for natural selection which can be used either to test selection on single gene or test selection on pair-wise genes. To evaluation the performance of the proposed statistics, we used software FREGENE to simulate the effects of natural selection on pair of genes and to compare our test with the results by simulations. Intensive simulations show that the test statistics work well. Finally, we performed genome-wide co-selection analysis using 1000 genome pilot 1 data. Since testing for all possible pair-wise co-selection across the genome in genome-wide co-selection analysis requires extremely large computation, we conducted pathway-based genome-wide co-selection analysis. We assembled 501 pathways from KEGG and Biocarta. The assignment of SNPs to a gene was obtained from NCBI human9606 database (version b129). We tested selection on all possible pair-wise of SNPs and constructed co-selection networks. The results will be presented in the conference.

1435/T

Selection Detection in Gene Families Utilizing Phylogenetics. *G. Wyckoff, M. Yang.* Div Molec Biol & Biochem, Univ Missouri -Kansas City, Kansas City, MO 64110.

As divergent gene regions have differing functional significance, several modes of evolution will simultaneously occur within a single gene, which may make positive selection very difficult to detect at the whole gene level. Thus to be able to detect positive selection more effectively, we divide genes into functionally independent segments. Protein domains are a natural candidate for this kind of analysis: they are functionally important, distributed across different genes, and they have a common evolutionary history. In addition, they are widely seen as evolutionarily conservative, so the number of mutations at the same locus are more limited making selective inference more reliable. We hypothesized that the positively selected members in protein domain families will display altered phylogenetic patterns compared with other members, and therefore will distort tree shape. We then utilize this pattern for the detection of outliers among domain members, and for the discernment of the number of gene families where positive selection is pervasive. While there have been continuous studies in phylogenetic tree shape in the areas of macroevolution and biogeography (Gould et al. 1977; Raup et al. 1973; Rosen 1978; Schopf 1979; Simberloff 1987), the employment of phylogenetic trees as a primary mechanism for detecting positive selection at the molecular level has always been, in our opinion, underutilized. Here, we have applied this methodology to a set of protein domains that are important to human evolution and human disease. We further estimate the number of protein domains where positive selection has likely affected a significant number of genes containing those domains.

1436/T

Correlation between Human and *Helicobacter pylori* ancestry in Colombian populations. *M.M. Torres[1], D. Cano[1], H. Groot[1], N. Ospina[1], L.E Bravo[3], M.B Piazuelo[2], P. Correa[2].* 1) Facultad de Ciencias , Universidad de los Andes, Bogota, Colombia; 2) School of Medicine, Vanderbilt University, Nashville, Tennessee,USA; 3) Facultad de Medicina, Universidad del Valle, Cali.Colombia.

Helicobacter pylori (*H.pylori*) is a human gastric bacterium involved in the peptic ulcers pathogenesis and constitutes a risk factor for gastric cancer. *H.pylori* strains from different geographic areas are associated with strong phylogeographic differentiation. Human DNA polymorphisms and DNA sequences from *H.pylori* have been used for the understanding of the human population migrations and can also explain the geographic differences in gastric cancer incidence. The main goal of the present study was to establish the genetic diversity of *H.pylori* isolates and their hosts in Colombian populations that have contrasting risk of gastric cancer and with different ethnic origins (Andean Mountains and Pacific coast) by mitochondrial DNA markers (HSV1-HSV2) and by multilocus sequence (7 housekeeping genes) of *H.pylori*. The mtDNA was sequenced from 86 subjects (65 from Pacific coast with somatic “mulato”phenotype and 21 from Andean Mountain with somatic “mestizo” phenotype). The ancestral genotypes of *H.pylori* were determined from 44 strains (40 from Pacific coast and 4 from Andean Mountains). We identified 79 unique mtDNA haplotypes clustered into amerind haplogroups (A-D) and African haplogroups (L0, L1, L2, L3). The 99% of population from Andean Mountain was of amerind origin and 1% European (Haplogroup H). The 74% of the Pacific coast populations were African in origin and the remainder 26% Amerind. The bacteria strains were assigned to populations according to their multilocus DNA sequences: those from pacific coast hosts yielded *hpEurope* (43%) and *hpAfrica1* (57%), all of those from Andean Mountains yielded *hpEurope*. None of the strains were hpAmerind. Finally, the great majority of the subjects with the Amerindian ancestry carried hpEurope genotype (14/20) and the African ancestry carried *hpAfrica1*genotype (16/23). Our results indicate that there is a high correlation between the ancestry of the host evaluated by mitochondrial DNA and the ancestral genotype of the *H.pylori* in the Andean Mountains. In agreement with previous reports, the low density or absence of hpAmerind genotype in our group reflects the tendency of this strain to disappear. The coastal populations is more heterogeneous, some carrying African and some European bacterial genotypes. This study also favors the hypothesis of Intrafamilial transmission of the *H.Pylori* . We confirm the potential usefulness of the study of the *H.pylori* genotype as a tool for inferring human migration.

1437/T

***CNTF* variations do to contribute to risk of POAG in Chinese population.** *J. Chen[1,2], L. Chen[2], Y. Zheng[1], J. Wang[1], S. Chiang[2], P. Tam[2], H. Chen[1,2], C. Pang[2], M. Zhang[1].* 1) Joint Shantou International Eye Center, Shantou University/The Chinese University of Hong, Shantou, Guangdong, China; 2) Department of Ophthalmology and Visual Sciences, The Chinese University of Hong Kong, Hong Kong Special Administration Region, China.

Purpose The ciliary neurotrophic factor (*CNTF*) can stimulate the regeneration of axons of retinal ganglion cells (RGC), loss of which is one of the major characteristics of glaucoma. Recently protective effect of the gene has been implicated in animal model of glaucoma, while its role in in primary open angle glaucoma (POAG) patients remains to be elucidated. Therefore we investigated the potential genetic involvement of *CNTF* variations in POAG among two Chinese Han cohorts. **Methods** The current study subjects included a cohort of 130 POAG patients and 134 controls recruited in Shantou, a city in southern China, and another cohort of 102 POAG patients and 103 controls recruited in Hong Kong. Screening of *CNTF* mutations was performed by direct DNA sequencing of polymerase chain reaction-amplified exons and splice regions. $\chi 2$ test was used for association analysis for any SNP detected. *In silico* tools were used for predicting the impact of any missense variant detected. **Results** In total, eight variants including two SNPs , and 6 novel variants, were found in the two Chinese cohorts. Among these variants two novel variants including an intronic mutation IVS1-21T>G and a non-synonymous mutation c.223G>C, were identified in patients with normal-tension glaucoma from Hong Kong cohort, and were absent in Shantou and Hong Kong controls. However, 2 variants in 5’-UTR namely c.-135G>T and c.-22C>T, and 3 non-synonymous coding variants namely c.407G>A, IVS1-6G>A (rs1800169) and c.545A>G (rs6266), were detected in both patients and controls from either Shantou or Hong Kong cohort. In particular, the intronic SNP rs1800169, which was predicted to result in a change in alternative splicing and consequently a truncated loss-function CNTF protein, was found in controls as well as in patients from both Shantou and Hong Kong cohorts. Furthermore, an insertion c.103_104insA causing frame-shift mutation was only detected in Shantou controls. None of the detected SNPs showed any statistical significance ($P > 0.05$) in the association analysis. **Conclusions** The existing of these *CNTF* variations, especially the loss-of-function mutations in control subjects from the two Chinese cohorts is probably a strong evidence that excludes *CNTF* as a disease-causing gene of POAG. The *CNTF* gene seems to have no or only a very low contribution to risk of POAG in Chinese. The current findings thus warranted further studies.

1438/T

Founder effect of LCA5 p.Q279X mutation in the Ashkenazi Jewish population. *PW. Chiang[1], E. Spector[1], J. Picker[2], WK. Chung[3].* 1) Pediatrics, UC Denver DNA Diagnostic Lab, Denver, CO; 2) Childrens Hospital Boston, Boston, MA; 3) Department of Clinical Genetics, Columbia University, New York, NY.

Leber’s Congenital Amaurosis (LCA) is a rare inherited eye disorder that leads to retinal dysfunction and visual impairment at birth or in the first few months of life. LCA is an autosomal recessive condition and is the most common cause of inherited child blindness with the prevalence of two to three per 100,000 births. There are at least 15 genes associated with the condition. The mutation detection rate for patients with a confirmed diagnosis of LCA is approximately 50% ~ 60%. Due to the breakthrough in gene therapy in patients with mutations in RPE65 (one of 15 genes associated with LCA) and the potential of gene therapy for patients with other LCA gene mutations, the clinical utility of molecular diagnosis is increasing. We have taken a novel approach to molecular diagnosis of LCA by sequencing the entire coding regions of all genes known to be associated with LCA. A total of 181 PCR reactions are simultaneously sequenced. To date, we have examined 77 patients. Two of the LCA patients carried the same homozygous mutation in the LCA5 gene, namely p.Q279X:c.835C>T. Both patients are of Ashkenazi Jewish decent and there was no known consanguinity in either family. Interestingly, the same mutation was also previously identified in a consanguineous family and in two other non- consanguineous LCA patients of Ashkenazi Jewish decent by other investigators. All five patients presented with severe LCA. This finding prompted us to determine the carrier frequency of the c.835C>T mutation in the Ashkenazi Jewish population. We estimate the carrier frequency to be ~ 1 in 100 based on our initial study of 96 individuals of Ashkenazi Jewish descent tested in our laboratory. A larger analysis of 1,000 normal individuals of Ashkenazi Jewish decent is in progress. The c.835C>T mutation has not been identified in LCA patients of other ethnic groups suggesting a founder effect in Ashkenazi Jews. Finding a common mutation in LCA patients of Ashkenazi Jewish decent has important clinical implications including: (1) molecular diagnosis of LCA patients of Ashkenazi Jewish decent will be more efficient, faster, and less expensive (1 PCR vs. 181 PCRs); (2) carrier screening can be offered to individuals of Ashkenazi Jewish decent; (3) due to the severity of the disease caused by this mutation and due to the founder effect in the Ashkenazi Jewish population, LCA5 should become a strong candidate for gene therapy.

1439/T

Asymmetric mutation processes in stochastic models of microsatellite allele length distributions in humans. *B. Fendler, G. Atwal.* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Microsatellites are frequent mutations throughout the genome, for which a short simple repeat sequence occurs. Genotyped microsatellite loci have been implicated in numerous diseases and can be used for linkage association mapping, making them useful biomarkers for causal genetic variants, phylogeny construction and forensic investigations. However, the observed distribution of repeat lengths in human populations is still not well understood from a quantitative perspective. Moreover, only the simplest dynamical models of allelic variability have been amenable to analytical methods, invoking stringent and unrealistic biological assumptions. We revisit and build upon the well known stepwise mutation model, and carry out extensive Monte Carlo simulations of the Moran model of microsatellite allelic evolution under a variety of different parameter regimes. In particular, we explore the effects of the interplay between drift and asymmetric mutation processes by independently varying the effective population size (N), the rate of deletion (η_d) and the rate of insertion (η_i). We also consider differing models of mutation processes that alter lengths by larger than one repeat unit, either by arithmetic or geometric progression. However, the arithmetic stepwise models do not lead to stationary distributions of allele lengths in the case of neutral evolution, and thus hampers the ability to infer mutation rates from population data. The neutral simulations of arithmetic mutation processes demonstrate that the allele length distributions evolve as Gaussian-like packets whose variance depends on N and whose mean increases with time when $\eta_i > \eta_d$ and decreases for $\eta_d > \eta_i$. We propose and simulate a geometric model of mutation in which the average allelic repeat length does not increase indefinitely even when $\eta_i > \eta_d$. This hypothesis is supported by earlier reported findings in which repeat increases were favored over decreases in the human CEPH reference family database. Fitting the simulations to experimental data by varying the mutation probabilities, we find mutation probabilities on the order 1×10^{-3}, within the accepted range of mutation rates (event/generation/loci).

1440/T

Genotype imputation in American Indians. *A. Malhotra[1], W.C. Knowler[1], L.J. Baier[2], C. Bogardus[2], R.L. Hanson[1].* 1) Diabetes Epidemiology and Clinical Research Section, NIDDK, Phoenix, AZ; 2) Molecular Genetics Section, NIDDK, Phoenix, AZ.

Current technologies allow rapid and extensive genotyping of common SNPs. However, none of the available genotyping platforms has complete coverage. One solution has been the development of methods to impute untyped variants using information on linkage disequilibrium with typed markers. These methods have been implemented in various software programs including MACH (Markov Chain Haplotyping). To impute genotypes, these methods require a suitable reference population, most often one selected from HapMap. However, some populations, such as American Indians, are not represented in HapMap. While it has been suggested that a combination of HapMap populations (e.g., the combination of Chinese and Japanese (CHB+JPT) and Caucasian (CEU) samples) may be useful for imputation in American Indians, it is not known how the accuracy of such imputation compares with using an American Indian reference. In the present study, we assessed imputation procedures using data obtained from a genome-wide association study (454,154 SNPs with minor allele frequency >0.05) conducted in 1266 Pima Indians. Due to computation time, we restricted our analysis to 6 randomly selected chromosomes. For each chromosome, genotypes were masked (either 1% or 20% of SNPs available for a given chromosome). The masked genotypes were then imputed using MACH and quality was assessed using two measurements: 1) the proportion of genotypes that were correctly imputed when the most likely genotype was taken, and 2) the average estimated r^2 between the true and imputed genotype. Using the HapMap reference populations, an average genotype error rate of 10.7% and r^2 of 0.77 were observed. In contrast, use of the original Pima Indian data resulted in an average error rate of 1.6% and r^2 of 0.99. We further explored the number of Pima Indian individuals (25, 50, or 100 randomly selected) that would be needed to get highly accurate imputed genotypes within this population. For these analyses, average genotype error rates were 3.0%, 2.2%, 1.7%, respectively for 25, 50, and 100 reference Pima individuals, with $r^2 > 0.97$ in all cases. In conclusion, our results suggest that HapMap reference populations do not provide accurate imputation of genotypes in American Indians. A possible solution would be to sequence a reference American Indian population. As the results suggest, a sample size as small as 25 individuals can provide high imputation accuracy.

1441/T

ABRAHAM'S CHILDREN IN THE GENOME ERA 2: A WORLDWIDE VIEW OF JEWISH DIASPORA GROUPS. *G. Atzmon[1,2], L. Hao[3], I. Pe'er[4], C. Velez[5], A. Pearlman[5], P.F. Palamara[5], E. Friedman[6], C. Oddoux[5], E. Burns[1], H. Ostrer[5].* 1) Department of Medicine, Albert Einstein College of Medicine, Bronx, NY; 2) Department of Genetics, Albert Einstein College of Medicine, Bronx, NY; 3) Center for Genome Informatics, New Jersey Medical School, University of Medicine and Dentistry of New Jersey, Newark, NJ; 4) Department of Computer Science, Columbia University, New York, NY; 5) Human Genetics Program, Department of Pediatrics, New York University School of Medicine, New York, NY; 6) The Susanne Levy Gertner Oncogenetics Unit, the Danek Gertner Institute of Human Genetics, Chaim Sheba Medical Center, 52621, Tel-Hashomer, and the Sackler School of Medicine, Tel-Aviv University, Tel-Aviv Israel.

Genome wide analysis of Jewish Diaspora groups that have formed over the past 2000 years provides an invaluable opportunity for understanding genetic origins and migrations and provides a framework for elucidating the genetic basis of complex disorders. Here, we have built upon our initial genome wide analysis of 7 ethnically diverse, healthy Jewish (European: Ashkenazi, Italian, Greek, Turkish, and Middle Eastern: Iranian, Iraqi, and Syrian) populations (AJHG online June 3rd, 2010) by analyzing members of an additional 9 Jewish communities (North African: Moroccan, Algerian, Tunisian, Djerban, and Libyan; and Georgian, Yemenite, Indian Bene Israel and Ethiopian). These 524 unrelated individuals were analyzed on Affymetrix v6 microarrays, then merged with results from the Human Genome Diversity Project and the Population Reference Sample studies that were comprised of 146 non-Jewish Middle Easterners (Druze, Bedouin and Palestinian), 30 northern Africans (Mozabite from Algeria), 1547 Europeans, and 653 individuals from other African, Asian, Latin American, and Oceanian populations. Principal component SNP and CNV analysis (PCA) and pairwise Fst distance showed that European, Middle Eastern, North African and Georgian Jewish populations formed a cluster clearly distinct from all major continental populations with each group demonstrating significant admixture with local populations. Yemenite, Ethiopian and Indian (Bene Israel) Jews fell outside this cluster. The North African Jewish cluster demonstrated proximity to the Sephardic Jewish. North African Jewish relationships showed a cline consistent with their geographic cline and the Tunisian Jewish population was statified into two subpopulations, one with proximity to Libyan and Djerban Jews and the other to Moroccan and Algerian Jews. Analysis of identity by descent revealed a high degree of segment sharing across most Jewish groups and endogamy within all of the Jewish populations that was especially high among the Indian and Djerban Jews. These results demonstrate the shared and distinctive genetic heritage of Jewish Diaspora groups that were formed during Classical Antiquity and indicate that both the flow of genes and the flow ideas has contributed to Jewishness.

1442/T

Resequencing of human type B scavenger receptor genes in Asia-Pacific populations. *K. Nakayama[1], A. Ogawa[1], Y. Yanagisawa[1], T. Gotoh[1], H. Miyashita[2], L. Munkhtulga[3], Y. Kagawa[4], S. Iwamoto[1].* 1) Divsion of Human Genetics, Jichi Medical University, Shimotsuke-City, Japan; 2) Jichi Medical University Hospital, Shimotsuke-City, Japan; 3) Health Science University of Mongolia, Ulaanbaatar, Mongolia; 4) Kagawa Nutrition University, Sakado, Japan.

CD36 and SR-BI belong to type B scavenger receptors that recognize various classes of lipoprotein and fatty acids. Genetic variations of CD36 and SR-BI were reported to be associated with lipid disorders, glucose intolerance, and hypertension in humans. In addition to the primary roles in lipoprotein metabolism, CD36 and SR-BI were implicated in other biological processes, for example, fat taste perception and malarial infection. Diversity of genes encoding CD36 and SR-BI (*CD36* and *SCARB1*) might have been shaped by natural selection relevant to their functions. In the present study, we investigated the variation of *CD36* and *SCARB1* in four Asia-Pacific populations. Resequencing of the whole coding exons and the flanking intronic region of *CD36* and *SCARB1* were performed against 380 individuals of Japanese, Mongolians, Thai and Palauans. A total of 23 polymorphic sites were observed in *CD36* and *SCARB1* and 15 of them were novel variants with allele frequencies of less than 5%. For *CD36*, Ser90Pro, which was the most prevalent loss-of-function variant of *CD36*, was common in Japanese (6%) but almost absent in other populations. Other loss-of-function variants of *CD36*, 329-330delAC and 1228-1239delATTGTGCCTATT, were found in Japanese and Thai with low frequencies (less than 1%) but were not detected in Mongolian and Palauans. For *SCARB1*, Thai uniquely possessed functional Gly2Ser variant and a novel 2bp deletion variant resulting in truncated proteins. Interestingly, among the four populations, variants altering amino acid sequences were most abundant in Thai. Since CD36 and SR-BI are host factors aiding infection and proliferation of malaria parasites, the functional variants of *CD36* and *SCARB1* may contribute to susceptibility to malaria as well as the variation in metabolic parameters in Thai population.

1443/T

A Wide STR Analysis of the Y Chromosome Haplogroups in Native American Populations. *V. Battaglia[1], N.M. Myres[2], U.A. Perego[1,2], V. Grugni[1], A. Achilli[1,3], J.E. Gomez-Palmieri[2], N. Angerhofer[2], S.R. Woodward[2], A. Torroni[1], O. Semino[1].* 1) Dip. Genetica e Microbiologia, Università di Pavia, Pavia, Italy; 2) Sorenson Molecular Genealogy Foundation, Salt Lake City, Utah, USA; 3) Dip. Biologia Cellulare e Ambientale, Università di Perugia, Perugia, Italy.

The settlement of the Americas represents the last major human colonization episode. It has been a topic of interest and controversy in numerous scientific fields, including genetics. While general archaeological, linguistic and molecular agreement on the Asian origin of Native Americans exists, the timing of their arrival, the routes followed, the number of population expansions/migrations involved, and the Asian source(s), have been, and still are, objects of discussion. Progress in the comprehension of the peopling of the Americas has been provided by studies on complete mitochondrial (mt)DNA (maternally transmitted) sequences, which document a higher level of molecular diversity and two distinct contemporary (15-17 kya) migration events from Beringia. While one entered the Americas following the Pacific coastline, the other moved through the ice-free corridor between the Laurentide and Cordilleran glacial sheets, suggesting they were derived from two separate founding populations. In comparison with mtDNA, the Native American Y-chromosome (Ycs) diversity is limited to two branches of haplogroups (hg) C and Q. Even though their resolution does not allow region/ethnic-specific sub-clades to be distinguished, the first hg is restricted to the North (Na-Dené speakers), while the second hg is spread from North to South, covering a wide part of the American continent. Here we report the preliminary analysis of 38 Ycs short tandem repeats (Y-STRs) from 2,025 samples belonging to the Sorenson Molecular Genealogy Foundation DNA database, with terminal paternal ancestry from a broad geographic area ranging from Mexico to Chile. Of these samples, 173 harbour the American specific hg Q1a3a-M3, which likely originated in the Beringian refuge during a long period of pause around the time of the LGM. The underlying molecular diversity indicates significant differences in haplotype (ht) distributions between North/Central and South Americans, suggesting different population histories in the two continental regions. In particular, two main ht branches have been observed: one pan-American and the other limited to samples from Peru, Bolivia, and Chile. These initial results are suggestive of a distinct Ycs founding lineage in South America with a discrete distribution which could likely represent the male counterpart of the coastline route previously identified through the mtDNA approach.

1444/T

A Novel Variant near *MTNR1B* Gene Affects Fasting Glucose Concentrations: Genetic Risk is Modulated by Obesity Status. *L.F. Been[1], J.L. Hatfield[1], A. Shankar[1], C.E. Aston[1,2], S. Ralhan[3], G.S. Wander[3], N.K. Mehra[4], J.R. Singh[5], J.J Mulvihill[1], D.K. Sanghera[1].* 1) Department of Pediatrics, University of Oklahoma HSC, Oklahoma City, Oklahoma; 2) General Clinical Research Center, University of Oklahoma HSC, Oklahoma City, Oklahoma; 3) Hero Dayanand Medical College & Heart Institute, Ludhiana, Punjab,India; 4) All India Institute of Medical Sciences, New Delhi, India; 5) Central University of Punjab, Bathinda, India.

Genome-wide association studies (GWAS) have identified variants in *G6PC2, GCK*, and *MTNR1B* affecting fasting blood glucose (FBG). In the pursuits of examining the role of these loci in our Asian Indian sample and also for searching causal variants in these genes, this study was undertaken. We genotyped five polymorphisms from these genes including *G6PC2* rs560887, *GCK* rs1799884, *MTNR1B* rs1387153, rs10830963, and a novel SNP discovered near 5' untranslated region in *MTNR1B*. These SNPs were studied in our diabetic case-control cohort comprising 2,222 subjects (1,201 cases and 1,021 controls). None of these SNPs was associated with type 2 diabetes (T2D) in this sample. Our data also could not confirm association of *G6PC2* and *GCK* with FBG phenotype. In *MTNR1B*, rs10830963 was associated with marginally increased HOMA-IR (insulin resistance) (β=0.13, p=0.026) and the novel SNP was linked to marginally decreased 2h glucose (β= -0.06, p=0.032) among controls. However, upon stratifying data by obesity, the minor allele carriers in the novel *MTNR1B* variant revealed a strong association with lower FBG levels in the non-obese group (β= -0.073, p= 0.002) compared to the obese group (β= 0.015, p=0.50). The effect was also significant in combined cohort (cases and controls) and remained confined to the non-obese group (β= -0.105, p=0.007). Our study for the first time reveals a strong association of a new variant in *MTNR1B* for affecting FBG levels in the absence of obesity. Future confirmation on a larger sample will be required to better define the role of this variant in glucose homeostasis.

1445/T

T45G Adiponectin Gene Polymorphism And Its Association With Hyperglycemia In Adult Filipinos Seen At The Philippine General Hospital - A Pilot Study. *E. Cutiongco[1], E. Paz-Pacheco[2], C. Josol[2], C. Sison[2], G. Jasul[2], F. Rocamora[1], A. Laurel[2].* 1) Institute of Human Genetics, National Institutes Health, University of the Philippines Manila; 2) Section of Endocrinology, Diabetes and Metabolism, Department of Medicine, College of Medicine, Philippine General Hospital, University of the Philippines Manila.

The gene encoding adiponectin is found in chromosome 3q27 where susceptibility loci for diabetes and metabolic syndrome have been mapped. Association of ADIPOQ gene single nucleotide polymorphisms (SNPs) with type 2 diabetes and impaired glucose tolerance or impaired fasting glucose has been found in some populations. The most commonly reported variant is the T/G polymorphism of SNP +45 in exon 2 which has been shown to be associated with diabetes and obesity in some Japanese, French Caucasians, Swedish Caucasians, Chinese, and Iranian subjects. However, studies among Pima Indians, another group of Japanese, and Korean subjects revealed conflicting results. Inconsistent data could be attributed to inter-ethnic differences in polymorphisms of the adiponectin gene. This is the first investigation on the association of T45G adiponectin gene polymorphism with hyperglycemia among Filipinos. A significant association may be exploited further in the search for novel approaches to the treatment and/or prevention of diabetes and the metabolic syndrome in Filipinos. Filipino subjects greater than 18 years old seen at the Philippine General Hospital were recruited and divided into hyperglycemic group and normoglycemic group using definitions recommended by ADA. Fasting plasma glucose, 2-hr post 75-gm anhydrous glucose random blood sugar, and serum adiponectin levels of eligible subjects will be taken. Direct sequencing was used to detect the T45G polymorphism of the adiponectin gene among the cases and controls. In this pilot study involving 36 normoglycemic and 32 hyperglycemic Filipinos, the T45G polymorphism compared to the homozygous GG allele which showed no significant association between this polymorphism and hyperglycemia (OR 1.03; CI 0.05-19.59). Interestingly, the homozygous TT appears to be a protective allele (OR 0.59 ; CI 0.03-0.93) compared to the T45G polymorphism.

1446/T

Association Between Estrogen Receptor-α Gene & Metallothionein-1 Gene Polymorphisms In Type 2 Diabetic Women Of Indian Population. *s.g.s.r. ganasyam shilpa reddy, ysr. murthy, T. Bhaskar.* Biochemistry, Institute of Genetics and Hospital for Genetic diseases, Osmania University ,Begumpet,Hyderabad 500016,andhra pradesh, India.

Background: Type 2 Diabetes Mellitus (DM) is a multifactorial disease where both genetic and environmental factors contribute to its pathogenesis. Estrogen plays an important role in type 2 DM pathogenesis. A number of polymorphisms have been reported in the estrogen receptor (ESR) α, including the Xba I and Pvu II restriction enzyme polymorphisms of ESR 1,which may be involved in disease pathogenesis. Metallothionein (MT) is a potent antioxidant that can affect energy metabolism. Very few studies have indicated the association between Estrogen Receptor-α and MT1A gene polymorphisms with type 2 DM. Methods: A total of 300 type 2 diabetic women and 100 age, sex matched controls, 50 healthy male controls were recruited. Using the PCR based RFLP method, the Pvu II and Xba I polymorphisms of ESR1 and rs 8052394, rs 11076161 in MT1A gene polymorphisms were analysed. Other parameters like lipid levels, serum superoxide dismutase activity (SOD), interleukin -6 and TNF α were analysed by ELISA method. Results: There was significant difference in Pvu II but not Xba I allele frequency between the type 2 DM and control groups (P=0.001 and P= 0.122). When the group was separated into men and women, the difference was significant in women (p<0.001) but not in men (p=0.854) with the Pvu II genotype. In addition, Pvu II genotype was associated with serum lipid changes. The frequency distributions of the G allele in SNP rs8052394 of MT1A gene were significantly associated with type 2 diabetic women. Serum levels of IL-6 and TNF-α were higher and serum SOD activity was significantly lower in the diabetic group than those in the control group. Conclusion: The above results are helpful to identify the Indian women with genetic predisposition to type 2 DM and to protect them from environmental risks.

1447/T

Frequency of 27-bp deletion mutation, another earwax determinant, in ABCC11 among the Japanese population. *A. Yamada[1,2], Y. Hori[1,2], Y. Ono[1,2], N. Matsuda[1,2], D. Starenki[2], N. Sosonkina[2], K. Yoshiura[3], T. Ohta[2], N. Niikawa[2].* 1) Sch Med, Sapporo Med Univ, Sapporo, Japan; 2) RIPHS, Health Sci Univ Hokkaido, Tobestu, Japan; 3) Dept Hum Genet, Nagasaki Univ Graduate Sch Biomed Sci, Nagasaki, Japan.

Human earwax is a dimorphic trait consisting of wet and dry types, which are determined mostly by a 538G/A SNP (rs17822931) in the ABCC11 gene. The wet type corresponds to GG or GA genotype, and the dry type to AA genotype. A 27-bp deletion (Δ27), a rare variant, downstream to the rs17822931 site in ABCC11 is another earwax determinant, acting as a loss-of-function mutation in a recessive manner. Either homozygotes for allele Δ27 or heterozygotes for Δ27 when the counterpart chromosome has allele A lead to the dry phenotype, regardless of cis-positioned allele G. Previous data of world-wide populations demonstrated that the highest allele-A frequencies (1.00-0.83) are observed in the East Asians and showed decreasing clines in its frequency from East Asia toward Europe and Southern Asia. The Δ27 allele was found only one in 334 Japanese, one in 50 Thai, two in 20 native North Americans, 9 in 30 Bolivia habitants, but none in Europeans and Africans. These data suggested that allele A origined in ancient Northeast Eurasians, while origin of the Δ27 remained unknown. Here we report results of a further study on the frequency of allele Δ27. Three such alleles were detected among 760 Japanese individuals and none in 397 Ukrainians: One Japanese is a homozygote for a haplotype A-Δ27 and the other a Δ27/WT and G/A double heterozygote. Thus, the allele frequency among the Japanese is 0.0019 (3/1520), being consistent with the previous data (0.0015, 1/334). Since a previously found Japanese has a G-Δ27 haplotype, a recombination occurred between the the rs17822931 and Δ27 cites, although the Δ27 might have arisen more recently than did allele A.

1448/T

The male gene pool of the contemporary Mesopotamia marsh population supports their Semitic origin. *N. Al-Zahery[1], J.A. Irwin[2], V. Battaglia[1], M.A. Hamod[3], V. Grugni[1], A.S. Santachiara-Benerecetti[1], O. Semino[1].* 1) Department of Genetics and Microbiology, Pavia University, Via Ferrata 1, 27100 Pavia, Italy; 2) Research Department, Armed Forces DNA Identification Laboratory (AFDIL), 1413 Research Blvd, Rockville, MD 20850, USA; 3) Department of Biotechnology, Faculty of Sciences, Baghdad University, Baghdad, Iraq.

The origin of the modern Mesopotamia marsh people, which are locally called “Ma’dan” or “Marsh’s Arabs”, is a question of great interest. Based on their life-style (living in reed houses, grazing of water buffalo and other aspects) and local archaeological sites, many historians and archaeologists believe they may have Sumerian ancestry. Although little is known about the origin of Sumerians themselves, two main hypotheses have been advanced in this regard. According to the first, Sumerians were a group of populations which migrated from the “South East” following a seashore route through the Arabian Gulf, and settled down in the southern marshes of Iraq. According to the second, the advancement of the Sumerian civilization is the result of migration from the mountainous area of Anatolia to the southern marshes of Iraq where they settled, adsorbing previous populations. In order to shed some light on the genetic origin of the Mesopotamia marsh population, we investigated the male gene pool of 145 DNA samples of modern Mesopotamia people, still living in marshes in the south of Iraq. The analyses of Single Nucleotide Polymorphisms (SNPs) and Short Tandem Repeats (STRs) of the paternally transmitted Male Specific region of the Y chromosome (MSY) revealed that more than 80% of marsh Y chromosomes belong to (Hg) J1-M267, the autochthonous haplogroup of Middle Eastern/Semitic speakers with possible recent expansion and/or founder effect reflected by the reduced STRs variability. In particular, 90% of them were assigned to the J1e-M267-PAGE08 sub-haplogroup, which is the predominant Y chromosome lineage among Middle Eastern Arab populations (Yemen, Qatar, UAE, and Levant). Thus, these findings testify, at least from the paternal side, a strong Semitic Arabian component in the contemporary Mesopotamia marshes population, whereas no clear Anatolian and/or South Asian genetic evidence has been detected.

1449/T

Natural Selection at High Altitude: Andean Patterns of Adaptation to an Extreme Environment. *A. Bigham[1], M.D. Shriver[2], L.G. Moore[3], F. Leon-Valerde[4], E.J. Parra[5], T.D. Brutsaert[6].* 1) Pediatrics, Univ Washington, Seattle, WA; 2) Department of Anthropology, Pennsylvania State University, University Park, Pennsylvania, USA; 3) Departments of Public Health Sciences, Anthropology and Obstetrics-Gynecology, Graduate School of Arts and Sciences, Wake Forest University, Winston-Salem, North Carolina, USA; 4) Departamento de Ciencias Biológicas y Fisiológicas, Universidad Peruana Cayetano Heredia, Peru; 5) Department of Anthropology, University of Toronto, Mississauga, Mississauga, Ontario, Canada; 6) Department of Exercise Science, Syracuse University, Syracuse, New York, USA.

High-altitude hypoxia, or the decrease in oxygen levels caused by lowered barometric pressure, challenges the ability of humans to live and reproduce. Human physiological responses to high-altitude have been extensively documented among long term high-altitude residents (i.e. Andeans and Tibetans). However, additional research is necessary to understand the genetic basis for the observed physiological traits. To this end, we performed a genome scan to identify selection nominated candidate genes or gene regions for high-altitude adaptation using the Affymetrix Inc. Genome-Wide Human SNP Array 6.0. We scanned across each chromosome to discern genomic regions with previously unknown function with respect to altitude phenotypes and examined groups of genes functioning in oxygen metabolism and sensing, such as the hypoxia inducible transcription factor (HIF) pathway, for evidence of directional selection. Candidate genes or gene regions were identified by comparing the patterns of variation between Andeans (n=49) and two low-altitude populations, Mesoamericans (n=39) and East Asians (n=90), using four tests for natural selection. The tests included the locus-specific branch length (LSBL), the log of the ratio of heterozygosities (ln*RH*), Tajima’s *D*, and a long-range haplotype test. Several genes including *NOS2A*, *PRKAA1*, *EGLN1*, and *TNF* as well as regions on chromosomes 12 and 15 showed significant evidence of natural selection. From these top candidates, ten single nucleotide polymorphisms (SNPs) were selected for further study and genotyped in a sample of n=141 Peruvian Quechua with associated resting and exercise phenotypic data. The subject participants included Q-SL subjects (Quechua sea-level) who were lifelong sea level residents transiently exposed to hypobaric hypoxia and Q-HA subjects (Quechua at high-altitude) who were lifelong residents of high-altitude. Significant associations were found for arterial oxygen saturation (SaO_2) and genotype at PRKAA1 and the candidate region on chromosome 15 (repeated measures ANOVA, $p<0.05$ after FDR correction). These markers, along with the group effect (i.e. born high or born low), display relatively large effects on SaO_2 with each explaining ~6-8% of the variance in this phenotype. Furthermore, the genotypic effects on SaO_2 are broadly similar between the Q-SL and Q-HA groups. The results provide key insights into the patterns of genetic adaptation to high altitude in Andean populations.

1450/T

Evidence of selection in the pigmentation genes SLC24A5 in Europeans, East Africans, and Southwest and Central Asians and SLC45A2 in East Asians and Native Americans. *M.P. Donnelly, W.C. Speed, A.J. Pakstis, J.R. Kidd, K.K. Kidd.* Department of Genetics, Yale University School of Medicine, New Haven, CT.

Skin pigmentation is one of the most recognizable human phenotypes and tends to vary on a latitudinal cline, even within Europe. The derived alleles of missense SNPs in SLC24A5 (rs1426654) and SLC45A2 (rs16891982) have both been implicated in light skin pigmentation among Europeans. We have collected data for these markers from 4474 individuals in 107 population samples. The derived alleles of both SNPs were observed at high frequencies throughout Europe, though the derived allele of rs16891982 is found at lower frequencies in Southern Europe. The derived allele of rs1426654 was also found at moderate to high frequencies 2 to 100% in East Africa, Southwest Asia, and Central Asia, whereas the derived allele of rs16891982 was seen at frequencies of 0 to 58% in these populations. At SLC24A5 a single allele of a 13-SNP (including rs1426654) haplotype covering ~146 kb accounts for ~95% of the chromosomes in Europe. At SLC45A2, we saw no significant LD around rs16891982. Using the REHH test, we found strong evidence of selection for the derived allele of rs1426654 in Europe as well as East Africa, Southwest Asia, and Central Asia where it had not previously been seen and were able to confirm the evidence of selection in East Africa using nHS. We saw no or very weak evidence of selection for rs1689192 using REHH or nHS among European and nearby populations. We did find previously unidentified evidence of selection in East Asians and Native Americans at a SNP about 15 kb upstream of SLC45A2. The allele showing evidence of selection at this SNP is found at high to moderate frequencies throughout the world except in Europe where it is virtually absent. Given its location it is likely part of or in LD with an upstream regulatory element upon which selection is/was acting. Taken together these results suggest several conclusions about the evolution of skin pigmentation in humans. First two SNPs shown to play a role in pigmentation in a region of the world can show different distribution patterns Second, it suggests that light skin among Europeans evolved both by means of natural selection and neutral factors. Finally, the evidence of selection in the upstream region of SLC45A2 in East Asians and Native Americans, suggests that though light skin pigmentation likely evolved separately in regions where light skin pigmentation is predominant, the evolution at some genes may have occurred independently through different variants. Funded in part by NIH Grant GM57672.

1451/T

Genome-wide scans of European population groups identify multiple loci with evidence for positive selection in Sardinians. *R. Kosoy[1], F. Macciardi[2,3], F. Taddeo[3], D. Cusi[4], S. Lupoli[5], H. Chen[6], M.L. Ransom[1], M.F. Seldin[1].* 1) Dept Biological Chemistry, Univ California, Davis, Davis, CA; 2) Department of Psychiatry and Human Behavior, Univ California, Irvine, Irvine, CA; 3) Genomics and Bioinformatics Unit, Univ of Milan - Fondazione Filarete, Italy; 4) School of Medicine. Dept. of Sciences and Biomedical Technologies. University of Milan, Italy; 5) INSPE, San Raffaele Scientific Institute, Milan, Italy; 6) Department of Genetics, Harvard Medical School, Boston, MA.

Identification of genes undergoing natural selection in various human populations may help identify genetic factors that increase or decrease the risk for specific diseases within that population. We applied multiple methods to detect signatures of positive natural selection using genome-wide scans of eight different European populations. The methods included Fst as a measure of population differentiation and two algorithms (XPEHH, and iHS), which detect the presence of extended identical haplotypes, arising due to a partial or a complete sweep in a population. In addition, we also used XPCLR, a likelihood method for detecting selective sweeps by modeling the multilocus allele frequency differentiation between pairs of populations. These methods were applied to 475,574 SNPs genotyped in English, Irish, Norwegians, Northern Italians, South Italians, Sardinians, Ashkenazi Jews and Arabs (number of individuals in each group between 70 and 780) in a total of 2035 individuals. A number of loci with strong evidence for positive selection were identified in the Sardinian population by pair-wise analyses with four other South European populations (Southern Italians, Northern Italians, Ashkenazi Jewish, and Arabic groups) and three North European populations (English, Norwegians, and Irish). In particular, multiple SNPs in 2p22.1 and 2q37.8 regions showed positive results with XPEHH scores > 0.9 and Fst scores > 0.14 for pair wise comparisons and with clearly defined boundaries. The first of the regions spans 440kb, and contains a part of a single gene, SLC8A1 (solute carrier family 8), previously suggested as a candidate for hypertension. The second region is 220kb long, and contains six genes, HDLBP, SETP2, FARP2, STK25, BOK, and THAP, the first of which codes for high density lipoprotein binding protein, whose likely function is in cell sterol metabolism. The pair-wise differences were strongest between Sardinian and other South European populations, suggesting that the natural selection at these genomic regions is largely confined to Sardinian population, and is not a part of the South Europe versus North Europe difference.

1452/T

Evolutionary origin of peptidoglycan recognition proteins. *A. Montano[1,2], F. Tsujino[2], N. Takahata[2], Y. Satta[2].* 1) Dept Pediatrics, St Louis Univ, St Louis, MO, USA; 2) Dept Biosys Sciences, School Advanced Sciences, The Graduate Univ. for Advanced Studies (SOKENDAI), Hayama, Japan.

Innate immunity is the ancient defense system of multicellular organisms against microbial infection. The basis of this first line of defense resides in the recognition of unique motifs conserved in microorganisms, and absent in the host. Peptidoglycans, structural components of bacterial cell walls, are recognized by Peptidoglycan Recognition Proteins (PGRPs). PGRPs are present in both vertebrates and invertebrates, and although some evidence for similarities and differences between them has been found, their evolutionary history and phylogenetic relationship have remained unclear. Such studies have been severely hampered by the great extent of sequence divergence among vertebrate and invertebrate PGRPs. Here we investigate the birth and death processes of PGRPs to elucidate their origin and diversity. We found that (i) four rounds of gene duplication and a single domain duplication have generated the major variety of present vertebrate PGRPs, while in invertebrates more than ten times the number of duplications are required to explain the repertoire of present PGRPs, and (ii) the death of genes in vertebrates appears to be almost null whereas in invertebrates it is frequent. These results suggest that the emergence of new PGRP genes may have an impact on the availability of the repertoire and its function against pathogens.

1453/T

The LPHN3 Common Haplotype Variant Predisposing to Attention-Deficit/Hyperactivity Disorder (ADHD) is Ancestral to the Protective Variant. *M. Muenke, M. Arcos-Burgos.* Med Gen Branch, NHGRI/NIH, Bethesda, MD.

Recently we identified (Latrophilin 3) LPHN3, a novel Attention Deficit/Hyperactivity Disorder (ADHD) susceptibility gene and showed that a LPHN3 common variant confers susceptibility to ADHD and predicts effectiveness of stimulant medication. LPHN3, a brain-specific member of the LPHN subfamily of G-protein coupled receptors, is expressed in ADHD-related regions, such as amygdale, caudate nucleus and cerebral cortex. Further, this ADHD susceptibility variant affect metabolism in neural circuits implicated in ADHD. Here by genotyping more than 300 SNP variants, including some spanning the LPHN3 genomic region associated to ADHD, in different species of monkeys (sanguinus (n=1), macaca mulatta (n=2)), and primates (gorilla gorilla (3), pan troglodytes (8), and pongo pygmaeus (2)) (a phylogeny covering ~70 millions years of evolution), we were able of reconstructing a phylogenetic tree showing that the LPHN3 variant of susceptibility is ancestral to the LPHN3 variant conferring protection against ADHD. Furthermore, the reconstructed phylogenetic tree suggests that the evolutionary splitting between these variants happens before modern humans separated from great apes. This phylogenetic scenario is in strong agreement with the suggested selective beneficial advantage of genetic variants conferring susceptibility to ADHD and consequently with the fact that the cluster of behaviors outlined by the ADHD syndrome are and/or were indeed very old and normal.

1454/T

Using STRs to shed light on the origin of endemic HLA-B alleles in Amerindian populations. *K. Nunes[1], R.S Francisco[1], C. Hurley[2], B. Tu[2], W. Klitz[3], E.J.M Santos[4], D. Labuda[5], R. Barrantes[6], A. Ruiz-Linares[7], D. Meyer[1].* 1) Universidade de São Paulo, São Paulo, Brazil; 2) Georgetown University, Washington, USA; 3) Public Health Institute, Oakland, USA; 4) Universidade Federal do Para, Belém, Brazil; 5) Université de Montréal, Montreal, Canada; 6) Universidad de Costa Rica, San José, Costa Rica; 7) University College London, London, United Kingdom,.

Compared to populations from other regions, Amerindian populations display low genetic variation and high inter-population diversity. An additional and striking pattern in Amerindians was revealed for HLA-B locus, which carries a large number of alleles which are found exclusively in this region, and are present at a relatively high frequencies. This pattern of variation is unusual, given that Amerindian populations typical harbor alleles which constitute a subset of those found in Asia. In the present study we use HLA-B flanking microsatellites to better understand the evolutionary history of HLA-B alleles endemic to the Americas. The STRs D6S2927 and D6S2928 from MHC region were genotyped in 241 individuals from 22 Amerindian populations (3 North American, 3 Central American and 16 South Americans). These samples were previously typed for the HLA-B gene by PCR-SBT and/or genotyped by PCR-SSOP, allowing the inference of haplotype frequencies for the compound marker consisting of HLA-B and flanking STRs We compared the distribution of STRs allele frequencies for endemic and cosmopolitan alleles, for each lineage of HLA-B. Overall, STR frequencies were similar for cosmopolitan and endemic alleles, with exception of the HLA-B*3543 for the locus D6S2928 (p = 3.99 e-09). The alleles of the 35 lineage usually have the allele 261 at the D6S2928 locus, however, the HLA-B*3543 presents the allele 257. The comparison between the sequences of HLA-B points to a possible recombination event between a lineage 35 allele and some other allele of lineage 15, giving rise to HLA-B*3543. This is corroborated by the fact that the alleles of lineage 15 generally present allele 257 at locus D6S2928. We conclude that: 1) The finding of the same STR alleles associated to more than one HLA-B allele suggests that the gene conversion occurs in the HLA-B alleles at a faster rate than the mutation rate of the STRs. 2) The absence of allelic profile alteration on the STRs between the cosmopolitan and endemic alleles confirms the hypothesis that the majority of the endemic alleles arise by genic conversion, involving a few nucleotides of exons 2 and 3 of the HLA-B gene. 3) The allele HLA-B*3543, seems to have arisen by recombination among a lineage 35 allele (possibly 350101g) and an allele of the lineage 15.

1455/T

Estimation of Selection Intensity of an *ABCC11* SNP Determining Earwax Type. *J. Ohashi, I. Naka, N. Tsuchiya.* Doctoral Program in Life System Medical Sciences, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8575, Japan.

Despite great progress in understanding the associations between genetic variants and traits in humans owing to genome-wide association study using a number of SNP markers, the effect of local adaptation on the difference in phenotype among human populations remains to be studied. A nonsynonymous single nucleotide polymorphism (SNP), rs17822931-G/A (538G>A; Gly180Arg), in the *ABCC11* gene determines human earwax type. The population frequency of rs17822931-A, which leads to dry earwax in a recessive manner, is much higher in Asians than in Africans, and the heterozygosity around rs17822931 is markedly reduced in Asian populations, implying that positive selection has acted on rs17822931-A in Asians. In this study, the selection intensity of rs17822931-A in East Asians was estimated by analyzing two microsatellite loci flanking rs17822931 in African (HapMap-YRI) and East Asian (HapMap-JPT and HapMap-CHB) populations. Under the assumption of a recessive selection model, computer simulations suggested that the selection intensity of rs17822931-A had been 0.01 in East Asians, and rs17822931-A is 40,120 years old. The present results provide a striking example that local adaptation has played a significant role in phenotypic diversification of human trait.

1456/T

The Thule Migration: Genetic Perspectives from the North Alaskan Slope. *M. Rzhetskaya, L. Armstrong, M.G. Hayes.* Northwestern University, Chicago, IL.

The nature of the Thule expansion from north Alaska to the Eastern Canadian Arctic appears to have been a very rapid migration, taking perhaps only 100-200 years for Alaskan Thule populations to reach their eastern terminus in Greenland. This migration is associated with a clear signal of near population replacement; the migrating Thule replacing, and perhaps interbreeding with, late Paleo-Eskimo populations - the Dorset. The Inupiaq speaking populations across northern North America are virtually monomorphic for mitochondrial haplogroup A (the remainder are D [<3%]), and this reduced genetic variability is not unexpected in small, isolated populations, such as the prehistoric Thule are likely to have been. Very little is known genetically of the North Alaskan Inupiat population, the local descendant of the most-likely source population for the subsequent Thule/Inuit inhabitants of the Eastern North American Arctic. Herein, we present our preliminary investigations into the mtDNA and haplotype frequencies of Inupiat populations from the Alaskan North Slope, and discuss the placement of this population in the geographical and temporal context of North American Arctic Prehistory.

Using standard methods, HVS1 of the mtDNA genome was sequenced using DNA extracted from saliva samples of consenting adults residing in four Alaskan North Slope communities. There is considerable variation among the four communities investigated although they hold the general North American Arctic pattern of haplogroup A being most common followed by haplogroup D. Most interestingly, the frequency of haplogroup D (14/102, or 13.7%) among the aggregate North Slope population is the highest observed among Inuit/Inupiaq speakers in the North American Arctic. When compared to the sub-haplogroup frequencies of their neighbors, the pattern of A2a and A2b frequencies is most similar to Siberian Eskimo. Haplogroup D3, which is found in among Greenland and Canada inhabitants, is found in North Slope populations, as is D2, which is found among Aleut and Siberian Eskimos and ancient Paleo-Eskimos. All Neo-Eskimo Eastern Arctic haplotypes are present at the North Slope, at least among contemporary populations, suggesting that the North Slope is a good source for Neo-Eskimo populations further east.

1457/T

Combining signatures of selection and disease-associated loci in three ethnic groups. *X. Sim[1], R.T.H. Ong[1,2], W.T. Tay[3], C. Suo[1], T.Y. Wong[3,4,5], E.S. Tai[5,6], K.S. Chia[1,5], Y.Y. Teo[1,5,7,8].* 1) Centre for Molecular Epidemiology, National University of Singapore, Singapore, Singapore; 2) NUS Graduate School for Integrative Science and Engineering, National University of Singapore, Singapore; 3) Singapore Eye Research Institute, Singapore National Eye Centre, Singapore; 4) Centre for Eye Research Australia, University of Melbourne, Australia; 5) Department of Epidemiology and Public Health, National University of Singapore, Singapore; 6) Department of Medicine, National University of Singapore, Singapore; 7) Department of Statistics and Applied Probability, National University of Singapore, Singapore; 8) Genome Institute of Singapore, Agency for Science, Technology and Research, Singapore.

Natural selection leaves an imprint on the genetic architecture of populations at the selected site and provides an insight to the evolutionary adaptations of the human genome. Often, the roles of environmental factors that shaped the selection or targets of the selection are not immediately clear. The study of positive selection therefore hinges on an understanding of the genotype-phenotype relationships and how these association signals could be rooted in regions of positive selection. Darwinian medicine has suggested that vulnerabilities to diseases are shaped by selection pressures and the process of selection has an important role in shaping the humans evolved defenses against infectious and immune diseases. Hence it is of great interest to combine evidences of selection and disease association to enhance the understanding of phenotype-genotype relationships in both chronic and infectious diseases. Different statistical methods have been developed to detect signatures of positive selection across the frequencies spectrum. While multiple populations are more useful to detect selection events that has risen to fixation in one population and not in another, a characteristic of recent signature of positive selection (at frequencies of 30% to 80%) is unusually extended haplotypes that rise in frequency rapidly as recombination has yet to break down the long haplotype while the selected variant is passed down preferentially. With the availability of genome-wide data on three ethnic groups in Singapore, Chinese, Malays and Asian Indians of 2500 individuals in each cohort and sophisticated statistical algorithms such as iHS and XP-EHH, we surveyed the genome for selection signatures and associations with common diseases such as Type II Diabetes and complex traits including lipids traits. Our findings suggest the overlapping of selection signatures and diseases-associated loci could elucidate differences in diseases susceptibility in different populations and the importance of environmental variations across populations.

1458/T

Natural selection and sex-specific demography shape patterns of genetic variation across the genome. *J.D. Wall[1], A.E. Woerner[2], F.L. Mendez[3], J.C. Watkins[4], M.P. Cox[2], M.F. Hammer[2, 3].* 1) Inst Human Gen, Univ California, San Francisco, San Francisco, CA; 2) ARL Division of Biotechnology, University of Arizona, Tucson, AZ; 3) Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ; 4) Department of Mathematics, University of Arizona, Tucson, AZ.

An analysis of six complete European genomes reveals that the ratio of X-linked to autosomal diversity deviates from the expected value of three-quarters. However, the direction of this deviation depends on whether a particular sequence is close to or far from the nearest gene. This pattern may be explained by stronger locally acting selection on X-linked versus autosomal genes, combined with larger effective population sizes for females than for males.

1459/T

Genetic Variation and Positive Selection at the WFDC locus in Hominids. *Z. Ferreira[1,3], A. Andres[1], W. Kretzschmar[1], J. Mullikin[1,2], W. Swanson[4], K. Gonder[6], S. Tishkoff[5], A. Stone[7], E. Green[1], B. Hurle[1], NISC Comparative Sequencing Program.* 1) NHGRI, NIH, Bethesda, MD; 2) NIH Intramural Sequencing Center, Bethesda, MD; 3) IPATIMUP Rua Dr. Roberto Frias, s/n 4200-465 Porto, PORTUGAL; 4) University of Washington Seattle, WA; 5) Departments of Genetics and Biology, University of Pennsylvania, Philadelphia, PA; 6) Department of Biological Sciences University at Albany, State University of New York, Albany NY; 7) Department of Anthropology, Arizona State University, Tempe, AZ.

The whey acidic protein (WAP) four-disulfide core domain (*WFDC*) genes encode protease inhibitors with roles in innate immunity and regulation of the endogenous protease kallikreins (*KLK*). Through a comparative genomics strategy that involved the re-sequencing of the *WFDC* centromeric sub-locus in twelve primates, it was previously shown that a striking number of contiguous *WFDC* genes and the neighboring seminal genes (semenogelin I and II - *SEMGI* and *SEMGII*) show strong patterns of positive selection in primates. It is unusual to find a series of tightly linked genes that all show robust patterns of positive selection, as hitchhiking effects make it difficult for successive selective sweeps of tightly linked loci. Despite this co-localization of positively selected genes, the *WFDC* locus does not stand out as particularly unusual in humans with respect to known genetic variation databases. We tested whether the levels and patterns of genetic variation within the *WFDC* locus differ among hominid species. We sequenced 19 genes of the *WFDC* locus, plus 54 evenly spaced non-coding regions in 71 humans from three HapMap populations and 68 western equatorial African chimpanzees from three *Pan* subspecies. A set of 47 unlinked and neutrally evolving loci was also surveyed to assess the general patterns of diversity. Overall, we generated ~23 and ~13 Mb of high-quality sequence data from humans and chimpanzees, respectively, enabling the identification of 541 human and 847 chimpanzee single-nucleotide polymorphisms (SNPs) and 487 human-chimp fixed differences. Ongoing research includes detecting recent positive selection events using classical neutrality tests and identifying both incomplete and complete sweeps as well as detecting differences at inter and intra-species level. Test significance is being assessed through coalescent simulations under different demographic scenarios. Unique features of this study include examining the dynamic nature of the *WFDC* locus in primates, capitalizing on the sheer number and verified demographic origin of the chimpanzee samples, and noting the lack of ascertainment bias in SNP collection. Our efforts offer insights on the evolutionary forces driving the rapid diversification of *WFDC* and *SEMG* genes in hominoids, and improve our knowledge about the biological dynamics of rapidly evolving genomic regions in primates.

1460/T

Patterns of genetic variation at CD-36 in diverse African populations: An examination of nucleotide variation at a malaria susceptibility locus. *F. Gomez[1,2], S.A. Tishkoff[1].* 1) Departments of Genetics and Biology, School of Medicine and School of Arts and Sciences, University of Pennsylvania, Philadelphia, PA; 2) Hominid Paleobiology Doctoral Program, Center for the Advanced Study of Hominid Paleobiology, Department of Anthropology,George Washington Univ, Washington, DC.

A central focus of human population genetics is creating a clear understanding of the evolutionary and demographic events that have shaped human genomic variation. It is clear that infectious disease has played an important role in driving human evolution and human population genomic variation. Additionally, malaria has arguably been one of the strongest selective forces during recent human evolution. In order to characterize signatures of natural selection due to malaria infection, and to identify potential functional variants, we have studied nucleotide variation at the *CD-36* locus. During a malarial infection, CD-36 that is expressed on vascular endothelial cells acts as a receptor for *P. falciparum* parasitized erythrocytes and is involved in the pathogenesis of cerebral malaria. Studies of nucleotide diversity at this locus have suggested that specific alleles may affect susceptibility to malaria. However, the data that describe allelic variation at this locus and its relationship to malaria susceptibility are not consistent across studies. Therefore, we have chosen to re-sequence *CD-36* in diverse African populations to better understand the scope of genetic variation at this locus and to identify variants that may influence susceptibility to malaria. A panel of ~300 individuals originating from Nigeria, Cameroon, Ghana, Tanzania, Kenya, and the Sudan were re-sequenced across an ~13 kb region of *CD-36*. Individuals from the Hap Map Chinese and European populations were also re-sequenced as comparative data. Using these data we have characterized levels of diversity and have used tests of neutrality to identify signatures of natural selection. We have also used these data to describe the frequencies of alleles that are known to cause CD-36 deficiency in African and Asian populations.

1461/T

Frequency spectrum estimation using low coverage sequence data. *S. Gopalakrishnan[1], P.L. St Jean[2], L. Li[2], M.G. Ehm[2], M.R. Nelson[2], S.L. Chissoe[2], J.C. Whittaker[3], V.E. Mooser[4], J. Novembre[5,6], S. Zöllner[1,7].* 1) Center for Statistical Genetics, Department of Biostatistics, University of Michigan, Ann Arbor, MI; 2) GlaxoSmithKline, Dept of Genetics, RTP, NC; 3) GlaxoSmithKline, Dept of Genetics, Harlow UK; 4) GlaxoSmithKline, Dept of Genetics, Swedeland, PA; 5) Department of Ecology and Evolutionary Biology, University of California Los Angeles, Los Angeles, CA; 6) Interdepartmental Program in Bioinformatics, University of California Los Angeles, Los Angeles, CA; 7) Department of Psychiatry, University of Michigan, Ann Arbor, MI.

Large sequencing projects, such as the 1000 Genomes project, provide us an opportunity to examine population genetics questions with sequence level data. However, the design of many of these projects involves low to moderate per-individual coverage. Under this design, the estimated genotypes for individuals are biased towards the reference sequence. Pooling data from all the samples can overcome this bias for sites with intermediate minor allele frequencies. This leads to an underestimation of the number of sites with low minor allele frequencies. It is important to accurately estimate the site frequency of these rare variants in order to make unbiased population genetic inferences. Here, we introduce a method to use complete sequence alignment information to estimate the site frequency spectrum of rare variants from low coverage data. At each site, we combine the likelihood of the aligned sequence data with theoretical population genetics priors in a Bayesian framework to estimate its contribution to the frequency spectrum. We combine evidence across all sites to estimate the site frequency spectrum. We use importance sampling to make the estimation computationally tractable at higher allele frequencies and large sample sizes. We test our method using simulation schemes with haplotypes simulated to mimic populations with various levels of diversity. We quantify the performance of our method using the Kullback-Leibler divergence between the true and estimated frequency spectra. In addition, we use resequencing data from a dataset with the exons of 202 genes sequenced at an average depth of 30x in ~ 15,000 individuals, as part of the QPOC study. We generate low coverage data by thinning the sequence data. We verify that we recover the original site frequency spectrum by applying our method to the thinned dataset. Using the true, corrected and uncorrected frequency spectra, we quantify the effect of bias in the frequency spectrum estimation on downstream population genetic inferences.

1462/T

Evidence of natural selection at genetic regions associated with HIV-1 control is geographically restricted. *Y.C. Klimentidis[1], B. Aissani[2], S. Shrestha[2].* 1) Section on Statistical Genetics, Department of Biostatistics, University of Alabama at Birmingham, Birmingham, AL; 2) Department of Epidemiology, University of Alabama at Birmingham, Birmingham, AL.

HIV susceptibility and pathogenicity exhibit both inter-individual and inter-group variability. The etiology of inter-group variability is still poorly understood, and could be partly linked to genetic differences between groups. These genetic differences may be traceable to different regimes of natural selection in the 60,000 years since human radiation out of Africa. Here, we examine population differentiation and haplotype patterns at several loci identified through genome-wide association studies on HIV-1 -control, as determined by viral-load setpoint in Caucasian and African-American populations. We use the genome-wide SNP dataset on the Human Genetic Diversity Panel of 53 world-wide populations to compare measures of FST and extended haplotype homozygosity (EHH) at these candidate regions to the rest of the genome. We find that the Europe-Middle East pair-wise FST in the associated regions is elevated compared to the rest of the genome, while the sub-Saharan Africa-Middle East pair-wise FST is very low, suggesting that genetic differentiation (diversifying/positive selection) occurred outside of sub-Saharan Africa, while balancing or purifying selection occurred in sub-Saharan Africa. We also find greater EHH, indicative of recent positive selection at these associated regions, among all population sub-groups except for sub-Saharan Africans and Native Americans. These findings corroborate findings from other studies suggesting recent evolutionary change at immunity-related regions among Europeans, and shed light on the potential genetic and evolutionary origin of population differences in HIV-1 control.

1463/T

Retrotransposons in the orangutan (*Pongo*) lineage: a new evolutionary tale. *M.K. Konkel[1], J.A. Walker[1], B. Ullmer[2], L.G. Chemnick[3], O.A. Ryder[3], R. Hubley[4], A.F.A. Smit[4], M.A. Batzer[1] for the Orangutan Genome Sequencing and Analysis Consortium.* 1) Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803, USA; 2) Department of Computer Science, Center for Computation and Technology (CCT), Louisiana State University, Baton Rouge, LA 70803, USA; 3) Conservation and Research for Endangered Species (CRES), Zoological Society of San Diego, San Diego, CA 92112, USA; 4) Institute for Systems Biology, Seattle, WA 98103, USA.

Orangutans *(Pongo)* are the only living Asian ape and are highly endangered. We investigated the mobile DNA composition (mobilome) of the orangutan draft genome sequence derived from a female of Sumatran origin *(Pongo abelii)*. Similar to other primate genomes, about half of the orangutan draft genome sequence is comprised of repetitive sequences. As expected, no DNA transposon activity was detected in the orangutan lineage. L1 (long interspersed element 1, LINE1) is the only active autonomous non-LTR retrotransposon in the orangutan lineage and shows a mostly linear evolution. The orangutan-specific L1 lineage appears to be derived from L1PA3. SVA elements have been active throughout the evolution of orangutans and appear to be currently undergoing retrotransposition. Similar to L1, the orangutan-specific SVA subfamilies show a mostly linear evolution. We found evidence of expansion of SVA and L1, with ~1800 and ~4700 orangutan lineage-specific insertions, respectively. This translates to a retrotransposition rate comparable to other sequenced primate genomes. In contrast, *Alu* elements appear to be relatively quiescent and have propagated at a very low rate in orangutans. The identification of polymorphic and population-specific *Alu* insertions indicates that *Alu* retrotransposition may be ongoing, albeit at a very low rate. In addition, we investigated the population structure within orangutans. For this purpose, we performed a Structure analysis with 37 orangutans - 18 Bornean *(Pongo pygmaeus)* and 19 Sumatran - using polymorphic retrotransposon markers. These elements were selected from the orangutan draft genome and also from Illumina paired-end reads from a Bornean orangutan. The orangutans of Bornean origin were clearly distinct from the Sumatran population with almost no evidence of ongoing admixture. In addition, Sumatran orangutans showed clear evidence of population substructure. The distinction of Sumatran from Bornean orangutans supports the relatively recent notion that Bornean and Sumatran orangutans represent separate species.

1464/T

Humans and other primates show varying selection pressures on Matrix Metalloproteinase 9 (MMP9), a gene responsible for placental invasion. *A. Lobell, M. Ruvolo.* Department of Human Evolutionary Biology, Harvard University, Cambridge, MA.

The ability to produce healthy offspring is critical to evolutionary fitness and is a phenotypic trait controlled largely by reproductive genes. Among humans, polymorphisms in the placentally-expressed Matrix Metalloproteinase 9 (MMP9) gene are associated with pathologies of pregnancy caused by insufficient transfer of nutrients across the placenta. While the negative health effects of these pathologies are well known, the evolutionary genetics of human placental invasion has not been characterized. Understanding the molecular evolution of human placental invasion is important because extremely deep placental invasion is a uniquely human trait that likely underlies key adaptations including increased brain size. Humans' deep placental invasion arose in the context of more ancient evolutionary changes that increased placental invasion in haplorhine primates (tarsiers, monkeys, apes, and humans). Our study investigates the forces that shaped MMP9 evolution in humans and other haplorhines in order to link adaptive change in placental invasion to its underlying genetic mechanisms. Using maximum likelihood analysis and a new full Bayesian test for positive selection on complete MMP9 coding sequences from 18 mammalian species, we demonstrate statistically significant signals of adaptive evolution ($\omega > 1$) in MMP9 only at the phylogenetic points in haplorhine evolution where increases in placental invasion arise. An intensification of positive selection is detected in regions critical to MMP9's enzymatic ability within the human/chimpanzee/gorilla clade and along the human lineage. These results suggest that increased placental invasion was the primary selection pressure acting on MMP9 in non-human primates and along the human lineage. However, an analysis of 10.5 Kb of resequencing data from 50 diverse human chromosomes does not find signals of recent adaptation for increased placental invasion in MMP9. Instead, patterns of variation in human MMP9 suggest the action of competing selection pressures that may be related to MMP9's role as a human oncogene.

1465/T

Frequencies of the Alzheimer's disease associated *TOMM40* poly-T allele in different ethnic groups. *M.W. Lutz[1], A.M. Saunders[1], D.G. Crenshaw[1], I. Grossman[2], D.K. Burns[1,2], T. Swanson[2], K. Whitfield[3], M. Hauser[4], A.D. Roses[1,2].* 1) Deane Drug Discovery Institute, Duke University, Durham, NC; 2) Cabernet Pharmaceuticals, Inc, Durham NC; 3) Psychology and Neuroscience, Duke University, Durham, NC; 4) Medicine, Duke University, Durham, NC.

A variable length poly-T polymorphism, rs10524523 (523), in *TOMM40* is significantly associated with age of onset of late-onset Alzheimer's disease (AD) for >85% of the Caucasian population. Phylogenetic analyses demonstrate that for Caucasians, *APOE4* is in LD with long (L, 21-30 T residues) 523 poly-T alleles 98% of the time but there are two distinct groups of polyT lengths in LD with *APOE3*: short alleles (S, 14-18 T residues) or very long alleles (VL, 30-39 T residues). Moreover, the L and VL poly-T alleles are associated with earlier age of onset of AD. Allele length frequency distributions for the poly-T were evaluated in diverse population samples as a first step towards translating the Caucasian findings to worldwide populations. Han Chinese, Japanese and Korean (Far Eastern, FE) and Ghanan population samples were genotyped for length of the 523 poly-T and for *APOE* genotype. The lengths of the poly-T alleles linked to *APOE4* were similar in the FE groups and Caucasians (21-30 T residues). In the Ghanan sample, there were no L alleles of the length that is most common in the other populations. For FE and Ghanans, there were also two distinct length distributions of poly-T tracts linked to *APOE3*. However, the length distributions of the 523 alleles (S and VL) connected to *APOE3* were shifted to shorter lengths in all of the FE populations. For Caucasians, the S alleles were rarely less than 14 T residues in length whereas S alleles of 11-13 T residues were common in the FE groups and the frequency of alleles of 15-16 residues was relatively reduced in these populations. The FE VL forms were generally 27-32 residues long whereas the length of the Caucasians VL alleles peaked at 33-37 residues. The Ghana sample was enriched for S alleles compared to Caucasians, perhaps linked to the frequent *APOE2* allele in this population, but there was an absence of the shorter length S alleles seen in FE groups. These data demonstrate that the distributions of 523 poly-T lengths differs in populations with different evolutionary histories. The size distributions may differ in various ethnic groups, but the risk of AD may be ultimately be predicted by the specif1c sizes of two 523 alleles inherited by each individual. Formal phylogenetic mapping of multiple ethnic groups is currently in progress.

1466/T

Detecting population structure and exploring demographic history. *C. Mair, V. Macaulay.* University of Glasgow, Glasgow, United Kingdom.

It is well established that genetic differences between human populations are associated with geographical distance. The population structure that such observations identify can be studied by various techniques. We will concentrate on principal components analysis (PCA), a dimension-reduction technique with a long history in population genetics, but which has become popular recently as a straightforward way of controlling Type 1 errors in association studies that arise because of (usually hidden) population structure. The emphasis here is different: to learn about the population structure itself by the method. By using the most significant components of single-nucleotide polymorphism (SNP) variation obtained through PCA, we will examine how the structure captured in these components allows us to estimate the parameters in simple demographic models. We will consider a population split model parameterized by split times T when ancestral populations fission into independent daughter populations, as well as an island model in which populations exchange migrants at a constant rate m arbitrarily far into the past. Do the first few principal components contain information to distinguish these models and, if so, can their respective parameters be well estimated? These questions will be addressed using SNP-like data sets simulated under the coalescent process.

1467/T

Human and great ape blood lipid profiles indicate novel metabolic differences that could influence physiological and cognitive processes. *K. Ramaswamy[1], P.A. Watkins[2], A.B. Moser[2], C.B. Toomer[2], S.J. Steinberg[2], H.W. Moser[2], M.W. Karaman[1], P.K. Dranchak[1], J. Hey[3], K.D. Sigmund[4], D.R. Lee[5], J.J. Ely[5], O.A. Ryder[6], J.G. Hacia[1].* 1) Department of Biochemistry and Molecular Biology, University of Southern California, Los Angeles, CA, 90033; 2) Hugo W. Moser Research Institute at Kennedy Krieger, and Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD, 21205; 3) Department of Genetics, Rutgers University, Piscataway, NJ 08854; 4) Department of Preventive Medicine, University of Southern California, Los Angeles, CA 90089; 5) Alamogordo Primate Facility, New Mexico, NM 88330; 6) Institute for Conservation and Research, Zoological Society of San Diego, Escondido, CA 92027.

It has been proposed that anatomical differences in human and great ape guts arose in response to the historic diets and energy demands of each species. To investigate functional genomic consequences of these differences, we compared their physiological levels of phytanic acid, a branched chain fatty acid that can be derived from the microbial degradation of chlorophyll in ruminant guts. Phytanic acid is a potent activator of PPAR and RXR transcription factors and can modulate the activity of lipid metabolic pathways. Despite their trace dietary phytanic acid intake, all great ape species had elevated red blood cell (RBC) phytanic acid levels relative to humans on diverse diets. Furthermore, chimpanzees, unlike humans, showed sexual dimorphism in RBC phytanic acid levels. We provide indirect evidence that great apes, unlike humans, derive significant amounts of phytanic acid from the hindgut fermentation of plant materials. This would represent a novel reduction of metabolic activity in humans and affect the interpretation of human and great ape transcriptomes. Furthermore, we observed species-specific differences in RBC plasmalogen (ether phospholipid) levels and molecular composition. Similar to phytanic acid, plasmalogens are metabolized by peroxisomes and, in addition, are critical for brain and male reproductive functions. We provide evidence that cross-species differences in plasmalogen metabolism are responsible our observations and likely affect the membrane composition of diverse tissues. Overall, we suggest that differences in branched chain fatty acid and plasmalogen metabolism among humans and great apes influence the functions of major organ systems and processes ranging from cognition to reproductive biology.

1468/T

Music as a novel marker in the study of prehistoric human migrations. *T. Rzeszutek[1], P. Savage[1], V. Grauer[2], S. Brown[1].* 1) Psychology, Neuroscience & Behaviour, McMaster University, Hamilton, Ontario, Canada; 2) Independent Scholar, Pittsburgh, PA.

The study of prehistoric human population history is often fraught with controversy owing to incongruent evidence among various markers of present-day genetic and cultural diversity. While archaeological evidence can be used to calibrate the conclusions drawn from present-day diversity, the fickle nature of the fossil record leaves some migration histories unresolved. Our work analyzes the potential of music - in particular, vocal music - to serve as novel migration marker, bolstering established migration work and shedding light on regions of the world whose settlement history is contested. One such migration is the recent expansion of Austronesian-speaking peoples across the Pacific within the last 6000 years. The dominant hypothesis posits a recent origin in Taiwan, with a rapid movement southwards and eastwards to populate Polynesia during the following 3500 years. While this model is strongly supported by both archaeological evidence and the present-day distribution of linguistic diversity, our goal was to analyze whether music could serve as a novel line of evidence in the study of Pacific prehistory. A critical concern regarding any migration marker is its time depth. In order to examine this for music, we analyzed correlations between musical diversity and mitochondrial-DNA diversity in 9 Taiwanese aboriginal tribes for which both types of data were available. A sample of 226 choral songs was analyzed using 39 binary characters representing significant structural features of music (e.g., rhythm, interval size, melodic contour, etc.). The musical samples were restricted to ritual musics, which constitute the most conservative (i.e., slowly changing) component of a culture's repertoire. Mantel tests showed a significant correlation between musical distance and genetic distance among these 9 tribes, suggesting that music may have a time depth comparable to widely-used genetic markers like mitochondrial DNA. This work demonstrates that music has the potential to enrich the conclusions drawn from other markers, and establishes methods for employing it as a tool in the study of prehistoric human movements throughout the world. At the same time, we want to capitalize on music's own unique dynamics of change over time and place, particularly its capacity for admixture. In other words, music might not only be able to support the narratives told by other migration markers but shed new light on the histories of population movement and cultural contact.

1469/T

Genetic Structure and Diversity of Gujjar Population of North India. *J. Saini, K. Matharoo, B. Ddoza, A.J.S. Bhanwer.* Department of Human Genetics, Guru Nanak Dev University, Amritsar, Punjab, India.

Molecular markers such as Alu insertion polymorphisms and RSP's can be used to define the differential Genetic structure or Genomic Diversity in Human populations. The present population study is targeted on Gujjar population of North India, which is a nomadic, pastoral tribe of North India with historical importance but uncertain origin. Gujjars are included in group of transhumant which migrate to Punjab during winters and back to Kashmir and Ladakh during summers. Their primary occupation is cattle rearing and marginal farming. Gujjars follow monogamy, clan (gotra) exogamy and caste endogamy. Gujjar population follows endogamy and thus has differential genetic structure due to separation from other populations. However, there is paucity of molecular genetic studies deciphering the genetic structure and diversity of Gujjar population in North India. Thus keeping in mind the whole scenario, the present study was designed to analyze the molecular markers in Gujjar population of North India. A total of 131 individuals of Gujjar population of North India were analyzed for Alu insertion polymorphism at PV92, ACE and APO loci; and Restriction Segment Polymorphism at ESR loci. Statistical analysis was done using SPSS 16.0. Heterozygosities for all three loci were calculated using the estimated allele frequencies for present population. Hardy Weinberg equilibrium was tested through $\chi 2$ goodness of fit test. Nei's measures of gene diversity, FST and FIS have been calculated. In present analysis all 4 studied loci (PV92, ACE, APO and ESR) were found to be polymorphic. The heterozygosity for all the loci ranged from 0.213-0.564. The average heterozygosity for each of the loci was substantially different from each other with ACE loci exhibiting maximum heterozygosity and APO exhibiting minimum heterozygosity of 0.564 and 0.213 respectively. Very high frequency value for (+) allele at APO insertion loci shows that this locus is reaching fixation in Gujjar population. Value of FIS indicates a maximum reduction of heterozygosity of 18.7% due to inbreeding ($P < 0.001$) at PV92 loci. This indicates that PV92 is derived from one common ancestor which led to substantial hidden inbreeding in Gujjar population. It was estimated from pair wise Genetic Distance analysis with other reported population from the region that Gujjar population has maximum genetic affinity with Jat Sikh population followed by Bania, Rajput and Brahmins.

1470/T

Risk of tuberculosis among close contacts to TB patients: the role of CCL5 and CCR5 gene polymorphisms and environmental factors. *M.R. Reichler[1], C.C. Luo[1], B. Chen[1], E. Sigman[1], F. Maruri[2], T. Sterling[3] for the TB Epidemiologic Studies Consortium.* 1) Division of TB Elimination, Centers for Disease Control and Prevention, Atlanta, GA; 2) Tennessee Department of Health, Nashville, TN; 3) Vanderbilt University, Nashville, TN.

Background: Single nucleotide polymorphisms (SNPs) in chemokine ligand 5 (CCL5) and chemokine receptor 5 (CCR5) have been implicated in susceptibility to HIV infection. Chemokines play an important role in host immune response to tuberculosis (TB), but the contribution of SNPs in chemokine genes to TB susceptibility is not known.

Methods: We enrolled U.S.-born contacts with latent TB infection or active TB disease with >180 hours of exposure to smear+, culture+ TB patients at 9 sites. Blood was collected and genotyped for CCL5 SNP IN 1.1 and CCR5 SNP Δ32. Epidemiologic data were collected by contact interview. Pair-wise analysis was conducted using X^2 tests with homozygous wild genotype as the referent group. This analysis was limited to contacts of black race/ethnicity.

Results: Among 285 contacts, 21 had active TB and 264 had a positive tuberculin skin test (TST+) but not active disease. Compared with TST+ contacts, contacts with active TB were more likely to have the homozygous mutant genotype for CCL5 IN 1.1 (OR=3.31, P=.13). There was no association for CCR5 Δ32 (OR=1.25, P=.76). For CCL5 IN 1.1, the association between mutant genotype and TB was significantly increased for contacts who used alcohol (OR=20.5, P<.001) or street drugs (OR=11.25, P=.04).

Conclusions: In this U.S. population, active TB in contacts after exposure to *M. tuberculosis* was associated with homozygous mutant genotypes for CCL5 gene SNP IN 1.1, particularly for contacts who used alcohol or street drugs. These findings suggest that the risk of active TB after *M. tuberculosis* exposure may be modulated by genes and environmental factors such as use of alcohol and street drugs.

1471/T

The Genetic Basis and Evolutionary History of PTC Bitter Taste Perception in Africa. *M.C. Campbell[1], A. Ranciaro[1], A. Froment[2], D. Zinshteyn[3], J.-M. Bodo[4], D. Drayna[5], P. Breslin[6,7], S.A. Tishkoff[1,3].* 1) Dept Gen, Univ Pennsylvania Sch Med, Philadelphia, PA; 2) Ecoanthropology and Ethnobiology Musée de l'Homme, Paris, France; 3) Department of Biology, University of Pennsylvania, Philadelphia, PA; 4) Ministry of Scientific Research and Innovation, Yaoundé, Cameroon; 5) National Institute on Deafness and Other Communication Disorders, Rockville, MD; 6) Monell Chemical Senses Center, Philadelphia, PA; 7) Department of Nutritional Sciences, Rutgers University, New Brunswick, NJ.

Bitter taste perception is an important dietary adaptation which may have evolved to prevent the accidental ingestion of harmful plant toxins. The ability to taste the bitter synthetic compound phenylthiocarbamide (PTC) is a highly variable trait in humans and is also correlated with the ability to detect naturally bitter substances in food. A large proportion of the phenotypic variance in PTC taste sensitivity has been attributed to genetic variability at TAS2R38, a bitter taste receptor gene, located on chromosome 7. In order to better understand the genetic basis and evolutionary history of PTC taste perception, we examined sequence variation within a 2975 bp region encompassing the 1002 bp coding exon of the TAS2R38 gene, in 588 individuals from more than 50 ethnically diverse populations from East and West Central Africa, as well as in a comparative sample of 132 non-Africans from different geographic regions. We also examined genotype-phenotype associations in a large subset of Africans, totaling 463 individuals, from the above sampled populations. Our analyses uncovered higher levels of nucleotide and amino acid haplotype variability, including an excess of rare non-synonymous polymorphisms, in Africans relative to non-Africans. The estimated time to the most recent common ancestor (TMRCA) of genetic variation within the TAS2R38 coding region is ~2.3 million years, consistent with the pattern expected under ancient balancing selection. The diverse amino acid haplotypes present in African populations were also associated with a broader range of PTC taste sensitivity than is typically observed outside of Africa. In addition, we found that several rare non-synonymous polymorphisms significantly modify PTC sensitivity in African populations, demonstrating the effect of single amino acid substitutions on bitter taste perception. This research provides further information regarding the genetic basis of phenotypic variation in Africa, and serves as a model system for understanding the influence of natural selection, as well as common and rare amino acid variation, on variable traits in humans.

1472/T

Ancient and recent demographic events influence mitochondrial DNA diversity in an immigrant Basque population. *M. Davis, S. Novak, G. Hampikian.* Department of Biological Sciences, Boise State University, Boise, ID.

The Basques are an ancient people, considered by many anthropologists to represent the oldest extant European population. Because of this, they have been the subject of numerous sociological and biological investigations. The Basque Diaspora, a relatively recent demographic expansion of the Basque population, has until now been overlooked in genetic studies. Samples were taken from 53 individuals with Basque ancestry in Boise, Idaho, and the mitochondrial DNA (mtDNA) sequence variation of the first and second hypervariable regions were determined. Thirty-six mtDNA haplotypes were detected in the sample. Comparing the genetic diversity in the Idaho sample with other Basque populations, signatures of founder effects were observed, consistent with both the recent and ancient history of Basque mitochondrial lineages. There has been a marked alteration of haplogroup frequency and diversity, and there is a slight reduction in other measures of diversity in the NW Basque population compared to the native Basque population. We have found a relatively high percentage of the Cambridge Reference Sequence (rCRS) haplotype for hypervariable regions I and II, which is absent in previous studies of Basque mtDNA, and rare in other Spanish populations. The amount of nucleotide diversity is consistent with a sample that is predominantly haplogroup H, which is especially common in the Basque regions of Europe, due to ancient migrations and expansions out of glacial refugia. This is the first report of mtDNA diversity in an immigrant Basque population, and we find that the diversity in NW Basques can be explained by the recent history of migration, as well as the phylogeography and diversity of the major European haplogroups.

1473/T

Defining population structure within Southern Africa to advance studies of human disease. *V.M. Hayes[1], D.C. Petersen[2], A.J. Schork[3], R-A. Hardie[2], R. Wilkinson[4], P. Venter[5], S.C. Schuster[6], N.J. Schork[3].* 1) Human Genomics, J. Craig Venter Institute, San Diego, CA; 2) Lowy Cancer Research Center and Children's Cancer Institute Australia, University of New South Wales, Randwick, NSW, Australia; 3) The Scripps Research Institute and The Scripps Translational Science Institute, San Diego, CA; 4) Blood Transfusion Services, Windhoek, Namibia; 5) Department of Health Sciences, University of Limpopo, South Africa; 6) Department of Biochemistry and Molecular Biology, Pennsylvania State University, State College, PA.

Although Africa is home to one-sixth of the world's population and is the epicenter of many globally significant infectious diseases, medical research tailored to African populations has been limited. As a result, the benefits of genome-wide association studies for defining human disease susceptibility, resistance and drug response, have not materialized for the continent. One of the main reasons for this limitation is that African representation in current DNA databases has largely been restricted to the Yoruba people of Nigeria. In an attempt to define the extent of genetic diversity within Africa, in 2010 the first Southern African genomes were sequenced, representing the diverse Niger-Congo B (Bantu) and Khoisan (click-speaking) linguistic groups. Whole genome and/or exome sequencing of five individuals resulted in the identification of 1.3 million novel DNA variants. In this study we use both current content genotyping arrays (>1 million SNPs) and the novel Southern African variant content (927,000 informative SNPs) to define the genetic diversity and population structure that exists within Southern Africa. It is clear from our studies that the Khoisan people not only represent an ancient divergent and broadly defined grouping with unique sub-population genetic structure, but are genetically distinct from the Bantu and Yoruba people. We also demonstrate a highly diverse Bantu population structure that is genetically distinct from the West African Yoruba. Ultimately, the smaller blocks of linkage disequilibrium in these older and diverse populations will provide an opportunity to overcome some of the limitations of current Eurocentric studies by facilitating disease-relevant casual variant identification. This data will drive host-genetic research efforts at the NIAID sponsored Genome Sequencing Center for Infectious Diseases at the JCVI.

1474/T

Western Eurasian Y chromosomes found in the Chinese Salar ethnic group. *Y. Lu, H. Li.* MOE Key Laboratory of Contemporary Anthropology, School of Life Sciences, Fudan University, Shanghai 200433 China.

Salar is a small Western-Turkish-speaking population living mostly in Qinghai province of China. The most similar languages to Salar are all far in Turkmenistan. Historical records suggested that they may be descendants of the Turkic nomadic tribes in Central Asia. In this study, 141 Salar Y chromosomes were analyzed for 39 SNP and 14 STR markers to investigate the potential imprints of their western ancestors. The most frequent haplogroup (hg) in this population sample is Hg R, comprising 40% of all Y chromosomes. Most of these Hg R samples belong to R1a1 (M17), which distributes in a wide geographic region including South Asia, East Europe, Central Asia, and South Siberia. Other four Western Eurasian haplogroups (G-2%, H-5%, I-3%, J-3%) were also found in Salar Y chromosome gene pool. These paternal lineages of Salar are absent in their East Asian neighbors but frequent in Central Asia. Y-STR-based analyses also grouped Salar to Central Asians. On the other side, Salar also has low frequencies of the East Asian specific Hg D and Hg O, suggesting possible gene flow from their neighboring populations. This Y chromosome study demonstrated that Salar well keeps the Western Eurasian paternal lineages of their Central Asian ancestors although they may have migrated to Central China for about 800 years.

1475/T

Alleles from SNPs at the SLC24A5 and SLC45A2 genes are associated with the presence of freckles and eye, hair and skin pigmentation in Brazil. *C.T. Mendes-Junior[1], N.C.A. Fracasso[2], L.R. Zanão[1], C.C.F. Andrade[2], M.S. Silva[1], L.A. Marano[2], C.E.V. Wiezel[2], E.A. Donadi[3], A.L. Simões[2].* 1) Departamento de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, 14040-901, Ribeirão Preto-SP, Brazil; 2) Departamento de Genética, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, 14049-900, Ribeirão Preto-SP, Brazil; 3) Divisão de Imunologia Clínica, Departamento de Clínica Médica, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, 14048-900, Ribeirão Preto-SP, Brazil.

SNPs from the SLC24A5 (solute carrier family 24, member 5) and SLC45A2 (solute carrier family 45, member 2) genes have been associated with variation in eye, hair, and skin pigmentation across worldwide populations. Protein products of both genes are involved in cross-membrane transport, and their expression in melanocytes are required to regulate melanogenesis and for melanosome maturation. The present study aimed at verifying the influence of the genetic diversity of such genes in the presence of freckles and determination of eye, hair, and skin pigmentation in a highly admixed population sample. The determination of eye, hair, and skin pigmentation of unknown samples found in crime scenes would be of great value for forensic caseworks. To achieve that goal, a SNP from the SLC24A5 gene [rs1426654 G>A (Ala111Thr)] and five SNPs spread across the SLC45A2 gene [rs732740 (intron 1 T>C), rs3776549 (intron 2 G>A), rs250417 (intron 4 G>C), rs16891982 (exon 5 G>C; Leu374Phe), and rs35394 (intron 5 A>G)] were evaluated in 150 unrelated individuals from the Ribeirão Preto area, a city located at the Northwestern region of the São Paulo State, Southeastern Brazil. DNA was extracted by a salting-out procedure and SNPs were genotyped either by PCR-RFLP or Allele-Specific PCR, followed by Polyacrylamide Gel Electrophoresis (PAGE). Even though SNP rs732740 was monomorphic (considering the 99% threshold), alleles from every remaining SNPs presented statistically significant associations with at least one of the four considered morphological features. It is noteworthy that most of the observed associations concerns SNPs rs1426654 (Ala111Thr) and rs16891982 (Leu374Phe), both of them located at the peptide-coding region of SLC24A5 and SLC45A2, respectively. For instance, alleles 111Thr (rs1426654) and 374Phe (rs16891982) were observed in association with fair pigmentation features (like pale skin, green/blue eyes, and/or blond hair), while alleles 111Ala 374Leu are associated with dark pigmentation. The present results corroborate previous findings and emphasize the role played by SNPs at SLC24A5 and SLC45A2 in the determination of pigmentation aspects of human populations. FINANCIAL SUPPORT: This study was supported by CNPq/Brazil (Grant 478843/2009-7).

1476/T

Geographic approaches to rare SNPs in human populations. *T.B. Mersha[1], R.A. Wilke[2].* 1) Cincinnati Children's Hospital Medical Center, Department of Pediatrics, University of Cincinnati, Cincinnati, OH, USA; 2) Division of Clinical Pharmacology, Vanderbilt University Medical Center, Nashville, TN, USA.

The simultaneous study of genomic loci across numerous populations will facilitate a better understanding of the role of genetic variation in different geographic regions. We examined global allele frequencies across all HapMap populations at 3.7 million SNPs to search for loci under recent selection pressure, with a goal toward identifying variants that contribute to ecotypic phenotypic divergence. By examining private SNPs in HapMap samples from geographically separated populations, we demonstrate that 463 loci (mapping to 38 genes) were fixed (delta= 1). These private loci included four non-synonymous coding SNPs: rs4536103 (NEUROG3), rs1385699 (EDA2R), rs11946338 (ARHGAP24), and rs4422842 (CACNA1B), and regulatory variants in four additional genes (IER5L, NPNT, SESTD1 and EXOC6B) were undergoing recent positive selection. Over all, very few genes in the human genome had extreme allele frequency differences among populations, and the bulk of variations at the nucleotide levels are not visible at the phenotypic level. We explore pathway-oriented strategies to identify signaling mechanisms that may have influenced human adaptation to different environments. These fixed SNPs contribute to variability in several cellular processes. As the clinical community moves toward application of genome-wide SNP scanning in large practice-based cohorts, our pathway-oriented approach has relevance to pharmacogenomic data. An improved understanding of these pathways in genomic regions of fixed loci may help explain race/ethnicity-specific variability in treatment outcome as ancient genes are exposed to modern drugs.

1477/T

Genetic Heterogeneity in Parents of cleft Probands and the Chromosome 8q24 cleft lip/palate associated region. *T. Murray[1], T.H. Beaty[1], J.B. Hetmanski[1], K.Y. Liang[1], I. Ruczinski[1], T. Wu[1], P. Patel[1], R.A. Redett[2], M.L. Marazita[3], J.C. Murray[4], R.G. Munger[5], A. Wilcox[6], R.T. Lie[7], Y.H. Wu-Chow[8], H. Wang[9], S. Huang[9], X. Ye[10], V. Yeow[11], S.S. Chong[12], S.H. Jee[13], K. Christensen[14], A.F. Scott[2].* 1) Dept Epidemiology, Johns Hopkins Univeristy, Baltimore, MD; 2) School of Medicine, Johns Hopkins University, Baltimore, MD; 3) Health Sciences, University of Pittsburgh, Pittsburgh, PA; 4) Children's Hospital, University of Iowa, Iowa City, IA; 5) Utah State University, Logan, UT; 6) Epidemiology Branch, NIEHS/NIH, Durham, NC; 7) University of Bergen, Bergen, Norway; 8) Chang Gung Memorial Hospital, Taoyuan, Taiwan; 9) Health Science Center, Peking University, Beijing, China; 10) School of Stomatology, Wuhan University, Wuhan, China; 11) KK Women's and Children Hospital, Singapore; 12) National University of Singapore, Singapore; 13) Epidemiology and Health Promotion, Yonsei University, Seoul, South Korea; 14) University of Southern Denmark, Odense, Denmark.

The 8q24 region showed the strongest evidence of linkage and association with non-syndromic cleft lip with or without palate (CL/P) in our recently published GWAS among trios of European descent, but was much weaker among Asians. Of the 78 SNPs in this region, 43 had signals; 20 had $p<10^{-8}$ among Europeans. In Asians, only 17 SNPs were nominally ($0.05>p>0.0001$) significant. By examining measures of SNP information content within our 13 recruitment sites (Europe, Asia, US) separately, we searched for clues which might help to explain the difference in statistical signal in 8q24. We conducted principal components analysis (PCA) on 38,188 SNPs in 3816 parents of CL/P cases, and also assessed the heterozygosity and the distribution of mating types at each SNP in the 8q24 region. We generated TDT p-values by site and compared the observed genotype distribution in CL/P offspring to the expected given the parents using a 2df chi-squared test. In addition to detecting strong differences between European and East Asia populations via PCA, we observed a genetic north-south cline extending from Korea through China to Singapore, while the Philippines clustered separately. Hierarchical clustering in the 8q24 region based on SNP heterozygosity revealed two major clades, European and Asian, which is consistent with PCA results. Within the Asian clade, the Philippines occupied a separate branch from other Asian sites. Clustering SNPs by heterozygosity showed all the SNPs with $p<10^{-6}$ in the TDT had high heterozygosity in Europeans, but low heterozygosity in Asians. SNPs with high heterozygosity in Asians showed no evidence of linkage and association. Additionally, clustering p-values from the TDT by recruitment site revealed Norway and Utah provided the strongest evidence of association, while all other sites were grouped together as a separate clade. Further TDT analysis of Norway and Utah as a combined group resulted in genome-wide significant p-values , while Maryland, Iowa, Denmark and Pittsburgh combined showed only nominal evidence ($0.01>p>0.000001$). Shandong, Hubei and Sichuan Provinces and Taiwan showed similar patterns of deviation of case genotype from expected across 8q24, while Korea, Philippines and Singapore clustered together. Although our results suggest the 8q24 CL/P-associated region is differentiated between Europeans and East Asians, there could be variants in this region which are associated with CL/P in Asians but were absent from our GWAS panel.

1478/T

Worldwide distribution kidney disease susceptibility alleles and haplotypes around the MYH9 gene. *T.K. Oleksyk[1], G.W. Nelson[2], P. An[2], W. Guiblet[1], J.B. Kopp[3].* 1) Department of Biology, University of Puerto Rico at Mayaguez, Mayaguez, PR., Puerto Rico; 2) Laboratory of Genomic Diversity, SAIC-Frederick, Frederick MD; 3) Kidney Disease Section, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda MD.

MYH9 was recently identified as renal susceptibility gene (OR 3-8, $p<10^{-8}$) for major forms of kidney disease disproportionately affecting individuals of African descent. The risk haplotype (E-1) occurs at much higher frequencies in African Americans (≥ 60%) than in European Americans (<4%), revealing a genetic basis for a major health disparity. We reconstructed *MYH9* haplotypes from 4 tagging single nucleotide polymorphisms (SNPs) spanning introns 12-23 using available data from HapMap Phase II, and by genotyping 938 DNAs from the Human Genome Diversity Panel (HGDP. The E-1 risk haplotype followed a cline, being most frequent within sub-Saharan African populations (range 50-80%), less frequent in populations from the Middle East (9-27%) and Europe (0-9%), and rare or absent in Asia, the Americas, and Oceania. The fixation indexes (F_{ST}) for pairwise comparisons between the risk haplotypes for continental populations were calculated for *MYH9* haplotypes; F_{ST} ranged from 0.27-0.40 for Africa compared to other continental populations, possibly due to selection. Uniquely in Africa, the Yoruba population showed high frequency extended haplotype length, as well as high population differentiation in HapMap Phase II data, also observable only in the Yoruba population from HGDP. The population-specific divergence in *MYH9* risk allele frequencies among the world's populations may prove important in risk assessment and public health policies to mitigate the burden of kidney disease in vulnerable populations.

1479/T

Diversity of KIR2DL4 in Amerindian populations. *M. Pincerati[1], J. Guerreiro[2], E. Santos[2], P. Martin[3], M. Carrington[3], D. Meyer[1].* 1) Universidade de São Paulo, São Paulo, Brazil; 2) Laboratório de Genética Humana e Médica, Universidade Federal do Pará, Belém, Pará, Brazil; 3) Cancer and Inflammation Program, Laboratory of Experimental Immunology, SAIC-Frederick Inc., National Cancer Institute-Frederick, Frederick, MD, USA.

The KIR2DL4 gene is unusual among KIR genes in that it exhibits structural characteristics of both an activating and an inhibitory receptor. KIR2DL4 is one of the KIR genes that shows a strong signature for balancing selection, frequently presenting higher diversity than expected under neutrality. Genetic polymorphisms in KIR2DL4 result in functionally distinct variants, which can influence expression of KIR2DL4. Based on the presence of 9 or 10 adenine residues at the end of exon 6, which encodes the transmembrane domain, two common allele groups, 9A and 10A, have been described. 9A alleles are not expressed on the cell surface due to the splicing out of the transmembrane domain. 10A alleles can be further subdivided into 10A-A allotypes and 10A-B allotypes, which cannot be detected on the cell surface. Analyses of these polymorphisms have shown that these allele groups are maintained at intermediate frequency in some populations, possibly by natural selection. Native American populations are of particular interest for studies of natural selection, since they occupy an extensive geographic territory with environmental heterogeneity. The aim of this study was to characterize the allelic variability of KIR2DL4 in Amerindian populations from the Amazon region, so as to investigate the role of natural selection in shaping variability at this locus. We sequenced exons 3, 5 and 7-9 of KIR2DL4 gene in 234 samples from 15 Amerindians tribes. We found 9 alleles, with the highest frequencies for *00501, *00102 (0.58 and 0.34 respectively) and the lowest frequencies for *011, *00801, *00201 and *00103, *002, *00502, *006. We also analyzed the distribution of 9A and 10A variants in our populations. In contrast to most populations already studied, there is a low frequency of 9A alleles in Amerindian populations (0.93 for 10A variant and 0.07 for 9A). In addition, the 10A-A subgroup (represented by *00501) and the 10A-B subgroup (represented largely by *00102) are maintained at high frequency in these populations. These results might indicate that for Amerindian populations, the 10A subgroups is more relevant than the 9A group. We also performed a Ewens-Watterson test to verify departure from neutrality of allele frequencies and found no evidence for natural selection acting on KIR2DL4 in the Amerindian population.

1480/T

Haploid and autosomal variation within a linguistic continuum of the Uralic-speaking people of Eurasia. *K. Tambets[1], S. Rootsi[1], M. Metspalu[1], B. Yunusbayev[1,2], E. Metspalu[1], M. Reidla[1], E. Khusnutdinova[2], L. Osipova[3], T. Kivisild[1,4], R. Villems[1].* 1) Estonian Biocentre and Dept. of Evolutionary Biology, University of Tartu, Tartu, Estonia; 2) Institute of Biochemistry and Genetics, Ufa Research Centre, Russian Academy of Sciences, Ufa, Russia; 3) Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences, Novosibirsk, Russia; 4) The Leverhulme Centre for Human Evolution, University of Cambridge, UK.

For about last two decades the examination of uniparentally inherited genetic marker systems revealing the variation embedded in mtDNA and Y chromosome has been the main tool in the studies of human genetic origins. Within few recent years the analysis of the genome-wide SNP data of individuals from different populations has started to give promising new insights in the field of human population genetics. The uniparentally inherited markers have shown slightly different demographic scenarios for the maternal and paternal lineages of North Eurasian, particularly of European Uralic-speaking populations. The geographical location of a population has evidently been the most important component that dictates the proportion of western and eastern mtDNA types in the gene pool of Uralic-speakers. Thus, the palette of maternal lineages of the Uralic-speakers resembles that of their geographically close European or Western Siberian Indo-European and/or Altaic-speaking neighbours, respectively. At the same time, the most frequent North Eurasian Y chromosome type N1c, that is also a common link between almost all Uralic-speakers, is with few exceptions rare, if present at all, among Indo-European-speakers of Western and Southern Europe. Here we combine genome-wide high density SNP data (650 000 SNPs, Illumina) with uniparentally inherited mtDNA and Y-chromosome variation of 16 Uralic-speaking populations to assess their place on the genetic landscape of North Eurasia. By the use of principal component and structure-like analysis on the autosomal data we show that the proportions of western and eastern ancestry components among the Uralic-speakers are determined mostly by geographical factors. The westernmost populations from Europe, both Uralic- and Indo-European speakers, are similar in their pattern of ancestry components and show low levels (<10%) of the eastern component. Conversely, the eastern ancestry component is dominant (60-70%) in the gene pool of the Siberian Uralic-speakers. In general, the genome-wide analyses corroborate the results of mtDNA analysis and do not reflect the common genetic characteristics between western and eastern Uralic-speakers at the level seen in case of N1c. Interestingly, among Saami from North Europe, who are often considered as =84outliers" in genetic studies, the dominant western component is accompanied by 30% of eastern component making them more similar to Volga-Uralic populations than to their closest neighbours.

1481/T

Fine-scale characterization of Genetic Structure in a Mexican Cohort. *H. Tang[1], N. Johnson[2], F. Zakharia[1], I. Romieu[3], M. Shriver[5], S. London[4].* 1) Dept Genetics, Stanford Univ Sch Med, Stanford, CA; 2) Dept. Statistics, Stanford University, Stanford, CA; 3) National Institute of Public Health, Cuernavaca, Mexico; 4) Epidemiology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, NC; 5) Dept. of Biological Anthropology, Penn State University, University Park, PA.

High-density genotype data has enabled extensive surveys of human population genetic structure at a global level as well as within the African and Eurasian continents, where geography is shown to be a major determinant of population structure. In contrast, genetic structure in recently admixed populations, such as the African Americans and the Hispanics, is influenced both by historical geographic isolation and by recent migration. Limited by the amount of data and by the available analytic tools, current understanding of population structure in these populations is largely limited to the estimation of the continental-level ancestry proportions. In this study, we report fine-scale characterization of genetic structure in a cohort of 500 parents-offspring trios recruited from Mexico City as part of a genome-wide association study of asthma. Using the genotypes of more than 500,000 SNPs, we estimate the relative contribution from the European, African and Native American ancestral populations. The ancestral origin of each chromosomal segment is inferred using a novel computational approach; accordingly, the genome of a single individual is partitioned into components that correspond to the continental-level ancestry. The European component of the genome in the Mexican individuals shows strongest affinity to Spanish / Portuguese populations from southwest Europe. In contrast, the Native American component of the Mexican genomes is much more heterogeneous: while the Native American ancestry of most Mexicans in our cohort can be traced to the indigenous groups of Nahua, Mixtec, and Tlapanec, residing in the State of Guerrero, a small group of individuals appear to have substantial ancestry from the Mayans of the Yucatan Peninsula, as well as from the Quechua and Aymara groups in Peru and Bolivia. Our study demonstrates the feasibility of characterizing sub-continental-level population structure in the Hispanics populations, as well as the importance of accounting for such population structure in the design and analysis of genome-wide association studies.

1482/T

Variation in IRF5-TNPO3, followed by STAT4 and HLA regions, are the Strongest Trans-Ethnic Risk Factors for Systemic Lupus Erythematosus (SLE). *T.J. Vyse[1], P.S. Ramos[2], K.M. Kaufman[3], J.A. Kelly[3], C. Gallant[4], A. Delgado[4], S.A. Chung[5], L.A. Criswell[5], R.P. Kimberly[6], M.E. Comeau[2], A.H. Williams[2], L. Russell[2], C.O Jacob[7], B.P. Tsao[8], M.E. Alarcón-Riquelme[3,4,9], K.L. Moser[3], P.M. Gaffney[3], J.B. Harley[3], C.D. Langefeld[2], International Systemic Lupus Erythematosus Genetics (SLEGEN) Consortium.* 1) Dept Rheumatology, Imperial College, London, United Kingdom; 2) Dept Biostatistical Sciences, Wake Forest Univ Health Sciences, Winston Salem, NC, USA; 3) Arthritis and Immunology Program, Oklahoma Medical Research Foundation, Oklahoma City, OK, USA; 4) Dept of Genetics and Pathology, Uppsala University, Uppsala, Sweden; 5) Rosalind Russell Medical Research Center for Arthritis, University of California, San Francisco, CA, USA; 6) Dept Medicine, University of Alabama at Birmingham, Birmingham, AL, USA; 7) Keck School of Medicine, Univ of Southern California, Los Angeles, CA, USA; 8) School of Medicine, Univ of California, Los Angeles, CA, USA; 9) Center for Genomic and Oncological Research, Pfizer-Universidad de Granada-Junta de Andalucia, Granada, Spain.

Despite a greater prevalence, there is a paucity of large-scale data on the genetics of SLE in African Americans, Hispanics and, to a lesser extent, Asians. Thus, the specific genetic variants predisposing to SLE and their overlap with European ancestry risk factors are largely unknown. We conducted a trans-ethnic study called Lupus Large Association Study 2 (LLAS2) consisting of 8436 SLE cases and 7554 controls of European (3977 cases and 3538 controls), African American (1679 cases and 1934 controls), Asian (1272 cases and 1270 controls) and Hispanic and Native American ancestries (1508 cases 812 controls). Selected from the SLEGEN European ancestry genome-wide association study (GWAS)(Harley et al, 2008), 7069 candidate SNPs were genotyped on an Illumina custom array; approximately 400 ancestry informative markers were included and principal components analysis and admixture estimates were computed. We computed ethnic-specific case-control association analyses adjusting for admixture and trans-ethnic meta-analysis using the weighted inverse normal approach. Strongest associated SNP in the region is reported. The most significant trans-ethnic variation is located in the IRF5-TNPO3 region (rs4728142, P=4.77x10-60), followed by STAT4 (rs11889341, P=4.66x10-53), HLA (rs3131379 in MSH5, P=1.17x10-52), ITGAM (rs9888739, P=5.38x10-31), BLK (rs13277113, P= 4.79x10-29), PTTG1 (rs2431697, P=6.55x10-24), TNIP1 (rs960709, P= 7.22x10-21), UBE2L3 (rs2298429, P=1.98x10-15), NMNAT2 (rs12146097, P=9.64x10-14), and TNFSF4 (rs10798269, P=5.69x10-12). Some regions are dominated by the European association (HLA, ITGAM, PTTG1 and NMNAT2), others by two or more ethnicities (IRF5-TNOP3, STAT4, BLK, TNIP1, UBE2L3, TNFSF4). Some regions share a similar pattern of association between two (IRF5-TNOP3, ITGAM) or more (STAT4, BLK, PTTG1, TNIP1, UBE2L3, NMNAT2) ethnic groups, while in others it is apparently discordant (HLA, TNFSF4). This is the first report of large-scale trans-ethnic mapping in SLE. This study shows that the IRF5-TNPO3 region is the strongest SLE trans-ethnic risk factor, and helps elucidate common versus ethnic-specific risk factors for SLE.

1483/T

Making a haplotype catalog with estimated frequencies based on SNP homozygotes: an application to the Japanese population. *Y. Yamaguchi-Kabata[1], N. Kumasaka[1], T. Tsunoda[2], A. Takahashi[1], N. Hosono[2], M. Kubo[2], Y. Nakamura[2,3], N. Kamatani[2].* 1) Ctr Genomic Med, RIKEN, Tokyo, Japan; 2) Ctr Genomic Med, RIKEN, Yokohama, Japan; 3) Human Genome Ctr, Inst Med Sci, Univ Tokyo, Tokyo, Japan.

Understanding the structure and frequencies of haplotypes is important for associating genetic polymorphisms with a given trait and for inferring the genetic genealogy of alleles in a population. SNP haplotypes can be determined without ambiguity when an individual does not have more than one heterozygous site in a given genomic region. In our previous study, using genome-wide SNP genotypes for 3397 individuals from the Japanese population, we examined the efficiency of determination of haplotypes and haplotype frequency estimation based on the proportion of SNP homozygotes in the sample. We applied this approach to a genome-wide SNP genotype data from about 19000 individuals from the Japanese population. As most Japanese fall into two main clusters (the Hondo and Ryukyu clusters), we selected individuals for both of the two clusters based on the result of PCA. Then we made a haplotype catalog for the Hondo Japanese from 18379 individuals. We compared the haplotype frequencies in the Hondo Japanese with the haplotype frequencies for the Ryukyu Japanese, which were roughly estimated from 504 individuals. Although haplotype frequencies in the Hondo and Ryukyu clusters were highly correlated in most genomic regions, we found the gene regions where the difference in haplotype frequencies was highly significant. There were functional biases in these highly differentiated genes; transporters and ion-channels were overrepresented. The catalog of real haplotypes with their estimated frequencies will be useful for identifying causative polymorphisms for a trait, which are linked to the most associated SNPs in a GWAS. Furthermore, the catalogue of haplotypes will be useful for a haplotype-based GWAS and detection of shared haplotypes that contain multiple variants that affect the trait.

1484/T

Association between Cystatin C polymorphisms and corpulence. *H. HOOTON[1,3], B. DUBERN[1,3,7], R. ALILI[1,3], F. ROUSSEAU[2], V. PELLOUX[1,3], C. HENEGAR[1,3,7], P. GALAN[4], S. HERCBERG[4], P. ARNER[6], T.I.A. SØRENSEN[5], K. CLEMENT[1,3,7].* 1) UMRS U872 équipe 7, INSERM, PARIS, France; 2) INTEGRAGEN, Paris, France; 3) University Pierre and Marie Curie, Paris, France; 4) INSERM U556, INRA U1125, CNAM EA3200, university Paris 13, nutritional epidemiology research unit, Paris, France; 5) Institute of preventive medicine, Copenhagen university hospital, centre for health and Society, Copenhagen, Denmark; 6) Karolinska institute, department of medicine, Huddinge, Lipid laboratory, Stockholm, Sweden; 7) Assistance publique hôpitaux de paris, paris France.

Background: Cystatin C, the most powerful endogenous inhibitor of cysteine protease Catepsin S, K and L, is a new adipose tissue synthesized molecule. Its expression rate in adipose tissue and circulating levels in blood are two to threefold higher in obese subjects than in lean controls. Work hypothesis: Our hypothesis is that common variants in Cystatin C gene could play a role in the evolution of BMI during lifetime. Methods: Tag SNPs were selected to obtain a full coverage of Cystatin C coding sequence (r^2= 0.8, MAF>5%) based on hapmap data. Tag SNPs were genotyped in 4300 subjects from the SUVIMAX study (BMI=23.78 +/-3.38 kg/m²), 750 women from the SPAWN cohort (BMI=21.73 +/-2.87 kg/m²) and 1500 Danish men from the Danish national birth cohort (DNBC) (BMI=27.00 +/-6.23 kg/m²). BMI measures at several time points were available in all three cohorts. Results: significant associations with time dependant BMI were found in each population ($p<0.05$). Concerning both SNPs, G/G carriers were less corpulent than A/G and A/A carriers in both the SUVIMAX population and the group of Danish men, but were more corpulent in the SPAWN cohort. Haplotype analysis revealed that AA carriers were more corpulent in the SUVIMAX and Danish groups and less corpulent in the SPAWN cohort than carriers of other haplotypes. This phenomenon is known as a flip-flop effect and has already been well described and discussed in the literature. Conclusions and perspectives: These results bring new insights into the role of Cystatin C in corpulence. Replication of these findings is ongoing in several other populations.

1485/T

Effect of Recent Admixture and Self-reported ancestry on genetic association for some susceptibility genes SLE among Hispanics. *J.E. Molineros[1], X. Kim-Howard[1], J.A. Kelly[1], B. Namjou[1], J.D. Reveille[5], L.M. Vila[5], J.C. Edberg[5], R. Ramsey-Goldman[5], M.A. Petri[5], E.E Brown[5], R.P. Kimberly[5], C.D. Langefeld[3], J.M. Anaya[4], M.E. Alarcon-Riquelme[1], K. Kaufman[1], J.B. Harley[2], S.K. Nath[1].* 1) Oklahoma Medical Research Foundation, Oklahoma City, OK; 2) Oklahoma City Veteran's Hospital, Oklahoma City, OK; 3) Wake Forest University, Salem, NC; 4) Universidad del Rosario-Corporación para Investigaciones Biológicas, Bogota Colombia; 5) University of Alabama, Birmingham, AL.

Systemic Lupus Erythematosus (SLE) is an autoimmune disease with complex etiology. Recently admixed populations like Hispanics have an increased risk of developing the disease compared with European-Americans. This suggests the hypothesis that differences in genetic background may contribute to ethnic differences in SLE susceptibility. OBJECTIVES. Estimate ancestry proportions for Hispanics; correlate geographic distribution across ancestry; assess ancestry versus phenotype association; and assess the effect of individual admixture on genetic association in 3 SLE associated genes. METHODS. To estimate population structure we ascertained 1245 SLE cases and 619 controls from 5 geographical regions (Argentina, Colombia, Peru, Puerto-Rico, Mexico). We selected 94 Ancestry Informative Markers (AIMs) from 7 populations from HGDP data (Pima-Maya (Native American [NA]), European (CEPH), African (YRI), Bantu, Pigmy, San, Chinese-Japanese). We selected SLE associated variants from ITGAM (rs1143679), IRF5 (rs2070197) and STAT4 (rs10181656) to test the effect of population stratification on genetic association. RESULTS. The 3 ancestral populations model had the best fit and aligned along the NA, African (YRI) and European ancestral populations. Mean ancestry of Hispanics was 36%(NA), 9%(African) and 55%(European), however, ancestry proportion varied by geographical origin. Puerto-Rico had the largest African ancestry (20 controls/18 cases), followed by Colombians (13 controls/14 cases). Peru had the largest NA ancestry (85 control/87 cases), followed by Mexico (53 controls/56 cases), Colombia (36 control/25 case) and Argentina (22 control/32 cases). Puerto-Rico had the largest European ancestry (71 control/73 cases). Allele frequencies varied across populations from 3%-22% on rs1143679; from 26% to 59% on rs10181656, and from 9% to 34% on rs207019. Overall there was no association between phenotype and ancestry proportion, but when we subdivided the samples into their geographical regions, the NA ancestry was significantly associated in Argentinean and Colombian samples. All markers were significantly associated to SLE, when corrected for ancestry (local and global). The uncorrected (corrected) p-values for ITGAM, STAT4 and IRF5 were $4.9x1^{0-10}$ ($7.96x10^{-7}$), $8.32x10^{-6}$ ($2.31x10^{-5}$), and $8.75x10^{-13}$ ($4.1x10^{-12}$) respectively. We discovered significant allele frequency differences between populations and its implications on admixture mapping for SLE gene identification.

1486/T

The Prevalence and Haplogroup-Specific Distribution of Homoplasmic/Near Homoplasmic Pathogenic Mitochondrial DNA Point Mutations in the General Population. *R. Bai, S. Suchy, J. Higgs, G. Richard, S. Bale.* GeneDx Inc, Gaithersburg, MD.

It has been reported that at least one in 200 live births is heteroplasmic for one of the ten pathogenic mitochondrial (mtDNA) point mutations in cord blood (Am J Hum Genet 2008, 83:254). To estimate the frequency of homoplasmic/near homoplasmic pathogenic mtDNA mutations in the general population, the published complete sequence of the coding region or entire mitochondrial genome of over 6000 individuals from worldwide populations, listed in the Mitomap database (www.mitomap.org), were analyzed. The sequences with missing bases or from known patients were excluded from this analysis. The frequency of each nucleotide change (variant) from the revised Cambridge Reference Sequence (rCRS) was calculated. The mtDNA haplogroup for each sequence was assigned and the frequency of each variant in a specific haplogroup was calculated. The above information for the 48 confirmed pathogenic mtDNA point mutations listed in the Mitomap database were collected and compared. The overall frequency of the 48 mutations is 2.11% (133/6305). The overall frequency of the six common Leber's Hereditary Optic Neuropathy (LHON) mutations (G3460A, G11778A, T14484C, C14482G, G14459A and C14568T) is 1.57% (99/6305), among which the frequencies of G3460A, G11778A and T14484C are 0.24%, 0.82% and 0.40%, respectively. The overall frequency of the DEAF-associated mutations (A1555G, 1494T, A7445G and 7472insC) is 0.35%(22/6305), with the frequency of the A1555G mutation being 0.25%. The mutations associated with Leigh syndrome (LS), Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes (MELAS), Myoclonic Epilepsy and Ragged Red Muscle Fibers (MERRF) or Maternally Inherited Cardiomyopathy (MICM) are rare, probably due to the typical low heteroplasmy of these mutations in blood that is undetectable by sequencing. The haplogroup (HG)-specific frequency for G3460A is 1.95% in HG-J, 0.92% in HG-H, 0.57% in HG-C and 0.41% in HG-K; for G11778A, 3.53% in HG-F, 3.43% in HG-T, 2.18% in HG-H, 1.82% in HG-G, and 1.58% in HG-non-k U, 1.46% in HG-J and 0.9% in HG-unclassified M and HG-V; for T14484C, 6.82% in HG-J, 4.35% in HG-Q and 1.16% in HG-W; for A1555G, 1.1% in HG-X, 0.9% in HG-H and HG-non-k U. In conclusion, asymptomatic carriers of homoplasmic/near homoplasmic pathogenic mtDNA point mutations are common in the general population; most of these mutations are LHON or DEAF-associated mutations. These mutations are preferentially associated with certain mtDNA haplogroups.

1487/T

A Common Spinal Muscular Atrophy (SMA) Deletion Mutation is Present on a Single Founder Haplotype in the U.S. Hutterites. *J.X. Chong[1], A.A. Oktay[1], Z. Dai[2], K. Swoboda[3], T.W. Prior[2], C. Ober[1,4].* 1) Human Genetics, The University of Chicago, Chicago, IL; 2) Pathology, The Ohio State University, Columbus, OH; 3) Neurology and Pediatrics, University of Utah School of Medicine, Salt Lake City, UT; 4) Obstetrics and Gynecology, The University of Chicago, Chicago, IL.

SMA is an autosomal recessive (AR) neuromuscular disease that occurs in ~1 in 10,000 European births, making it the second most common lethal AR disease in European populations. In U.S. whites, ~1 in 40 are carriers. SMA is caused by the homozygous loss of Survival Motor Neuron 1 (*SMN1*) gene function due to a deletion or gene conversion event in 95% of cases; carrier and affection status are determined using quantitative measures of *SMN1* exon 7 copy numbers. A few cases of SMA had previously been reported in the Hutterites (Boycott et al. 2008; AJMG 146A:1088), a North American founder population that descended from <90 ancestors, but the carrier frequency in this population was unknown. In this study, DNA samples from one South Dakota Hutterite (S-leut) and 3 Montana Hutterites (L-Leut) with SMA due to the homozygous deletion of *SMN1* (as determined by a competitive PCR dosage assay), were genotyped with Affymetrix SNP arrays. A 9-generation pedigree connects these 4 individuals to their nearest common Hutterite ancestors (b. 1770's). The 4 individuals were all homozygous for a stretch of ~790 SNPs spanning 10 Mb across the locus, indicating a single founder haplotype. Using Affymetrix genotypes in an additional 1402 Hutterites, representing a population-based sample of South Dakota Hutterites (age 6-93 yrs), we identified 171 individuals who carried at least 9.3 Mb of the founder haplotype. Carrier status was confirmed in 63 representative individuals from the pedigree by the competitive PCR assay, yielding an overall SMA mutation frequency of 0.063 in the South Dakota Hutterites. The corresponding carrier frequency of 1 in 8 (12.5%) is the highest ever reported for SMA and the highest carrier frequency reported to date for an AR mutation in the Hutterites of South Dakota. However, because the genetic structure of the *SMN1* locus makes it prone to mutation and this study would not have identified *de novo* SMA mutations on different haplotypes, this carrier frequency is likely an underestimate of the true carrier frequency in the Hutterites. This is the first population-based study to directly assess SMA carrier status and to use haplotype analysis to determine carrier status in a founder population. The extremely high carrier frequency for this severe disease in the South Dakota Hutterites underscores the need for widespread carrier screening in this population.

1488/T

Type II procollagen gene analysis and cephalometric linear measurements of the mandible: A comparative study. *s. morcos[1], a. el-kadi[1], t. mansour[2].* 1) FACULTY OF DENTISTRY, SUEZ CANAL UNIVERSITY, ISMAILIA, EGYPT; 2) cancer institute, CAIRO UNIVERSITY, CAIRO, EGYPT.

The present study was designed in order to estimate the relationship between both COL2A1 gene and mandibular morphology on different skeletal classes of malocclusion and also to evaluate this genetic marker as predictors of mandibular growth. The study was carried out on 30 patients both males and females of different skeletal Classes, where the patients were selected from the out-patient clinic of Faculty of Dentistry, Suez Canal University, with age range (18-30) years without any congenital anomalies in the craniofacial structures. The selected patients were divided into 3 groups according to their skeletal Class, as each group included 10 patients. For each patient, lateral cephalometric view was taken and blood sample was aspirated. The cephalometric linear measurements of the mandible were recorded from lateral cephalogram. DNA was extracted from the blood sample and then genetic analysis was done using PCR-RFLP (polymerase chain reaction - restriction fragment length polymorphism). The cephalometrice linear measurements of the mandible were compared with the genetic analysis results, statically analyzed and tabulated. The following conclusions could be drawn from the current study: 1- There was a relation between Type II procollagen (COL2A1) gene and both total mandibular length (Cd-pg) and mandibular body length (Go-Gn) and this relation was significant for both males and females.

1489/T

Population genomics and haplotype construction from diverse human genomes. *J.M. Kidd[1], S. Musharoff[1], B.M. Henn[1], F. De La Vega[2], C.D. Bustamante[1].* 1) Department of Genetics, Stanford University, Stanford, CA; 2) Life Technologies, Foster City CA.

Human population genomics studies have become a reality due to advances in short-read sequencing technology. In these studies, variant discovery entails mapping to a reference sequence, followed by identification of variant sites. This results in a set of genotypes for a given individual at positions along the genome. Phased haplotypes can be inferred from these genotypes either via parent-offspring trios or from phasing algorithms that incorporate allele frequency information from the same or similar populations. Alternatively, short phased haplotype segments can be reconstructed from the underlying paired-end sequence reads without relying on the sequencing of additional genomes for trio-based or allele frequency-based phasing methods. We apply this haplotype construction approach using paired end sequences to 12 diverse individual human genomes that have been sequenced with the SOLiD technology using libraries with a fragment size of 1 kb - 3 kb. Using the linking mate pair information, we physically phase a subset of heterozygous variants in these sequenced individuals and utilize the resulting segments to explore estimates of divergence within and between individuals of diverse ancestry.

1490/T

The African American risk allele for focal segmental glomerulosclerosis (FSGS), and hypertension-associated end-stage kidney disease (H-ESKD). *G. Genovese[1,2], S. Tonna[2,3], R. Lazarus[4], M. Pollak[2,5].* 1) Department of Mathematics, Dartmouth College, 6188 Kemeny Hall, Hanover, NH 03755; 2) Renal Division, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115; 3) Epigenetics in Human Health and Disease Laboratory, Baker IDI Heart and Diabetes Institute, Melbourne, Australia; 4) Channing Laboratories, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, United States; 5) Division of Nephrology, Dept of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA 02215, United States.

Genetic variation at the MYH9 locus has been shown to be responsible for the high incidence of focal segmental glomerulosclerosis (FSGS) and hypertension-associated end-stage kidney disease (H-ESKD) among African Americans. We performed a genome-wide association using 56 African Americans affected by biopsy-proven FSGS and compared the results with 1800 African Americans controls provided from Illumina. We determined continental ancestry in a 1MB region around MYH9 and still, the ancestry corrected case-control comparison showed that variants within a large region of 80kb, containing part of MYH9, associate with increased FSGS risk. This region spans multiple linkage disequilibrium blocks and variants associating with disease within this region are in linkage disequilibrium with variants which have shown signals of natural selection. Previous studies identified the E-1 haplotype as the best predictor for disease (~60% frequency in controls and ~88% frequency in our cases, p<10e-8). Our data suggests that a different haplotype defined by the risk alleles for SNPs rs2239785 and rs136187 (~31% frequency in controls and ~65% in our cases, p<10e-13) is more strongly correlated with disease and the frequency discrepancy for this haplotype cannot be explained alone by the discrepancy at the E-1 haplotype. This suggests that the association signal is actually stronger than thought by previous studies which might have underestimated the genetic effect of the MYH9 locus.

1491/T

Accuracy of Ancestry Informative Markers (AIMs) for the estimation of individual ancestry in admixed populations. *J.M. Galanter[1], C. Gignoux[1], M. Aldrich[1], D. Torgerson[1], J.G. Ford[2], S. Nazarui[2], J.R. Rodriguez-Santana[3], J. Casal[2], A. Torres-Palacios[2], J. Salas[4], R. Chapela[4], H. Geoffrey Watson[5], K. Meade[6], M. LeNoir[7], W. Rodriguez-Cintrón[3], P.C. Avila[8], A. Bigham[9], M. Shriver[9], E. González Burchard[1,10].* 1) Department of Medicine, University of California, San Francisco, San Francisco, CA; 2) Veterans Caribbean Health Care System 10 Casia Street San Juan, PR 00921; 3) Centro de Neumologia Pediátrica Torre Medica Auxilio Mutuo Suite 215 Ave. Ponce de Léon No. 735 San Juan, Puerto Rico 00917; 4) Instituto Nacional de Enfermedades Respiratorias Mexico City, Mexico; 5) James A. Watson Wellness Center 5709 Market St Oakland, CA 94608; 6) Children's Hospital Oakland Research Institute 5700 Martin Luther King Jr Way Oakland, California 94609; 7) Bay Area Pediatrics Ste 1, 2940 Summit Street Oakland, CA 94609-3410; 8) Division of Allergy/Immunology Northwestern University M-316, McGaw Pavilion, 240 E. Huron, Chicago, IL 60611; 9) Department of Anthropology Penn State University 512 Carpenter Building State College, PA; 10) Department of Biopharmaceutical Sciences University of California, San Francisco, San Francisco, CA.

Introduction Ancestry informative markers (AIMs) have been used as a cost-effective way to estimate individual ancestral proportions in admixed populations such as African Americans and Latinos. We determined the accuracy of individual ancestry estimates derived from smaller AIMs panels compared to ancestry estimates using all genomewide data as the gold standard. Methods Latino participants of Mexican (n = 271) and Puerto Rican (n = 324) originwith asthma were recruited from the San Francisco Bay Area, New York City, Puerto Rico, and Mexico City. Genotyping was performed using the Affymetrix 6.0 GeneChip Array; after applying standard QC filters 729,685 markers remained for analysis. We used the intersection of Illumina-550 and Affymetrix 6.0 as our set of potential SNPs to encourage universal applicability of our marker panels. Ancestry information for each SNP was measured via pairwise In calculations. AIMs panels of 18, 36, 75, 150, 300, 600, 1200, and 2400 unlinked markers were selected. Individual ancestry was estimated using the program ADMIXTURE, specifying a three population model. Ancestral populations consisted of HapMap Yorubans and CEPH Europeans, as well as Maya and Nahua Native Americans. We compared differences in ancestry estimated with different size AIMs panels with ancestry estimated from genomewide markers. Mean and standard deviation of the difference in ancestry estimation between AIMs and genomewide data were calculated. Results There was an inverse correlation between the number of AIMs used to estimate ancestry and mean and standard deviation of the error in ancestry estimation. Using AIMs, African ancestry was consistently overestimated, while the major ancestral component (European in Puerto Ricans and Native American in Mexicans) was systematically underestimated. Using 300 or fewer AIMS consistently produced a standard deviation of ancestry estimation error of 10% or greater. Discussion Our results illustrate significant error in the estimation of individual ancestry using AIMs. There is both systematic bias resulting in overestimation of African ancestry (and underestimation of other continental ancestry) and random error. Such error is inversely proportional to the number of AIMs used. These findings may have implications for genetic association studies where ancestry is used to control for population stratification as well as for studies examining associations of individual ancestry estimates with a phenotype.

1492/T

Genome wide scan identifies loci associated with gait speed and handgrip in older Amish. *J.E. Hicks[1], J.R Gilbert[1], L. Jiang[2], A.C. Cummings[2], D.R. Velez Edwards[3], P.J. Gallins[1], L. Caywood[1], L. Reinhart-Mercer[1], D. Fuzzell[2], C. Knebusch[2], R. Laux[2], C.E. Jackson[4], M.A. Pericak-Vance[1], J.L. Haines[2], W.K. Scott[1].* 1) Hussman Institute for Human Genomics, University of Miami, FL, USA; 2) Center for Human Genetics Research, Vanderbilt University, Nashville, TN, USA; 3) Vanderbilt Epidemiology Center, Vanderbilt University, Nashville, TN, USA; 4) Scott & White, Temple, TX.

Gait speed and handgrip strength are physical performance measures that assess lower and upper body strength, respectively. Both are strong predictors of incipient disability and mortality in older adults. Identification of polymorphisms affecting these traits would result in new potential avenues to promote healthy aging. Adults over 65 were enrolled in a population based door-to-door survey of Amish communities in Indiana and Ohio. The Anabaptist Genealogy Database was used to connect all 814 participants in a 13-generation pedigree. Genotyping was performed using the Affymetrix Genome-Wide Human SNP Array 6.0. Gait speed was measured by timing subjects on a 10ft walk. Grip strength was measured in each hand (three trials) with a dynamometer. Of the 814 participants, 519 had valid measured gait speeds, and 473 had valid measured grip strength. After quality control, 630,309 autosomal SNPs were analyzed for association with gait speed and grip strength using the GRAMMAR-GC method (accounting for pedigree relationships in a linear mixed model) in the GenABEL package. The data were adjusted for age, sex, height, and cognitive status in gait speed analysis, and age, sex, BMI, and cognitive status in grip strength analysis. Analysis identified 641 SNPs associated with gait speed at $p<10^{-3}$. The most significant result was at rs11050430 ($p=4.9x10^{-6}$) on 12p11, near TMTC1. A 137kb LD block on 14q21 containing 25 highly correlated SNPs was also strongly associated (smallest $p=5.3x10^{-6}$ at rs7154025, near LRFN5 and FBXO33A). This region was previously linked to successful aging (of which gait speed is a component) in a subset of this sample. Another 31kb LD block on 20q11 (10 SNPs) showed significant association (smallest p=8.4x10-6 at rs6069151). Grip strength analysis identified 551 SNPs associated at $p<10^{-3}$. The strongest association was on chromosome 3q29, near HES1 (at rs7629288, $p=5.5x10^{-6}$). Other SNPs showing strong association were rs2852230 ($p=6.6x10^{-6}$) on 11q23 within an intron of GRIK4. Another SNP on chromosome 11 showed association ($p=7.6x10^{-6}$). Analysis of these regions for functional variants that explain the association will aid in understanding the underlying biological processes of disability. The overlap of association with gait speed and linkage of successful aging on chromosome 14 suggests that analysis of continuous intermediate phenotypes will assist in identifying genes underlying successful aging in the Amish.

1493/T

Pigmentation in Britain: Regional patterns and genotype-phenotype correspondence. *E.C. Røyrvik[1], W.F. Bodmer[1,2].* 1) Clinical Pharmacology, University of Oxford, Oxford, United Kingdom; 2) Cancer and Immunogenetics Laboratory, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, United Kingdom.

Large differences in external pigmentation are some of the most striking features in human populations, and some of the most notable of these differences are found in hair and eye color in Northwest Europe. These phenotypes are genetically determined, and many associated genetic variants have been identified. To investigate to what extent pigmentation phenotype is predictable from genotype, we have genotyped 2842 locally rooted individuals from different regions of Britain for a subset of these variants, and use their frequencies to estimate hair and eye color in these areas. The results are compared to observed frequencies from two large mid-19th and early 20th century surveys of 9470 British people from the same or adjacent areas.=AC The SNPs used include rs12913832 - a major determinant of brown versus non-brown eye color, and major variants of *MC1R* that cause red hair. Pairwise comparisons of color categories in the current and 19th/early 20th century local populations were conducted to investigate differences, either diachronic or geographical, between them. There are significant differences between different areas of Britain in terms of pigmentation. North Wales has a significantly higher proportion of dark eyes than the rest of the island, although South Wales also has a comparatively high count. Red hair is approximately equally frequent in all regions; the other main hair shades show a tendency to separate Wales and Southwest England from other areas at a significant level, in particular from East England. We find good general accord between the observed and estimated color frequencies in any given area. Small but regular underestimates of the observed values by the estimated values are likely due to the contribution to any given color category of alleles that are unknown or have a lesser effect, or to uncertainties in effect size of known variants. Our results indicate that average regional hair and eye color have remained relatively stable in Britain over the past century, and support that it is possible to predict normal pigmentation phenotypes from genotype data.

1494/T

Frequency distribution of NOS3, MIF, TNF-α and MPO gene polymorphisms in healthy Southeastern Anatolian Population. *T. Sever[1], S. Oguzkan Balci[1], M. Pehlivan[2], S. Budeyri[2], A. Bayram[3], A. Aydeniz[4], V.N. Ulgezer[1], S. Pehlivan[1].* 1) University of Gaziantep, Faculty of Medicine, Department of Medical Biology, Gaziantep, Turkey; 2) University of Gaziantep Faculty of Medicine, Department of Hematology, Gaziantep, Turkey; 3) Zirve University, Gaziantep, Turkey; 4) University of Gaziantep Faculty of Medicine, Department of Physical Medicine and Rehabilitation, Gaziantep, Turkey.

Objectives of Study: Endothelial nitric oxide synthase (NOS3), macrophage migration inhibitory factor (MIF), tumor necrosis factor-alpha (TNF-α) and myeloperoxidase (MPO) are important enzyme to chronic inflammatory disease, cancer, etc. The aim of this study was to determine NOS3 (+894, intron 4 VNTR), MIF (-173), TNF-α (-238, -308, -857) and MPO (-463) polymorphisms in healthy Southeastern Anatolian population and to show whether there was any difference by comparing with the results belonging to healthy individuals in various populations studied. **Methods:** The allele frequencies and distribution of genotype corresponding to seven polymorphisms in 4 different genes NOS3 (+894, intron 4 VNTR), MIF (-173), TNF-α (-238, -308, -857) and MPO (-463) were analyzed in healthy Southeastern Anatolian individuals. The study was conducted on 150 healthy random Turkish individuals which were living in Southeastern Anatolian at least three generations. The study was approved by the local ethical committee and informed consents were obtained from the patients. Genomic DNA was extracted from peripheral blood using the salting out procedure. Genotyping were performed by using PCR and/or PCR-RFLP method. **Results:** The observed genotype counts was deviated from those expected according to the HWE for NOS3 (+894) (p=0.01) and TNF-α (-857) (p=0.003) whereas NOS3 (intron4 VNTR), MIF (-173), TNF-α (-238, -308), MPO (-463) was not deviated (p>0.05). The results were compared according to the literature data if there is any difference between the healthy population of Southeastern Anatolian with the populations of 6-13 different countries. TNF-α (-308) polymorphism was different from Thailand, Brazil, India, United Kingdom, Chine, Japan, Tawain, Korea and Zimbabwe. TNF-α (-857) polymorphism TT genotype was found higher than that of other populations. NOS3 (+894) polymorphism was found to different than the others. NOS3 (intron 4 VNTR), MIF (-173), TNF-α (-238) and MPO (-463) polymorphisms were similar to the other populations. **Conclusion:** This is the first study in Southeastern Anatolian healthy population and the results show that deviations occur due to the fact that Southeastern Anatolian is the transition region of migrations. The data can be used for antropological comparisons, as well as for association studies with different diseases and for use in transplant stuation involving acute and chronic rejection.

1495/T

Pairwise sequence differences among private and shared haplotypes in a two-population model. *Z.A. Szpiech[1], N.A. Rosenberg[1,2].* 1) Center for Computational Biology and Bioinformatics, University of Michigan, Ann Arbor, MI; 2) Department of Human Genetics, University of Michigan, Ann Arbor, MI.

When one population is selected over another as a basis for genetic variation discovery, it is of interest to consider the properties of haplotypes that go undiscovered because they are private to an unsampled population. To consider a scenario in which only one of a pair of populations is sampled, we develop a modeling approach for examining the properties of private and shared haplotypes in two populations descended from a common ancestral population. In particular, in a coalescent model, assuming the infinite alleles model as the mode of mutation in a non-recombining region, we examine the smallest nontrivial case, with sample size four. In this case, two haplotypes are drawn from each population and three distinct haplotypes are observed, one of which is common to both populations, one of which is private to one population, and the third of which is private to the other population. Given this configuration, we calculate the probability that the pairwise sequence difference between the two private haplotypes is greater than either pairwise sequence difference between a private haplotype and the shared haplotype. Although we find that regardless of the mutation rate and divergence time between populations, the pair most likely to have the greatest sequence difference is the pair of private haplotypes, the largest difference often occurs between one of the private haplotypes and the shared haplotype, especially for closely related populations. Thus, if only one population is used for discovery of haplotypes in this simple scenario, a region of the parameter space exists where there is a reasonable probability that undiscovered haplotypes will be quite distinct from the shared haplotypes that can be identified without using both populations in variation discovery.

1496/T

Survey of genetic correlation in the human genome of 17 population groups. *RTH. Ong[1,2], X. Sim[2], H. Xu[2], Q. Fan[3], X. Liu[1], X. Wang[2], ES. Tai[3,4], M. Inouye[5], C. Suo[2], CC. Khor[2,6], ML. Hibberd[3,6], TY. Wong[3,7,8], JY. Lee[9], BG. Han[9], YS. Cho[9], CP. Simmons[10], SJ. Dunstan[10], KS. Chia[2,3], YY. Teo[2,3,11].* 1) NUS Graduate School for Integrative Science and Engineering, National University of Singapore, Singapore; 2) Centre for Molecular Epidemiology, National University of Singapore, Singapore; 3) Department of Epidemiology and Public Health, National University of Singapore, Singapore; 4) Department of Medicine, National University of Singapore, Singapore; 5) Immunology Division, Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia; 6) Genome Institute of Singapore, Agency for Science, Technology and Research, Singapore; 7) Singapore Eye Research Institute, Singapore National Eye Centre, Singapore; 8) Centre for Eye Research Australia, University of Melbourne, Australia; 9) Korea National Institute of Health, Seoul, Korea; 10) Wellcome Trust Major Overseas Program, Oxford University Clinical Research Unit, Vietnam; 11) Department of Statistics and Applied Probability, National University of Singapore, Singapore.

Through the use of commercial genotyping platforms with incomplete genome coverage, long stretches of linkage disequilibrium (LD) have been critical to the success of many genome-wide association studies in enabling the detection of statistical signals of phenotypic variation. The detected polymorphisms in these studies are often not the causal variant, but markers in LD with the casual variant, making the success of replication and meta-analysis dependent on the similarity of LD patterns in the different human populations used. Regions of high LD however confound fine-mapping studies as it becomes difficult to distinguish between the causal variant and neighboring markers in high or perfect LD with the causal variant. Here, we describe the results of assessing LD variations with the varLD metric across 17 population groups; consisting of 11 populations from Hapmap Phase 3, 3 populations from the Singapore Genome Variation Project, and populations from Korea, Sweden and Vietnam. Our findings indicate that the integration of LD differences between populations could be used to refine the boundary of region containing the causal variant, narrowing the search space and thus reducing the cost of genotyping and sequencing during fine-mapping studies. In addition, regions with strong LD differences between populations are associated with genomic regions with copy number variations, differential signals of selection and variations in recombination rates due to evolutionary and migration history of these populations. These results are relevant to the design and analysis of fine-mapping studies, where candidate gene regions from genome-wide association studies could be ranked and identified to maximize the likelihood of finding the common causal variants of disease.

1497/T

Estimating the ages of two founder mutations that increase the risk of colorectal cancer in the Spanish population. *E.M. Jewett[1], N.A. Rosenberg[1,2,3], S. Gruber[4,5,2], G. Capellá[6,7,8,9].* 1) Center for Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI; 2) Department of Human Genetics, University of Michigan, Ann Arbor, MI; 3) The Life Science Institute, University of Michigan, Ann Arbor, MI; 4) Department of Internal Medicine, University of Michigan, Ann Arbor, MI; 5) Department of Epidemiology, University of Michigan, Ann Arbor, MI; 6) Laboratori de Recera Translacional, Hospitalet de Llobregat, Spain; 7) Institut Català d'Oncologia, Hospitalet de Llobregat, Spain; 8) Institut d'Investigació Biomèdica de Bellvitge, Hospitalet de Llobregat, Spain; 9) Programa de Consell Genètic en Càncer, Institut Català d'Oncologia, Spain.

Estimating the ages of alleles provides a basis for learning about the history of disease mutations. Here we apply a version of the allele age estimation method of Schroeder et al. (Mol Biol Evol 26:995-1016, 2009) to estimate the ages of two disease alleles of the *MLH1* mismatch repair gene that have recently been discovered in the Spanish population. The method first obtains a count of the number of recombination events that have occurred on a genealogy in the region surrounding a disease mutation. This count is used to estimate the length of the genealogical tree for the set of haplotypes on which the mutation has been observed. Coalescent simulations that evaluate the relationship between genealogy length and genealogy height are then used to determine the most likely height of the gene tree, which is taken as an estimate of the age of the mutation. We estimate that the two *MLH1* mutations arose 75 and 15 generations ago (95% confidence intervals 53-122 and 12-22 generations, respectively). The recent ages estimated for both mutations are consistent with their localized distributions within two distinct geographical regions of Spain.

1498/T

Aplication of authenticity criteria in mitochondrial studies on archaic bone remains from a prehispanic muisca population. *N.P. Jara[1,2], M. Díaz[1], V. Villegas[2], C. Lopez[1], D. Torres[2], J. Bernal[2], A. Gómez[2], I. Briceño[1,2].* 1) Universidad de La Sabana, Bogotá. Campus Universitario del Puente del Común, Km. 7, Autopista Norte de Bogotá; 2) Laboratorio de Genética Pontificia Universidad Javeriana.

Introduction: Ancient DNA studies can support hypotheses regarding ancient populations; molecular studies can analyze the local population's genetic composition, minimizing biases introduced by later migrations, demographic expansions, mutations, and bottleneck effects. These analyses must be performed with special care due to the low DNA concentrations and contamination risk; therefore it is necessary to establish protocols to guarantee reproducibility and veracity of results. Objective: The present study aims to establish a protocol to obtain ancient DNA from 16 pre-columbian bone samples found in an excavation performed in the area "Candelaria La Nueva", Bogota, Colombia, dated in the period "Muisca Tardìo". Methods: Four founder mitochondrial DNA Amerindian haplotypes were analyzed by high resolution restriction enzyme analyses, obtaining fragments between 121 and 186 base pairs (bp). Different analyses were performed following a strict control of authenticity criteria regarding laboratory conditions, including: positive and negative controls, reproducibility of results and verification of particular characteristics present in ancient DNA. Results: Results obtained from the bone samples showed the exclusive presence of haplogroup A in the studied population. This data support the statement of the archaeologists of a single biological population in space and time. The distribution of this haplogroup in a 100% frequency supports the hypothesis of Chibcha genetic affiliation. Conclusion: The present study is a contribution to the study of genetic diversity in archaic American populations, allowing the integration of geographic and historic data with genetic characterization techniques associated with linguistic, ethnographic and glottochronology patterns. Following the protocol proposed in the present study allows the fulfillment of authenticity criteria for ancient samples, with the avalaible techniques, with the avalaible techniques Key words: Mitochondrial DNA, Archaic DNA, Muisca, Chibcha.

1499/T

The Initial Peopling Of The Americas: An Ever-Growing Number Of Founding Mitochondrial Genomes From Beringia. *U.A. Perego[1,2], N. Angerhofer[1], M. Pala[2], A. Olivieri[2], H. Lancioni[3], B.H. Kashani[2], V. Carossa[2], J.E. Ekins[1], A. Gómez-Carballa[4], G. Huber[5], B. Zimmermann[5], D. Corach[6], N. Babudri[3], F. Panara[3], N.M. Myres[1], W. Parson[4], O. Semino[2], A. Salas[5], S.R. Woodward[1], A. Achilli[2,3], A. Torroni[2].* 1) Sorenson Molecular Genealogy Foundation, Salt Lake City, UT, USA; 2) Dip. Genetica e Microbiologia, Università di Pavia, Pavia, Italy; 3) Dip. Biologia Cellulare e Ambientale, Università di Perugia, Perugia, Italy; 4) Unidade de Xenética, Departamento de Anatomía Patolóxica e Ciencias Forenses; and Instituto de Medicina Legal, Facultade de Medicina, Universidade de Santiago de Compostela, Santiago de Compostela, Galicia, Spain; 5) Institute of Legal Medicine, Innsbruck Medical University, Innsbruck, Austria; 6) Servicio de Huellas Digitales Genéticas, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina.

Genetic evidence based on mitochondrial DNA (mtDNA) has recently revealed the existence of additional founding lineages that have contributed to the first peopling of America's double-continent in addition to the more popular five Native American haplogroups (A2, B2, C1, D1 and X2a), and has demonstrated as well the need for additional sampling and analysis to be performed for some of the already known but poorly characterized lineages. One paradigmatic example is represented by the pan-American haplogroup C1. Two of its sub-branches (C1b and C1c) harbor ages and geographical distributions that are indicative of an early arrival from Beringia about 15-17,000 years ago, concomitantly with the other currently accepted Paleo-Indian founders. However, the estimated age of C1d - the third Native American subset of C1 - is only 8-10,000 years, which is suggestive of a much later entry and spread in the Americas. In this study, we shed light on the origin of this enigmatic Native American branch of C1 by completely sequencing a large number of C1d mitochondrial genomes from a wide range of geographically diverse, mixed and indigenous American populations. The revised phylogeny shows that the age previously reported for C1d was heavily underestimated and indicate that C1d is ancient enough to be among the founding Paleo-Indian mtDNA lineages. Moreover, our results reveal that there were two C1d founder genomes for Paleo-Indians that most likely arose early (~16kya), either in the dynamic Beringian gene pool, or at a very initial stage of the Paleo-Indian southward migration. This brings the recognized maternal founding lineages of Native Americans to the unexpected number of 15, and indicates that the overall number of Beringian or Asian founder mitochondrial genomes will probably continue to increase as more Native American haplogroups reach the same level of phylogenetic resolution as we obtained here for C1d. Additionally, we have confirmed a nearly identical geographic distribution pattern for haplogroup C1d when comparing samples collected in the general mixed population with those from native tribal groups, as it was also reported previously for haplogroups X2a and D4h3. This substantiates the validity of searching large public mtDNA databases (such as the one available through the Sorenson Molecular Genealogy Foundation, www.SMGF.org) for novel founder candidates able to reveal unknown details concerning the ancient human history of the Americas.

1500/T

Detecting heteroplasmy from high-throughput sequencing of complete human mtDNA genomes. *M. Stoneking, M. Li, A. Schoenberg, I. Nasidze.* Evolutionary Genetics, MPI-EVA, Leipzig, Germany.

Heteroplasmy, the existence of multiple mtDNA types within an individual, has been previously detected using mostly indirect methods (e.g., multiple peaks in Sanger sequencing electropherograms) and focusing on just the hypervariable segments of the control region. High-throughput (HT) sequencing should enable studies of heteroplasmy across the entire mtDNA genome at much higher resolution, as many independent reads are generated for each position. However, the higher error rate associated with the HT platforms must be taken into consideration, to avoid false detection of heteroplasmy. We used simulations and control phiX174 sequence data to design criteria for accurate detection of heteroplasmy with the Illumina GA platform, and replicate data and artificial mixtures to test the criteria. We then applied these criteria to a dataset of 131 complete mtDNA genome sequences from five Eurasian populations that had been generated using a parallel tagged approach and sequenced to an average coverage of 67-168X. Based on simulations with this amount of coverage we have good power with our criteria to detect heteroplasmies at a frequency of 10% or higher with no false positives expected. We identified 37 heteroplasmies at 10% frequency or higher at 34 sites in 32 individuals, or 24% of the individuals studied. Three sites were heteroplasmic in two different individuals, which is significantly more than expected ($p<0.001$) if heteroplasmies are occurring randomly. The mutational spectrum does not differ between heteroplasmic mutations and polymorphisms in the same individuals, but the relative rate of mutation at heteroplasmic mutations is 24.95, which is significantly higher than the average relative rate of mutation of 2.72 estimated for all mutable sites in the human mtDNA genome ($p<0.001$). Finally, the ratio of nonsynonymous to synonymous (N:S) changes for heteroplasmies in the protein-coding genes was significantly greater than the N:S ratio for polymorphisms in the same individuals ($p<0.05$). Overall, the increased relative rate of mutation for heteroplasmies suggests that they play a role in generating neutral diversity in human mtDNA, whereas the elevated N:S ratio for heteroplasmies suggests that they are also subject to negative selection. Our results indicate that with appropriate criteria for avoiding false positives due to sequencing errors, HT platforms can provide novel insights into genome-wide aspects of mtDNA heteroplasmy.

1501/T

Mitochondrial DNA Control Region Mutational Events Based on Empirical Data. *S.R. Woodward[1], T.T. Steele[1], B.C. Burdett[1], R. Hughes[1], U.A. Perego[1,2], N. Angerhofer[1], A. Achilli[2,3], N.M. Myres[1].* 1) Sorenson Molecular Genealogy Foundation, Salt Lake City, UT, USA; 2) Dip. Genetica e Microbiologia, Università di Pavia, Pavia, Italy; 3) Dip. Biologia Cellulare e Ambientale, Università di Perugia, Perugia, Italy.

Two peculiar features of mitochondrial DNA (mtDNA) make it a very informative and widely employed instrument in studying human migrations and evolution: it is inherited exclusively along the maternal line and it lacks recombination. While it is difficult to determine the ancestral origin of mutations that occur on various locations of diploid nuclear DNA, the sequence variation observed in any mtDNA genome is the result of the sequential accumulation of new mutations along the radiating maternal lineages. Therefore, present-day molecules stand as a literal biological record of the mutational events that took place through the evolutionary history of mankind across the world. Numerous studies during the past two decades have proposed different mutation rates based on either a phylogenetic approach or on empirical data, based on control region, coding region, or complete genome sequences. However, disagreement persists on the accuracy of effective rates. One of the major limitations with a phylogenetic approach is the unavailability of actual genealogies to accurately quantify a mutation fixation rate. With regard to studies utilizing empirical data, the major limitation has been the limited size of the datasets used for such analyses. In the current study, we tried to address this issue by using data extrapolated from the Sorenson Molecular Genealogy Foundation (SMGF, www.SMGF.org) correlated database of genealogical and genetic data. As of June 2010, the publicly accessible portion of the SMGF database includes 73342 mtDNA control region haplotypes which are linked to 313121 maternal ancestors, showing a total of 140827 insertions, 39120 deletions, and 584216 substitutions. Extension and correlation of the pedigree data associated with each mtDNA sequences revealed the presence of approximately 25000 generational events characterized by nearly 1000 mutational events. An accurate analysis of these data allowed us to discuss differences/matches on the calibrations of the mtDNA molecular clock obtained from "pedigree or near-time" and "phylogenetic or distant" approaches.

1502/T

Molecular characterization of abnormal hemoglobin chromatograms detected by the Bio Rad Variant II Turbo HPLC systems in Korean. *E. Cho, S.G. Lee, E.H. Lee, E.J. Kim, I.H. Yu.* Laboratory Medical Genetics, Greencross reference laboratory, Yongin, Korea.

Background: Patients with hemoglobin (Hb) variants may produce false HbA1c measurement. This study aimed to detect the common Hb variants in Korean population, to evaluate their effect on the determination of HbA1c and to characterize them by molecular methods. Methods: A total of 20,000 samples referred to Greencross reference laboratory from June 2009 to July 2009 were submitted for Hb variant analysis by the Bio-Rad Variant II Turbo (VII) which uses high-performance liquid chromatography (HPLC) method. Patients identified with Hb variants were re-tested by the Bio-Rad Variant II in thalassemia mode (VII-TM). We carried out DNA studies of the β and α1 globin genes by PCR and sequencing of all the coding exons. In the cases with high Hb F (>10%) and normal results by β globin gene sequencing, we performed the α and β globin gene dosage analyses using multiplex ligation-dependent probe amplification (MLPA, MRC-Holland) method. Results: Among the 20,000 cases, 20 cases (0.1%) had variant peaks and 31 cases (0.16%) had increased Hb F (>5%). Eight cases had variant peaks between the A1c and A0 peaks. Among them, five cases were identified as Hb G-Coushatta (β22Glu®Ala), (mean retention time, 0.71 min at VII , 2.74 min at VII-TM) and three cases were identified as Hb Ingelwood (β142Ala®Thr) (mean retention time, 0.82 min at VII, 2.05 min at VII-TM). One case had peaks between the Hb LA1c and A1c (retention time: 0.52 at VII, 1.04 at VII-TM) which were identified as Hb North Manchester (β51Pro®His) and one case had a very high peak at Hb F (46.3%), which were identified as Hb Beckman (β135Ala®Asp). Eight cases had peaks at S-window, two cases had peaks at variant window and P3, respectively. One case with high Hb F(>10%) were revealed to have heterozygous deletion of the total β-globin gene and partial δ gene deletion spanning about 9.4Kb. In patients with Hb variants, HbA1c was measured lower at the VII than VII-TM. Conclusion:In conclusion, the incidences of Hb variant and high Hb F concentrations were estimated to be 0.1% and 0.16%, respectively. The most common Hb β variants in Koreans were Hb G Coushatta and Hb Ingelwood.

1503/T

Genetic Characterization of Indigenous Populations in Mexico, Mazahua and Zapotecs, with reference to phylogenetic relationships among indigenous North- and Meso-American populations. *S. Ueda[1], F. Mizuno[1], J. Gojobori[2], L. Wang[3], K. Ohnishi[1], S. Sugiyama[4], V. Acuna[5].* 1) Dept Biological Sci, Univ Tokyo, Grad Schl Sci, Tokyo, Japan; 2) Hayama Center for Advanced Studies, Graduate University for Advanced Studies, Hayama, Japan; 3) Institute of Genetics and Developmental Biology, Chinese Academy of Science, Beijing, China; 4) School of Foreign Studies, Aichi Prefectural University, Aichi, Japan; 5) Instituto Nacional de Antropología e Historia, Mexico City, Mexico.

Most indigenous American people share common mitochondrial DNA lineages that define four primary haplogroups (A, B, C, and D), now further characterized as A2, B2, C1, and D1. Mesoamerica is a region extending approximately from central Mexico to Honduras and Nicaragua, and indigenous American populations in Mesoamerica are characterized by high frequencies of haplogroup A with moderate to low frequencies of haplogroup B. To the contrary, southwest indigenous populations in North America exhibit high frequencies of haplogroup B and very low frequencies or the complete absence of haplogroup A. We here investigated the genetic structure of two indigenous American populations in Mexico, Mazahua and Zapotecs. The Mazahua inhabit the northwestern portion of the State of Mexico and northeastern area of Michoacán, while the Zapotecs do the southern state of Oaxaca and its neighboring states. We determined mitochondrial DNA sequences for 25 and 88 individuals of Mazahua and Zapotecs, respectively. All individuals examined belong to any of haplogroup A, B, C, or D. We compared their haplogroup frequencies for all pairs of populations including other indigenous American populations around Mesoamerica previously examined. In addition, we also calculated the nucleotide diversity (the mean of pairwise nucleotide differences per site) within each population and the genetic distances (net values of nucleotide substitutions) between the populations using nucleotide sequences of the mtDNA hyper-variable regions (HVR). Phylogenetic network was constructed for the mtDNA haplogroups obtained, while a neighbor-joining phylogenetic tree and principle component analysis was conducted using the HVR sequence. Based on these molecular phylogenetic indices, we discuss genetic structure of indigenous Mazahua and Zapotecs populations and genetic similarity to other indigenous populations in Meso- and North- America.

1504/T

Adaptations to high altitude in Ethiopia. *G. Alkorta-Aranburu[1], D. Witonsky[1], C. Beall[2], A. Di Rienzo[1].* 1) Human Genetics , University of Chicago, Chicago, IL; 2) Anthropology Department, Case Western Reserve University, Cleveland, OH.

High-altitude (HA) resident human populations differ biologically from lowlanders; however, the biological bases of their differences are not well understood. The possibility of genetic adaptation to HA is supported by the high heritability of hemoglobin (Hb) concentration in Andean and Tibetan highlanders; a locus associated with higher oxygen saturation in Tibetans and positively selected haplotypes of EGLN1, PPARA and EPAS1 associated with lower Hb concentration in Tibetans. Epigenetic adaptation can also be hypothesized since invasive cancer clones show both genetic and epigenetic modifications due to hypoxia and nutrient deprivation.

To learn more about human adaptations to HA, we extended genomic analysis to two Ethiopian ethnic groups: the Amhara and the Oromo with, respectively, thousands of years and 500 years of residence at HA. Amhara populations at 3500-4000 and 1500 meters, and Oromo populations at 4000 and 1500 meters were sampled. HA samples show lower oxygen saturation and higher Hb concentration; but when ethnic groups are compared, Oromos show greater altitudinal differences. Then, methylation levels at ~27000 CpG sites in 17 HA and 17 low-altitude (LA) Oromos and Amharas were collected using Illumina's HumanMethylation27 BeadChips. While methylation levels differ significantly between Amharas and Oromos, no evidence for epigenetic differences between HA and LA within the same ethnic group is observed. Next, genome-wide genotype data were collected in 48 HA and 48 LA Amharas, and 24 HA and 24 LA Oromos using Illumina's 650Y or 1M SNP arrays. When allele frequency differences between HA and LA Ethiopians are compared to expectations, the Amharas and, especially, the Oromos show a significant excess of high-FST values. Interestingly, some of the genes with high-FST SNPs are good candidates for hypoxia driven adaptation based on their biological function; e.g., the Parkinson's disease gene SEMA5A that promotes angiogenesis by increasing endothelial cell proliferation, migration, and decreasing apoptosis. In addition, these data allow us to test whether the variants and genes associated with Hb concentration and/or signals of adaptation to HA in Tibetans also play a role in Ethiopian adaptations to HA. We replicate in the Oromo one EPAS1 SNP significantly associated with Hb concentration in two Tibetan cohorts.

1505/T

A cross-population comparison extended haplotype-based homozygosity score test to detect positive selection in genome-wide scans. *R. Fan[1], M. Zhong[2], K. Lange[3].* 1) Dept of Statistics, Texas A&M Univ, College Station, TX; 2) Dept of R436, GPRD, Abbott Laboratories, Abbott Park, IL; 3) Dept of Human Genetics, David Geffen School of Medicine, Univ of California, Los Angeles, CA.

Positive selection may shed on complex disease and human evolution. Previously we proposed a powerful score test, Extended Haplotype-based Homozygosity Score Test (EHHST), to detect positive selection in genome-wide scans. We adopted extended stretches of homozygosity as a surrogate indicator of recent positive selection and tested the haplotype version of Hardy-Weinberg equilibrium (HWE). In this article, we developed a cross-population comparison test (xp-EHHST) to detect chromosome regions in which there is no significant excess homozygosity in one population but homozygosity remains high in another population, i.e., the HWE is roughly true in one population but hardly valid in the other. We assumed that every population has a random sample of unrelated individuals, each typed on a large number of single nucleotide polymorphisms (SNPs). A pooled t-test statistic was constructed to compare the measurements of homozygosity of the two samples around a core SNP. The detected strong signals highlighted the candidate regions that undergo positive selection in one of the two populations. We conducted the simulation study to evaluate the performance of the new test in terms of type I error and power, and then applied it to HapMap Phase II data.

1506/T

Increased risk of Crohn's disease resulting from genetic hitchhiking at IBD5. *C.D. Huff[1], D. Witherspoon[1], Y. Zhang[1], C. Gatenbee[1], S. Kugathasan[2], H. Hakonarson[3,4,5], A. Whiting[1], C. Davis[1], W. Wu[1], J. Xing[1], W.S. Watkins[1], M. Bamshad[6], K. Bulayeva[7], T. Simonson[1], L.B. Jorde[1], S.L. Guthery[8].* 1) Department of Human Genetics, Eccles Institute of Human Genetics, University of Utah, Salt Lake City, UT, U.S.A; 2) Department of Pediatrics, Emory University School of Medicine and Children's Health Care of Atlanta, Atlanta, GA, USA; 3) Center for Applied Genomics, Children's Hospital of Pennsylvania,Philadelphia, Pennsylvania, United States of America; 4) Division of Human Genetics, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, United States of America; 5) Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, United States of America; 6) Department of Pediatrics, University of Washington, Seattle, WA 98195; 7) Vavilov Institute of General Genetics, Russian Academy of Sciences, Moscow, Russia; 8) Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, Utah, USA.

We present genetic evidence that adaptation to an agricultural diet may have increased the risk of Crohn's disease in Europeans. IBD5 is a 250 kb haplotype on chromosome 5 that is associated with an increased risk of Crohn's disease in Europeans. The OCTN1 gene is centrally located on IBD5 and encodes a transporter of the antioxidant ergothioneine (ET). The 503F variant of OCTN1 is strongly associated with IBD5 and is a gain-of-function mutation that increases absorption of ET. Here, we present several lines of evidence supporting the hypothesis that 503F is a dietary adaptation to early farming in the Fertile Crescent. The decay of linkage disequilibrium (LD) around 503F indicates that the mutation originated approximately 12,500 years ago, which corresponds with the origin of agriculture in the Fertile Crescent. The iHS test for recent positive selection yielded statistically significant results for 503F in the European CEU HapMap population (p= 0.0007) and European HGDP populations (p£0.01). Evidence of a strong selective sweep among Europeans has important implications for understanding the nature of disease association with IBD5. Our simulation studies demonstrate that selective sweeps in the nuclear genome often increase the frequency of mildly deleterious alleles via genetic hitchhiking. This process results in disease association with a favorable allele, even when the favorable allele has no direct influence on disease risk. In a sample of 836 Crohn's disease cases and 1262 controls, we identified signals of disease association on the IBD5 haplotype that are consistent with genetic hitchhiking. These results suggest that genetic hitchhiking increased the frequency of disease-causing alleles at a location distal to OCTN1, accounting for the association between 503F and Crohn's disease.

1507/T

Characterization of deleterious variation by using allelic age. *A. Kiezun, I. Adzhubei, S. Pulit, P. de Bakker, S. Sunyaev.* Division of Genetics, Brigham & Women's Hospital, Harvard Medical School, Boston, MA.

Characterization of deleterious genetic variation is important for evolutionary and medical genetics. Medical sequencing studies aim at detecting the direct effect of functional allelic variants on phenotypes. Most functional variants are expected to be evolutionary deleterious but identification of these alleles is challenging. Current approaches to analyze deleterious variation use site frequency spectrum, polymorphism-to-divergence ratio, evolutionary conservation and protein structure. We present a new method to characterize deleterious variation. The method uses allelic age conditioned on frequency. The effect described by Maruyama in 1974 predicts that, at a given frequency, neutral variants are older than either deleterious or advantageous variants. Because low frequency non-synonymous variation is expected to be enriched in deleterious alleles, the effect predicts that non-synonymous variants are, on average, younger than synonymous or non-coding variants of the same frequency. Our analysis of simulations and real data supports this prediction. Allelic age can be estimated from frequency, intra-allelic variability, LD, or shared haplotypes. The data from the 1000 Genomes Project enables new approaches for estimating relative allelic age: mutation and recombination "clocks". The mutation clock estimates, for each variant, the number of likely younger (less frequent and completely linked) variants. The recombination clock estimates the length of the haplotype block on which the variant exists. The two clocks can be combined to improve power. Analysis of the 1000 Genomes Project pilot dataset agrees with theory and simulations. The mutation and recombination clocks show that non-synonymous alleles are younger than synonymous alleles. Consistently with simulations, the mutation clock is more sensitive, and the effect is stronger for damaging variants than for benign non-synonymous variants. The effect disappears at higher frequencies, because few deleterious variants reach higher frequencies. Consistently with the Maruyama effect, population-specific variants are more damaging than variants shared between populations (thus older), when conditioned on frequency. As expected, however, benign variants are not significantly more population specific than synonymous variants. We will present characterization of deleterious variation using the Maruyama effect as seen in 1000 Genomes data and discuss its potential applications.

1508/T

Contrasting patterns of sequence variation in pigmentation and neutral loci indicate population-level effects of selection. *H. Norton[1], M. Hammer[2].* 1) ARL-Biotech, Univ Arizona, Tucson, AZ; 2) Department of Ecology and Evolutionary Biology.

While demographic processes affect all regions of the genome in a similar manner, selection should preferentially affect levels of diversity and the site frequency spectrum (SFS) in genic regions relative to neutral regions. Here we compare patterns of variation in ten pigmentation candidate loci to a neutral dataset comprised of 61 loci located in regions of high recombination and far from genes. Both the genic and neutral loci were sequenced in the same panel of 72 African (Biaka, Mandenka, and San) and 80 Non-African (Han Chinese, French Basque, and PNG Highlander) chromosomes. As expected, mean levels of diversity are significantly lower in genic relative to neutral regions (π_{genic} = 0.00072; $\pi_{neutral}$ = 0.00113; p < 0.01). Because it is often assumed that pigmentation loci have been affected by purifying selection within Africa and by positive directional selection outside of Africa, we compared the SFS of pigmentation and neutral loci in these regions. We observe a trend towards an excess of rare or singleton alleles (exhibited as negative Tajima's D and Fu and Li's D values) consistent with either purifying or directional selection in the genic regions. However, as a class pigmentation loci do not significantly differ from the neutral loci in either Africans ($TD_{pigmentation}$ = -0.80, $TD_{neutral}$ = -0.82, p = 0.92; $FLD_{pigmentation}$ = -1.08, $FLD_{neutral}$ =-1.03 p = 0.90) or non-Africans ($TD_{pigmentation}$ = -0.38, $TD_{neutral}$ = -0.04, p = 0.16; $FLD_{pigmentation}$ = -1.00, $FLD_{neutral}$ =-0.60 p = 0.36). At the population level, only the French Basque showed a significant difference between pigmentation loci and the neutral regions for Tajima's D ($TD_{pigmentation}$ = -0.30, $TD_{neutral}$ = 0.35, p < 0.05). However, despite the failure of pigmentation loci as class to appear distinct from neutral loci, several individual loci stood out from the neutral regions in population-level comparisons. The gene MATP exhibits an excess of high frequency derived alleles in the French Basque (FWH = -4.11, p < 0.01) consistent with expectations under positive directional selection, while the gene MITF exhibits an excess of rare alleles in the Mandenka population (TD = -1.46, p < 0.05) indicating a role for purifying selection. These results suggest that while selection pressure on pigmentation loci may differ between Africans and non-Africans, the effects of selection on individual genes may have varied among populations within broader geographic regions.

1509/T

Resequencing of the catechol-O-methyltransferase gene, *COMT* revealed non-neutral processes associated with schizophrenia. *H. Shibata[1], M. Uchida[1], H. Goto[2], S. Mano[3], O. Takenaka[4], Y. Fukumaki[1].* 1) Medical Inst Bioregulation, Kyushu Univ, Fukuoka, Japan; 2) Center for Comparative Genomics and Bioinformatics, Pennsylvania State Univ, State College, PA; 3) The Inst of Statistical Mathematics, Tokyo, Japan; 4) Primate Research Inst, Kyoto Univ, Aichi, Japan.

Schizophrenia is a common psychiatric disease with the relatively strong genetic background (λ_s = 10). Because of the early onset and the loss of sociality in patients, the susceptibility alleles of schizophrenia are expected to be selected out through generations. However, the lifetime prevalence is stably high at ~1% in any of the populations surveyed. To explain this paradox, we hypothesized that the schizophrenia susceptibility alleles are maintained by the non-neutral process such as balancing selection. In this study, we analyzed the nucleotide diversity in the *COMT* (catechol-O-methyltransferase) gene as a schizophrenia susceptibility gene. We resequenced the entire coding regions and the upstream regions of > 6 kb potentially affecting the gene expression in 72 humans (24 Japanese, 24 Caucasians, 24 African Americans) and 24 chimpanzees. Using the dataset, we examined neutrality in the evolution by the summary statistics approach such as Tajima's method. In sliding window analysis, we identified two subregions consistently showing signatures of balancing selection in different human populations. One is the subregion surrounding a common nonsynonymous SNP, rs4680 of which significant association has been frequently reported with schizophrenia (*TD* = + 2.10, *p* < 0.05, Caucasians; *TD* = + 1.46, Japanese; *TD* = +1.75, African Americans) and the other is intron 5, approximately 2 kb downstream from rs4680 (*TD* = + 2.14, *p* < 0.05, African Americans; *TD* = + 1.98, Japanese; *TD* = + 1.48, Caucasians). There is another common SNP, rs4818, of which highly significant association has been reported with schizophrenia, located in the same window with rs4680. We conclude that haplotypes defined by the two SNPs in the *COMT* gene, has been maintained by balancing selection shared by different human populations and that the balancing selection may be associated with schizophrenia as well as human brain functions.

1510/T

Signals of local adaptation in human populations. *T.S. Simonson[1], C.D. Huff[1], Y. Zhang[1], W.S. Watkins[1], D.J. Witherspoon[1], J. Xing[1], S.R. Woodward[2], L.B. Jorde[1].* 1) Department of Human Genetics, University of Utah, Salt Lake City, UT; 2) Sorenson Molecular Genealogy Foundation, Salt Lake City, UT.

Humans have adapted to various environmental conditions. Genome-wide scans of positive selection have provided a first glimpse into locally adapted regions of the genome. It is possible that many more examples of positive natural selection exist in populations that have not yet been surveyed. In order to enhance the current understanding of population-specific adaptation, we performed genome-wide selection scans for 14 previously unstudied world-wide populations from Africa, Europe, Asia, and the Americas using the iHS statistic. Five of the seven selection candidates previously reported in the HapMap populations (Sabeti 2007) were identified in at least one additional population in our sample at the 0.02 significance level. Two of the previously reported regions were found in three or more of the populations we sampled: 1) the genic region containing *PDE11A* (phosphodiesterase 11A), first identified in Europe, Japan, and China, was also significant in Bolivia, Mongolia, Thailand, and Tibet; 2) the genic region containing *SLC30A9* (solute carrier family member 9), first identified in Japan and China, was significant in India, Pakistan, and Iraq. In contrast to finding many HapMap selection candidates in our additional populations, more than two-thirds of our selection candidate regions were not significant at the 0.05 level in any of the HapMap populations. We find that positive selection candidates vary considerably among these populations, highlighting novel signals of natural selection. These results suggest that distinct human populations sampled here have differentially adapted to specific environments.

1511/T

A Genome-Wide Search for Signals of High Altitude Adaptation in Tibetans. *S. Xu[1,2], S. Li[3], Y. Yang[3], J. Tan[3], H. Lou[1], W. Jin[1], L. Yang[1], X. Pan[3], J. Wang[1], Y. Shen[4], B. Wu[3,4], H. Wang[1], L. Jin[1,2,3].* 1) Computational Genomics, CAS-MPG Partner Institute for Computational Biology, Shanghai, Shanghai, China; 2) Key Laboratory of Computational Biology, CAS-MPG Partner Institute for Computational Biology, Chinese Academy of Sciences, Shanghai 200031, China; 3) State Key Laboratory of Genetic Engineering and Ministry of Education (MOE) Key Laboratory of Contemporary Anthropology, School of Life Sciences and Institutes of Biomedical Sciences, Fudan University, Shanghai 200433, China; 4) Children's Hospital Boston, Harvard Medical School, Boston, MA 02115, USA.

The Tibetan Plateau, known as "the roof of the world" and with an average elevation of over 4,500 meters, is the highest plateau in the world. It is almost certain that biological adaptability has contributed to the success of Tibetans in occupying the Tibetan Plateau, because traditional technology could not buffer them from the unavoidable environmental stress of severe lifelong high-altitude hypoxia. However, efforts so far have not been successful to identify specific genetic loci contributing to high-altitude adaptation. We conducted a genome-wide study of 1,000,000 genetic variants in 46 Tibetans (TBN) and 92 Han Chinese (HAN) for identifying the signals of high altitude adaptations (HAA) in Tibetan genomes. We discovered the most differentiated variants between TBN and HAN at chromosome 1q42.2 and 2p21. HIF2A and HIFPH2, both related to hypoxia-inducible factor (HIF), were found most differentiated in the two regions, respectively. Strong positive correlations were also observed between the frequency of TBN dominant haplotypes in the two gene regions and altitude in East Asian populations. Linkage disequilibrium and further haplotype network analyses of world-wide populations suggested the antiquity of the TBN dominan haplotypes and long-term persistence of the natural selection. Finally, a "dominant haplotype carrier" hypothesis could describe the role of the two genes in HAA. Our results indicate that HIF2A and HIFPH2 are most likely responsible for HAA of Tibetans. We suggest that the natural selection on the two genes should be ancient, long-term persistant and ongoing.

1512/T

Research into Association between Periodontal Diseases and Mannose-Binding Lectin Gene Polymorphisms. *K. Erciyas[1], T. Sever[2], K. Ustun[1], S. Pehlivan[2].* 1) Gaziantep University, Faculty of Dentistry, Department of Periodontology,Gaziantep, Turkey; 2) Gaziantep University, Faculty of Medicine, Department of Medical Biology, Gaziantep, Turkey.

Mannose-Binding Lectin (MBL) is an acute phase reactant with an important duty in activating the classical complement pathway and phagocytes in natural immunity. This study aimed to investigate whether there was any difference in the MBL gene polymorphisms between individuals with different periodontal diseases and healthy individuals and to evaluate its relationship with periodontal clinical parameters. This study was conducted on 167 voluntary individuals in total, individuals diagnosed with Gingivitis (39), Aggressive (37) and Chronic (57) Periodontitis as well as periodontally healthy individuals (34). Periodontal conditions of all individuals in the study were detected by measuring probing depth, clinical attachment level, plaque index, and gingival index. MBL gene in the DNA samples isolated from peripheral blood was investigated using the PCR-RFLP method. The results were analyzed comparing both patient groups and the control group and clinical parameters. When the MBL gene polymorphism was compared in terms of allele and genotype frequencies in control and patient groups, no significant association was observed, whereas when compared in terms of Hardy-Weinberg Equilibrium (HWE), a deviation was observed in the Aggressive Periodontitis group only. Moreover, no significant association of the MBL polymorphism could be detected in terms of age, sex and clinical parameters. In conclusion, it was detected that there was no significant association between MBL gene polymorphism and Periodontal diseases in terms of allele and genotype frequencies or clinical parameters but it was necessary to work on more samples in Control and Aggressive Periodontitis groups as presence of some deviation from HWE was detected in Aggressive Periodontitis patient group.

1513/T

POPULATION GENETICS STUDY OF MEXICAN MESTIZO POPULATION: APPLICATIONS IN FORENSIC SCIENCES AND PARENTAL ASSESSMENTS. *R. Gomez[1], C. Santana[2], G. Norris[2], A. Majluft[3], M.A. Meraz-Ríos[4], M.C. Revilla[5], F. Castañeda[6], I. Solano[6], S. Martínez-Salas[7], M.P. Cabrales-Romero[8], P.F. Lucio-Montero[7], S. Xihuitl[2], A. Keiman[2], R. Quezada[2], M.L. Munoz[6], Santana C.* 1) TOXICOLOGY, CINVESTAV-IPN, Mexico City, Mexico. Av. Instituto Politécnico Nacional N=BA 2508, Col. San Pedro Zacatenco, C.P. 07360, México D.F., México; 2) BIMODI (Biología Molecular Diagnóstica) laboratory, Querétaro, Qro., México; 3) Unidad de Investigación Médica en Trombosis y Aterogenesis, HGR Gabriel Mancera, IMSS#1 Carlos McGregor Sanchez Navarro, México D.F., México; 4) Department of Molecular Biomedicine, Cinvestav-IPN, México D.F., México; 5) Unidad de Investigación Médica en Enfermedades Metabólicas del Centro Médico Nacional Siglo XXI, IMSS, México, D. F., México del Instituto Mexicano del Seguro Social; 6) Department of Genetic and Molecular Biology, Cinvestav-IPN. México D.F., México; 7) Laboratorio Multidisciplinario de Investigación, Escuela Militar de Graduados de Sanidad, Secretaría de la Defensa Nacional, México D.F., México; 8) Department of Cell Biology, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México (UNAM).

Mexican Republic is a country with diverse historical, cultural and linguistic characteristics, due to the pluriethnic legacy from a large number of native Amerindian groups, the Spaniard colonization (1519), the arrival of African slaves, and many other historical events such as the French intervention (1861-1867). The application of DNA fingerprinting in forensic/criminal cases and paternity testing is increasing worldwide. Most DNA typing applications have legal and ethic implications. Therefore there is a particular need to generate descriptive population statistics as well as forensic and paternity parameters, in order to have a reliable database. A Mexican mestizo population study (n=1640), was performed on the loci D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, FGA and a portion of the X-Y homologous gene Amelogenin for gender determination using AmpFISTR Identifiler® kit and capillary electrophoresis. We determine allele and genetic frequencies, and evaluate STR technology for developing a Mexican DNA database with forensic and paternity aims using Genètix, Arlequin, Fstat, and Powerstat (Promega corporation) softwares. We found that D2S1338, D18S51, and FGA are the most polymorphic and informative loci. In summary, overall 15 loci show a high forensic efficiency with a power of discrimination close to 1, with the capacity to genetically discriminate one individual in one quintillion (1/1018). We observed an important Hardy-Weinberg departure, only 3 out of the 15 loci are in equilibrium, with a high inbreeding coefficient. The Mexican mestizo population analyzed data appears significantly different (p=0 in each one of the 15 loci) to a previously published Spaniard population data. In conclusion, this database potentially validate the use of these 15 STR loci to established the forensic identity and parentage testing for legal purposes, and offers a powerful tool for genetic variation analysis.

1514/T

Comparison of TPH2 sequence variability among Taiwan aboriginal populations: Application on association study of schizophrenia. *Y.M.J. Lin[1], S.C. Chao[2], C.H. Chen[3], H.S. Sun[4].* 1) Institute of Biomedical Sciences, National Chung Hsing University, Taichung Taiwan; 2) Department of Obstetrics and Gynecology, National Cheng Kung University Hospital, Douliou Branch, Yunlin, Taiwan; 3) Institute of Human Genetics, Tzu-Chi University, Hualien, Taiwan; 4) Institute of Molecular Medicine, National Cheng Kung University Medical College, Tainan, Taiwan.

Genetic differences exist among human ethnic populations address an important issue in biomedical studies, and has been considered to be a distinct problem for evaluating risk factors and the prognosis/treatment of various diseases. Taiwan has heterogeneous groups of people including several aboriginal populations that account for 1.5% of present Taiwan's population. Aborigines in Taiwan suffer from the disparity of health behavior and medical access with the major Chinese Han population. For example, higher prevalence of some psychiatric diseases, like alcoholism, drug abuse and suicide have been reported in aborigines. Therefore, the well characterized genetic feature of candidate target gene in particular ethnic group is very important for genetic studies of these diseases in Taiwan aboriginal groups. Tryptophan Hydroxylase 2 (TPH2), the rate-limiting enzyme for the biosynthesis of serotonin in the central nervous system, is the most promising candidate gene in CNS-related diseases including psychiatric disorders. This study was designed to characterize genetic information of TPH2 in Taiwan aboriginal groups. Five Single Nucleotide Polymorphisms (SNPs) of TPH2 that were previously identified in Taiwan's Han Chinese population are selected to be characterized in three aboriginal populations, Ataya, Amis and Bunun. Our results showed that most of the SNPs exist in all populations with different allelic frequencies except the functional C2755A was only observed in Amis. Based on the TPH2 genetic information, we further revealed that Ataya individuals are more distant from Amis and Bunun, and this result is consistent with previous studies of genetic relationship between Taiwan aboriginal groups. Take advantage of profiled TPH2 genetic information and based on the potential role of serotonin in schizophrenia etiology, we also pretest the association between TPH2 gene and schizophrenia in Amis and Ataya. Preliminary result showed significantly haplotypic association between TPH2 and schizophrenia in Ataya, but not in Amis. Results from this study provide an essential profile that will be useful for further genetic studies related to TPH2 in Taiwan aboriginal groups.

1515/T

MEFV, TNFRSF1A and CARD15 mutation analysis in Behcet's Disease. *A. Mory[1], Y. Baruch[2], E. Dagan[1,3], M. Rozenbaum[4,5], F. Massimo[2], R. Gershoni-Baruch[1,5], I. Rosner[4,5].* 1) Inst of Human Genetics, Rambam HealthCare Campus, Haifa, Israel; 2) Dept of Clinical Medicine, University of 'La Sapienza', Rome, Italy; 3) Dept of Nursing, University of Haifa, Haifa, Israel; 4) Dept of Rheumatology, Bnei-Zion Medical Center, Haifa, Israel; 5) The Ruth and Bruce Rappaport Faculty of Medicine, Technion-Institute of Technology, Haifa, Israel.

Given the pathological similarities between Behcet's disease (BD), Familial Mediterranean fever (FMF), TNF receptor-associated periodic syndrome (TRAPS) and Crohn's disease (CD) we studied the rate of mutations and polymorphisms in MEFV, TNFRSF1A and CARD15 in Israeli BD patients compared to an ethnically matched control population. Fifty-four BD patients (11 Jews and 43 Arabs), evaluated with respect to the entire spectrum of BD disease manifestations, were granted a systemic severity score for BD. An association between BD manifestations and MEFV, TNFRSF1A and CARD15 variants was analyzed. Twelve patients (20.7%) displayed a single MEFV mutation and four patients (7.4%) had two mutated FMF alleles. Two patients (3.8%) carried a CARD15 variation and none carried a TNFRSF1A polymorphism. The frequency and distribution of mutated alleles between patients and controls was comparable (p=0.27). No statistically significant differences between carriers and non-carriers with respect to disease manifestations and severity score were calculated. Arab patients were diagnosed earlier than Jewish patients (25.8±11.6 and 37.2±10.7, respectively, p= 0.06). The overall MEFV high carrier frequency in our cohort of BD patients seems to be attributed to their Mediterranean extraction rather than related to BD. The propensity of Arab patients (79.6%) in a cohort of BD patients from Northern Israel is highlighted in face of their proportion (20%) in the general population lending further support to arguments that favor a genetic component for BD.

1516/T

Analysis of 17 Y-chromosomal STR loci in the Iranian population. *N. Amini[1], M. Sharafi Farzad[1], M. Tavallaie[2], S. Zeinali[1,3].* 1) Kowsar Human Genetic Research Center, Tehran, Iran; 2) Genetic Research Center, Baqiyatallah Medical Science University, Tehran, Iran; 3) Dept of Mol. Med. Biotech Research Center, Pasteur Institute of Iran, Tehran.

Introduction One of the smallest human chromosomes is the Y chromosome with an average size of 60 Mb. Exchanges is limited to small pseudoautosomal regions of the X-Y pair between X and Y chromosomes in the meiosis. The Y chromosome in most of its length is male-specific and effectively haploid and is transmitted from father to his son unchanged unless a mutational event takes place. Y chromosome-specific STRs have proved to be an important tool in paternity cases, especially when the alleged father is deceased, as well as forensic and non-forensic fields. Materials and methods Blood samples were collected from 129 randomly selected, unrelated Iranian males, following procedures that are in accordance with Promega kit and FTA Cards. All 17 (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385a/b, DYS438, DYS439, DYS437, DYS448, DYS458, DYS456, DYS635, and Y-GATA-H4) markers were co-amplified using the AmpFlSTR Y-filerTM PCR Amplification kit(Applied Biosystems, USA).The amplified products were separated by capillary electrophoresis on ABI Prism 3130 XL Genetic Analyzer. The sample run data were analyzed by GeneMapper IDX Software V. 1.0. Allelic frequencies were estimated by direct counting. To determine of haplotype diversity and the other parameters we use Arlequin software ver 3.1 and also the online AMOVA tool from YHRD.org3.0 for y-STR haplotyping. Results Allele frequencies in our study are similar to reported allele frequencies in Iranian population on Y-STR haplotype databases. We observed 123 different haplotypes. Total haplotype diversity was 0.9996. In this study we found some new mutation as off-ladder which most of them are located on DYS458 marker and tow duplication in DYS392 and DYS393 markers. Among the 17 markers analyzed, DYS385 and DYS389II were calculated to be the highest and DYS448 lowest values for allelic range. Conclusion we used 17Y-STR markers and providing further population data for Iranian men.The Y-chromosome structure in men from Iran is very diverse. Combination of Y chromosome 17 STR loci may be used as a powerful tool for individual identification and parentage analysis in the Iran male population.

1517/T

Signatures of founder effects, admixture and selection in the Ashkenazi Jewish population. *S. Bray[1], J. Mulle[1], A. Dodd[1], A. Pulver[2], S. Wooding[3], S. Warren[1].* 1) Department of Human Genetics, Emory University School of Medicine, Atlanta, GA; 2) Department of Psychiatry and Behavioral Sciences, Johns Hopkins School of Medicine, Baltimore, MD; 3) The McDermott Center for Human Growth and Development, University of Texas Southwestern Medical Center, Dallas, Texas.

The Ashkenazi Jewish (AJ) population has long been viewed as a genetic isolate, yet despite decades of population genetic studies, we still lack definitive evidence of founder events or positive selection. Here we analyzed a large AJ cohort and found higher linkage disequilibrium (LD) and identity-by-descent, as expected for an isolate. However, paradoxically we also found higher genetic diversity relative to Europeans, a sign of an older or more admixed population but not of a long-term isolate. Further analysis indeed revealed higher than expected levels of admixture with Europeans, likely accounting for the increased genetic variation. Moreover, we demonstrate that admixture also correlates with high LD, suggesting that the AJ population has undergone gradual admixture with neighboring populations, increasing both its genetic diversity and LD, yet still maintaining some shared haplotypes from founding events. Additionally, we applied extended haplotype tests to determine whether positive selection can account for the level of AJ-prevelant diseases. We identified genomic regions under selection that account for lactose and alcohol tolerance in the AJ population, and while we found evidence for positive selection of some loci responsible for AJ-prevalent genetic diseases, the higher incidence of the majority of these loci is likely the result of genetic drift. Thus, the AJ population shows some evidence of past founding events, however, admixture and selection have also strongly influenced its current genetic structure.

1518/T

Effective Prediction of HLA Alleles Using SNP Data for a Han Chinese Population. *S.W. Chang[1], P.L. Chen[2,3], T.C. Chang[3,4], C.S.J. Fann[1].* 1) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 2) Department of Medical Genetics, National Taiwan University Hospital, Taipei, Taiwan; 3) Division of Endocrinology & Metabolism, Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan; 4) Department of Medicine, College of Medicine, National Taiwan University, Taipei, Taiwan.

Genetic variation at classical human leukocyte antigen (HLA) loci has played an important role in the regulation of fundamental molecular or cellular processes and susceptibility to various diseases. Direct typing of HLA alleles can provide insights into complex associations among genomic structure, SNP variation and variation at respective alleles. However, the excessive cost has compelled researchers to adopt more economical approaches. A new statistical methodology developed by Leslie et al. (2008), along with HapMap individuals typed at classical HLA loci and SNPs within the MHC region, was shown to successfully predict allelic status with up to 95% accuracy. It was claimed that by utilizing a database of 300 haplotypes from African and Caucasian family trios, a set of ~100 SNPs was satisfactory for predicting HLA alleles at all loci in any population. In this study, we modified Leslie et al.'s algorithm and reshaped the models to allow for more flexibility in SNP selection and for more efficient prediction. We used Illumina HumanHap550K and Affymetrix 500K SNP data within the MHC region and predicted alleles at 6 HLA loci (HLA-A, -B, -C, -DPB1, -DQB1 and -DRB1) for the sample of 210 unrelated Han Chinese individuals residing in Taiwan. Our results indicated that a single panel of 164 SNPs typed across the region was sufficient for predicting HLA alleles at 6 loci with up to 98% accuracy at 4-digit resolution in the Han Chinese population. In comparison to previous findings, we observed that different sets of prediction SNPs were specific for different populations. We validated the methodology with a cohort of 227 unrelated Han Chinese individuals. Sensitivity, specificity, and the false discovery rate were evaluated. In conclusion, with a small number of SNPs genotyped in a population, the effective prediction algorithm provides a low-cost alternative to direct typing of HLA alleles. Moreover, the specific prediction SNP sets in different populations may aid in future medical diagnosis and drug prescription when HLA allele information is essential.

1519/T

Population differentiation as a test for soft sweeps on standing genetic variation. *H. Chen[1,2], N. Patterson[2], D. Reich[1,2].* 1) Dept Gen, Havard Med Sch, Boston, MA; 2) Broad Institute of MIT and Harvard, Boston MA.

Recent studies have suggested that adaptations to environmental change in human populations in the past 10,000 years may have occurred in part as a "soft sweep", that is selection on standing genetic variation. A number of statistical methods have been developed for detecting selective sweeps on newly arising genetic variants but a concern is that almost all have low power for soft sweeps. This is because the population genetic model for selective sweeps underlying these methods assumes that causal mutants under selection started from a single copy ("hard sweeps"), and soft sweeps generate a genetic polymorphism pattern that is strikingly different from classical signatures of hard sweeps. We propose a method that can detect soft sweeps efficiently, which is based on analysis of the haplotype differentiation patterns across populations. We incorporate haplotype patterns from reference populations (which presumably did not experience selection) as an approximation of the haplotype structure around the selected standing variant before selection. We then use population genetic theory to explicitly model the effect of a soft sweep on the allele frequencies of the nearby SNPs (single nucleotide polymorphisms). This approach can capture the characteristic genetic polymorphism patterns caused by soft sweeps and it achieves much better power compared with existing methods. We apply our method to several genome-wide SNP array data sets, including comparisons of Africans to non-Africans, and also northern to southern Europeans. Our method assumes that the selected variants are genotyped in the data set and thus can be expected to work substantially better on resequencing data. In addition to providing a new method for scanning for soft sweeps, our work also demonstrates that investigating the impact of natural selection in multiple populations simultaneously can provide insights about selection that would not be accessible in any one population.

1520/T

Collection of peripheral blood mononuclear cells obtained from Amerindians and other Mongoloid minority groups. *I. Danjoh[1], K. Saijo[1], S. Oota[2], K. Fukami-Kobayashi[2], Y. Nakamura[1].* 1) Cell Engineering Division, RIKEN BioResouce Center, Tsukuba, Ibaraki, Japan; 2) Bioresource Information Division, RIKEN BioResource Center, Tsukuba, Ibaraki, Japan.

<Objectives>

Ancestors of Amerindians migrated from Eurasian continent to North American continent via Bering Strait, and then migrated to South American continent over 10 thousand years ago. If human geneticists tried to find out their origin in Eurasia and to trace their migration path(s) with genetic methods, it has been difficult to obtain sufficient number of tissue or cell samples. Dr. Sonoda who has been a professor at Kagoshima University in Japan and Dr. Tajima who is the director of Aichi Cancer Center Institute in Japan had spent nearly 30 years to collect blood samples from more than 3,000 individuals of Mongoloid minority groups around the world, mainly the individuals living in South America. This cell collection was donated to RIKEN BioResource Center. No other cell banks in the world possess such a collection.

We are establishing B lymphoblastoid cell lines (BLCL) by Epstein-Barr virus (EBV) infection using the donated samples. We have recently started to provide with the BLCL together with the information such as age, gender, tribes, and localities of the individuals who each BLCL was derived from, and also relationship between individuals.

To evaluate how Amerindian populations diverse with each other, we analyzed genetic similarities between tribes and localities using this collection.

<Methods>

We analyzed short tandem repeat (STR) polymorphism regarding 8 different loci using PowerPlex 1.2 Systems (Promega). The BLCL derived from 200 Amerindians and 152 Japanese were subjected to the analysis. Allele frequencies were compared between Amerindians and Japanese, and among Amerindian groups.

<Results and Conclusion>

Significant differences of allele frequency were observed between Amerindians and Japanese. In addition, difference was observed among Amerindians such as between individuals in the Andes and Amazon area. Japanese group showed similarities with Amazon area. These data provide basic information on genetic bias for each tribe among Amerindian populations.

1521/T

Human genetic differentiation from HapMap data. *E. Elhaik, A. Chakravarti.* McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD.

Apportionment of human genetic variation has long established that most human variation is within groups and that the additional variation between groups is small but greatest when comparing continental populations. These studies have used Wright's F_{ST} that apportions the standardized variance in allele frequencies within and between groups in a hierarchical manner. High values of F_{ST} are unlikely in humans due to genetic drift and migration and are consequently used to identify genes undergoing directional or heterotic selection. The availability of the HapMap data from phases I - III now allows us to reexamine these questions. We analyzed data on ~3 million autosomal, X-linked, Y-linked, and mitochondrial SNPs from the HapMap database on 602 samples from 8 populations and a common subset of ~1 million autosomal and X-linked SNPs that have been genotyped in all populations. We identified two major features of the data. First, only a paucity (12%) of the total genetic variation is among populations of different continents and even a lesser (1%) amount among populations of the same continent. These data are remarkably consistent with the early observations of Lewontin in 1972. Second, we demonstrate that, although the overall distribution is similarly shaped (inverse J), the distribution of F_{ST} varies significantly by mean allele frequency. Since the mean allele frequency is a crude indicator of allele age, these distributions mark the time-dependent change in genetic differentiation. The change in mean F_{ST} of these distributions is linear in mean allele frequency suggesting the nature of allele frequency dynamics. These observations are true for autosomal, X-linked, and mitochondrial SNPs, but not Y-linked SNPs. These results suggest that investigating the extremes of the F_{ST} distribution for each allele frequency class may be more efficient for detection of selection. Consequently, we demonstrate that such extreme SNPs are more clustered that that expected from linkage disequilibrium for each allele frequency class. These genomic regions are likely candidates for natural selection.

1522/T

Demography and History Shape Risk Factors in a Large Genome-Wide Study of U.S. Hispanics. *C. Gignoux, M. Via, D. Hu, C. Eng, D. Torgerson, E. Gonzalez Burchard.* UCSF, San Francisco, CA.

In the U.S., asthma prevalence, morbidity and mortality are highest in Puerto Ricans and lowest in Mexicans, although both are considered part of the same ethnic group - "Hispanic" or "Latino." Although there are many potential explanations for disease discrepancies between ethnic groups, including social and environmental factors, the genetic diversity of Latino populations provides the intrinsic variability necessary to untangle the genetic component of disease susceptibility. Here we demonstrate the importance of demography and the heterogeneous process of admixture in the Genetics of Asthma in Latino Americans (GALA) study, which includes 700 parent-child trios and 500 controls from both Mexico and Puerto Rico genotyped on the Affymetrix 6.0 platform. For each individual, we leveraged genome-wide polymorphism data to estimate ancestry at each locus to infer ancestry, and increase the genetic information beyond simply genotypes. Genomic admixture mapping yielded a unique association between locus-specific European ancestry and disease risk for each population within GALA. Specifically, Puerto Ricans with European ancestry at 16p12 are protected against asthma, while Mexicans with European ancestry at 7q31 are at increased risk for asthma (both $p<5x10^{-4}$). In order to further characterize these loci, and to understand why European ancestry confers varying risk in Puerto Ricans and Mexicans, we extend beyond traditional continental admixture models to investigate the distribution of ancestral European haplotypes in modern-day Hispanic/Latinos. Moreover, we investigated the biogeographical origins of population-specific risk variants in the Americas giving rise to modern-day Hispanic/Latino populations, and in doing demonstrated the importance of incorporating demographic inference in a genome-wide association studies of diverse human populations. .

1523/T

Genetic Admixture and Population Substructure Patterns in Hispanic Populations: Implications for Disease-Gene Association Studies in Schizophrenia. *S. Gonzalez[1, 2], A. Compean[3], A. Figueroa[3], L. Morales Smith[4], B. Cheng[2], R. Leach[2], J. Ge[5], R. Chakraborty[5], A. Ontiveros[6], H. Nicolini[7], M. Escamilla[1].* 1) Dept. of Psychiatry, Texas Tech University Health Science Center, El Paso, TX; 2) Dept. of Cellular and Structural Genetics, University of Texas Health Science Center, San Antonio, TX; 3) Department of Computer Science, University of Texas-Pan American, Edinburg, TX; 4) UT-Health Science Center at San Antonio, Regional Academic Health Center, Edinburg, TX; 5) Institute of Investigative Genetics, University of North Texas Health Science Center, Fort Worth, TX; 6) Instituto de Informacion de Investigacion en Salud Mental, Monterrey, Mexico; 7) Medical and Family Research Group, Caracci S.C., Mexico City, Mexico.

Genetic admixture and population substructure patterns were examined among three distinct geographical Hispanic populations to determine if sample sets from them could be combined for future population-based disease-gene association studies of schizophrenia. A panel of 75 ancestral informative markers (AIMs) was used to genotype unrelated control samples from the Lower Rio Grande Valley of Texas, USA (N=110), Mexico City, Mexico (N=25) and Monterrey, Mexico (N=21). Genetic structure was evaluated via AMOVA. F_{ST} analysis revealed no significant differences among the three Hispanic control groups (F_{ST}=0.0027, P-value = 0.088) or at any individual AIM locus after adjustment for multiple testing. A subset of 55 AIMs was used to compare genotypes between the control samples and schizophrenia subjects. Sample sizes for affected subjects were 38, 55, and 42, respectively for the three populations. Significant differences were detected among cases vs controls within each Hispanic population (F_{SC}=0.0099, P-value < 0.05) and among affected subjects across the three population groups (F_{ST}= 0.0128, P-value < 0.05). These results can be explained by significant differences in six of the 55 AIMs, after multiple testing adjustments. This implies that there is an ancestral contribution to the schizophrenia subgroups within as well as between these three Hispanic populations. All of these observations were consistent with the STUCTURE analysis of the data. Together, these imply that the three populations can be merged to investigate disease-gene association for schizophrenia because of no significant differences of genetic variation between the control samples of these populations. However, since significant ancestry differences were noticed between cases and controls as well as between populations within the cases, any disease-gene association statistic should be adjusted for ancestry specific admixture differences of each subject of these populations.

1524/T

Population Genetic Analysis of a Large Ashkenazi Jewish Cohort. *S. Guha[1], J. Rosenfeld[1], I. Pe'er[2], A. Darvasi[3], A. Malhotra[1], T. Lencz[1].* 1) Division of Psychiatry Research, The Zucker Hillside Hospital, The Feinstein Institute for Medical Research, North Shore - Long Island Jewish Health System, New York, NY; 2) Department of Computer Science, Columbia University, New York, NY; 3) Department of Genetics Institute of Life Sciences, Hebrew University of Jerusalem, Givat Ram, Jerusalem, Israel.

The abundance of high resolution, genome wide SNP data has provided novel insights in to the origin and structure of many population isolates. Traditionally these isolated populations with less genetic diversity have been considered ideal for whole genome association mapping in disease genetics. The Ashkenazi Jewish population represents a unique population for study based on its recent (<1000 year) history of a limited number of founders, population bottlenecks, and tradition of endogamy. While population history has been inferred from examination of Y chromosome and mitochondrial DNA, few population genetic studies in Ashkenazim have utilized genome-wide autosomal markers. Utilizing a large sample (n>1000) of Ashkenazi healthy controls from the Hebrew University Genetic Resource (HUGR), genotyped with the Illumina 1M platform, we compared Ashkenazi subjects to other neighboring populations (European and Asian), and sought to identify fine-scale population structure within this cohort. Analysis in STRUCTURE was conducted using 810 European substructure ancestry informative markers (ESAIMs) to compare the Ashkenazi subjects with European, Middle Eastern and Central Asian populations derived from HGDP-CEPH database. The optimal solution (k=5) separated the Ashkenazi Jewish cohort from European, Central Asian, and Middle Eastern populations, and revealed a cline within the Ashkenazi population. The principal component analysis with ~ one million markers only for Ashkenazi subjects suggest strong correlation with the diversity at Major Histocompatibility Complex (MHC) locus (6p22.1 - 6p21.3) at the top 5 principal components. Other than MHC locus, a region on chromosome 4p16.1 also emerged. Findings are relevant for understanding Ashkenazi population history, as well as for the interpretation of disease mapping studies conducted in this population.

1525/T

An unbiased SNP array for population genetics using the Axiom™ Genotyping Solution. *E. Hubbell[1], S. Mallick[2,3], N. Patterson[3], D. Reich[2,3].* 1) Affymetrix, Santa Clara, CA. 95051; 2) Harvard Medical School Department of genetics, Boston, MA 02115; 3) Broad Institute of Harvard and MIT, Cambridge, MA 02142.

A major goal in analyzing human genetic variation is to use the data to learn about human history: population expansions, contractions and migrations. While array genotyping of hundreds of thousands of SNPs simultaneously is in theory a powerful tool for these studies, a limitation is that arrays to date have also been designed for medical genetics, with the SNPs chosen in a very complicated way that results in bias in inferences about history.

Following the Keinan et al. Nature Genetics 2007 paper, a clean ascertainment strategy is to discover SNPs in a comparison of two chromosomes from the same individual of known ancestry. This should produce a panel of SNPs whose false-negative rate is unaffected by their frequency in the population, so that allele frequency spectrum analysis can be carried out simply after making a simple correction for discovery in two chromosomes. We are screening a set of such panels for validation in the Axiom genotyping assay to produce markers that achieve good genotyping performance. The validated set, from which we expect to have a commercially available array by early 2011, will be a union of such panels of SNPs, one for each individual of diverse ancestry used in discovering SNPs. We aim to obtain at least one hundred thousand SNPs from each ascertainment to produce powerful inferences about history.

An important feature of the array is that we are extraordinarily careful regarding the technical details of validating SNP performance. The validation procedure we use is blinded to prior knowledge about which probes have worked well in previous Axiom screening assays, as using this information would result in a higher success rate for probes that have been previously tried on SNP arrays, re-introducing complex biases into the ascertainment. Similarly, the oligonucleotide probes are designed based entirely on flanking sequence from the same individual, to avoid bias due to the ancestry of the human reference sequence. Finally, to avoid ascertainment bias based on population frequency, we only use information from replication of the sample in which a SNP was discovered and samples outside the human population (chimp and gorilla) to verify acceptable separation of genotype clusters.

1526/T

The Genetic Canvas of European Roma. *M. Karmin[1], M. Baldovi[2], N. Jeran[3], S. Cvjetan[4], M. Reidla[1], T. šaric[3], J. šarac[3], M. Cenanovic[6], A. Leskovac[5], D. Marjanovic[6], H.D. AuguŽtin[3], A. Ficek[2], G. Chaubey[1], S. Rootsi[1], V. Ferak[2], R. Pavao[3], E. Metspalu[1], D.M. Behar[1], R. Villems[1].* 1) Estonian Biocenter and Department of Evolutionary Biology, University of Tartu, Tartu, Estonia; 2) Comenius University, Bratislava, Slovakia; 3) Institute for Anthropological Research, Zagreb, Croatia; 4) Mediterranean Institute for Life Sciences, Split, Croatia; 5) Vinca Institute of Nuclear Sciences, Belgrade, Serbia; 6) Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia.

According to linguistic evidence, the Indian exodus of the ancestors of the European Roma took place most probably around the end of the first millennium. By 13th - 15th centuries, different groups of Roma had spread throughout Europe. Virtual lack of written records prior to their arrival to Europe has left us with scarce knowledge about their historical migratory routes. Therefore, valuable insight may come from archaeogenetic studies. Here we report the results of a combined mtDNA, NRY and autosomal study of the genetic variation of Roma (Gypsies). We have studied variation of close to 600 Roma mtDNAs from six European countries, including 60 complete mitochondrial genomes from various populations of European Roma, India and the Near East. The most common Indian-specific maternal lineage among Roma is M5a1b1. Reaching from 5% to 35% in different Roma communities, it is, at the same time, present in various linguistically, socially and geographically different populations of India. The analysis of complete mitochondrial genomes shows that U3b1 and X2e1 lineages found in Roma have their closest relatives in the Near East and X2d1 lineages in the Caucasus, suggesting that at least a part of their West Eurasian-specific matrilineages has been picked up by the Roma *en route* before reaching Europe. We have analyzed over 250 Y-chromosomes from different Roma communities for their Y-STR and Y-SNP variation. Our results confirm previous studies, identifying NRY hg H1a as a clear sign of the Indian-specific contribution within the Y-chromosomal pool of Roma. Whole genome analysis of Roma, carried out using Illumina BeadArrays in the context of Eurasian and North African populations, revealed that Roma individuals exhibit varied levels of shared South Asian - West Eurasian ancestry. Taken together, our mtDNA, Y-chromosomal and GW analysis of European Roma populations in the context of Indian, Middle Eastern and European populations offers new insights into the demographic history of Roma populations.

1527/T

Detecting Ethnic Differences in DNA Using Inter-chromosomal Paralogous Regions. *R. McCullough[1], R. Tim[1], Å.O.H. Nygren[1], C. Deciu[1], D. van den Boom[2], M. Ehrich[2].* 1) 1Sequenom Center for Molecular Medicine, San Diego, CA; 2) 1Sequenom, San Diego, CA.

The identification of genetic markers that are able to distinguish different ethnic groups is of ongoing interest. While ethnic differences have recently been characterized using single nucleotide polymorphisms (SNPs) and copy number variations (CNVs), no current method is accepted for ethnic classification of DNA. We previously studied the allelic stability of inter-chromosomal paralogous regions and identified over 12,000 paralogous regions in the genome that have high sequence similarity between two different chromosomal loci. We used single base extension in combination with MALDI TOF mass spectrometry analysis to determine the allelic ratios of these loci. Each resulting mass spectrum displays two mass signals representing the two chromosomal locations. The intensity ratio of the two mass signals reflects the ratio between the two chromosomes. Hence we expect a 50/50 ratio when analyzing a normal genome. We analyzed a total of 1862 of such inter-chromosomal paralogous regions in a set of nearly 400 genomic DNA samples. Upon analysis, the resulting data from some regions showed variable degrees of bias between the chromosomes. An unsupervised cluster analysis found that the samples clustered into groups according to their ethnic origin. The subset of markers (n=12) which exhibited the strongest ability to discriminate between ethnic groups was confirmed in a larger population of close to 800 samples in four self-declared ethnic groups. We used next generation sequencing to explore the potential underlying mechanisms which could lead to this ethnic bias. We found in this analysis previously uncharacterized SNPs that were located in the primer binding regions. In addition we explored another potential cause for the observed ethnicity related allelic bias. We performed copy number variation analysis in the regions, since if only one of the regions is duplicated, this could also lead to the observed effect. Although we did detect some new sequence polymorphisms, we did not identify any SNPs that are strongly correlated to the ethnicity of the sample. In the analysis of copy number variations we did find that the regions we are interrogating are subject to copy number changes, which are also strongly correlated with ethnicity. Our data suggests that using paralogous regions located at least partially in CNV regions is a promising tool for developing a simple and cost effective assay for the exploration of the ethnic background of a DNA sample.

1528/T

A genome-wide survey of the history of the Roma people. *P. Moorjani[1], N. Patterson[2], M. Bonin[3], O. Riess[3], D. Reich[1,2,4], B. Melegh[5].* 1) Dept Gen, Harvard Medical School, Boston, MA; 2) Broad Institute, Cambridge, MA; 3) Department of Medical Genetics, University Tuebingen, Tübingen, Germany; 4) Harvard School of Public Health, Boston, MA; 5) Department of Medical Genetics, University of Pécs, Pécs, Hungary.

Previous linguistic studies, as well as studies of mitochondrial DNA and the Y chromosome, have suggested that the European Roma or Romani people are a group of endogamous founder populations that have inherited genetic material from South Asians. However, no previous studies have been able to characterize the date and proportion of mixture. To understand the origin and population history of the Roma, we have analyzed data for about a million single nucleotide polymorphisms (SNPs) and confirm evidence for their South Asian origin. We prove that the Roma, like other Indian populations, inherit ancestry from both 'Ancestral South Indians' (ASI) and 'Ancestral North Indians' (ANI) that are closely related to West Eurasians. To estimate the date of the mixture, we develop a novel method that uses summary statistics of admixture linkage disequilibrium. We estimate that the average number of generations since mixture is 29 ± 2 generations or about 800 years ago. In addition, we characterize the effect of founder events by calculating a genomic measure of individual homozygosity. We observe that the Roma have significantly higher autozygosity compared to other European populations, which likely contributes to the higher prevalence of recessive disorders seen in this population.

1529/T

Brazilian Afro-derived communities: genetics and history reconstruction. *S. Oliveira[1], CC. Gontijo[1], CEG. Amorim[1,2], GGBL. Ribeiro[1], G. Falcão-Alencar[1], MAF. Pedrosa[1], RCP. Toledo[1], MR. Luizon[3], RW. Pereira[1], RM. Neto[1], LB. Ferreira[4], AL. Simões[3], CT. Mendes Júnior[3], MN. Klautau-Guimarães[1].* 1) Laboratório de Genética, Universidade de Brasília, Brazil; 2) Programa de Pós-graduação em Genética e Biologia Molecular, UFRGS, Brazil; 3) Universidade de São Paulo, Campus Ribeirão Preto, Brazil; 4) Cátedra Unesco de Bioética, Universidade de Brasília, Brazil.

During the slavery period in South America, fugitive or freed slaves founded rural semi-isolated Afro-derived communities as a form of resistance against slavery. Some of these communities still exist in South America, nourishing the same traditions from their foundation time, and in Brazil they are known as Remanescentes de Quilombo. Yet, the genetic constitution of these Brazilian Afro-derived communities is barely known. The aim of this study was to obtain an overview of the current genetic composition, especially concerning African, European and Amerindian parental contributions, in four Brazilian Afro-derived communities - Mocambo, Rio das Rãs, Kalunga and Riacho de Sacutiaba. We investigated biparental markers - 16 autosomal Ancestry Informative Markers (AIMs) and 8 STRs - and uniparental markers - 12 Y-STRs, mtDNA and Y haplogroups, and 6 X-linked Indels - in over 280 individuals from the four communities listed. African parental contribution based on autosomal AIMs was the highest for all the communities evaluated herein, although Amerindian and European contributions were also present. Regarding autosomal STRs, some differences were observed in comparison to AIMs, but no clear pattern was noticeable. Data from the Y chromosome, both STRs and haplogroups, indicate a high European contribution and a virtually absent contribution from the Amerindian parental group. Differently, data from the female profile (mtDNA) showed a high African contribution and an absence of European lineages in our samples. This result was further confirmed by the data from the X chromosome, which showed an absence of European contribution and a high African contribution in these four populations. The data presented herein indicate that, despite years of contact, these communities still maintain a higher African ancestry than urban and rural Brazilian populations of admixed descent. Yet, our data has strongly suggested that the admixture process has indeed occurred over generations. We could also observe that men were the major responsible for the insertion of European alleles, and women for the insertion of Amerindian ones. This is supported by historical data of asymmetrical matings among the parental groups in Brazil. This study was funded by CNPq, CAPES and Finatec.

1530/T

Inference of Recent Demographic Events Based On Haplotype Sharing. *P.F. Palamara, G. Aponte, I. Pe'er.* Department of Computer Science, Columbia University, New York, NY.

Much progress in Population Genetics has been achieved under the assumption of unlinked markers. While this assumption is reasonable for ancient demography, relationship between contiguous markers can assist investigation of recent demographic changes. Furthermore, models for the modern diversification of a population are currently motivated by large-scale sequencing projects, where the decision of sequencing a small set of representative individuals may significantly reduce the costs. Patterns of Linkage Disequilibrium and, more recently, shared long-range haplotypes identical-by-descent (IBD), have been recognized as a signature of recent demographic events. We use these to reconstruct population parameters of the extremely recent demographic history. We develop a model to quantify the expectation on IBD sharing for different demographic scenarios. Specifically, we estimate the proportion of the Genome expected to be spanned by IBD segments in a particular length interval in the coalescent process, as a function of a population's demographic parameters. The expectation has a closed form in a Wright Fisher model, and an infinite (approximable) sum in more complex models. We use this expectation for several length intervals to guide the inference of a population's demographic parameters. Formally, we employ a hill climbing procedure in order to minimize the deviation between expected and observed levels of IBD sharing across multiple intervals of segment lengths. The accuracy of our method is confirmed using realistic coalescent simulations, where the model is used to recover a population's demographic history such as recent effective population size or bottleneck parameters (e.g. bottleneck timing, size of current and ancestral populations under exponential expansion). We apply our method to two real data sets of densely typed individuals: 1,200 Ashkenazi Jewish (AJ) subjects from the US and 56 Maasai subjects from Kenya. We reconstruct a severe bottleneck event in the AJ population about 23 generations before present, followed by the fast expansion of few hundred ancestral individuals to hundreds of thousands of contemporary N_e. This is consistent with both historical records and estimates of sample sizes for population sequencing in AJ. An opposite trend is observed in the Massai samples, for which our model suggests a very large ancestral population quickly decreasing to current few hundreds.

1531/T

Evaluation of the HapMap dataset as reference for the Greek population. *P. Paschou[1], I. Karagiannidis[1], A. Tsirigoti[1], A. Stampoliou[1], V. Papadopoulou[1], V.G. Manolopoulos[2], J.R. Kidd[3], K.K. Kidd[3], P. Drineas[4].* 1) Dept. of Molecular Biology & Genetics, Democritus University of Thrace, Alexandroupoli, Greece; 2) Laboratory of Pharmacology, Medical School, Democritus University of Thrace, Alexandroupoli, Greece; 3) Dept. of Genetics, School of Medicine, Yale University, New Haven CT, USA; 4) Dept. of Computer Science, Rensselaer Polytechnic Inistitute, Troy NY, USA.

The HapMap project has provided a unique tool for the analysis of human genetic variation, providing reference information for allele frequency and genotype distributions as well as linkage disequilibrium patterns of Single Nucleotide Polymorphisms (SNPs) across the entire genome. The latest release of HapMap phase 3 data provides genotypes for millions of SNPs in 11 populations from around the world, with Europe being represented by the CEU (originating from Northwestern Europe) and the TSI populations (Tuscan Italians from Southern Europe). Although initial studies support the fact that the CEU can be used as reference for the selection of tagging SNPs in other European populations, a critical step in the design of genetic association studies, this hypothesis has not been extensively studied across Europe and in particular in Southern Europe. We set out to explore the extent to which the HapMap populations can be used as reference for a previously unstudied population of South-Eastern Europe, the Greek population. To do so we studied genomic variation in 1,813 SNPs, genotyped by our group in 56 individuals of Greek origin, and compared them to the CEU and TSI genotypes (1,813 SNPs from the CEU HapMap dataset and 1,205 from the TSI dataset). The studied SNPs are spread over 13 autosomal chromosomes and 26 regions, ranging in size from 120Kb to more than 4Mb. Genotype, allele frequency, and pairwise LD measures were compared across all three populations. PCA was used in order to identify those markers that are responsible for the observed inter-sample variance. Tagging SNPs were selected in the CEU and TSI samples and their transferability to the Greek population was tested, using both the r2 metric as well as the efficiency of genotype imputation of the non-selected SNPs. Our results demonstrate that, although the CEU population can to some extent be used as reference for the Greek population, it is preferable to use as reference a European population of closer genetic ancestry, like the TSI. These results are applicable in medical genetics, in order to inform the design of genetic association studies, as well as in studies of evolutionary relationships of Southern European populations.

1532/T

Inference of unexpected genetic relatedness among HapMap Phase 3 individuals. *T.J. Pemberton[1], C. Wang[2], J.Z. Li[1], N.A. Rosenberg[1,2].* 1) Department of Human Genetics, University of Michigan, 100 Washtenaw Avenue, Ann Arbor, Michigan 48109 USA; 2) Center for Computational Medicine and Bioinformatics, University of Michigan, 100 Washtenaw Avenue, Ann Arbor, Michigan 48109 USA.

The International Haplotype Map Project ("HapMap") has provided a widely used resource on human genetic variation. Phase I and Phase II of the HapMap project generated genotype data across >3 million SNP loci in 270 individuals representing four populations. Phase III provides dense genotype data generated by the Illumina Human 1M and the Affymetrix SNP 6.0 platforms on ~1.5 million SNPs. Release 3 of Phase III of the HapMap is comprised of 1,397 individuals from 11 populations, including 250 of the original 270 Phase I and Phase II individuals and an additional 1,127 individuals. While some known relationships among the Phase III individuals have been described in the data release, the genotype data that are currently available provide an opportunity to empirically ascertain previously unknown relationships. We performed a systematic analysis of genetic relatedness and were able to not only confirm previously described relationships, but also to detect additional, previously unidentified pairs of close relatives in Release 3 of Phase III of the HapMap. The inferred relative pairs make it possible to propose standardized subsets of unrelated individuals for use in future studies in which relatedness needs to be clearly defined.

1533/T

Using community-based participatory research methods to link gene discovery and translation in a large founder population in rural mid-Michigan. *D.L. Schutte[1,4,6], J.D. Bonner[4], R.A. Fisher[3], J. Rivard[2], C. Wei[5], Q. Lu[5], K.H. Friderici[2,3,6], J.L. Elfenbein[7], E. Wilch[6], B.C. Schutte[2,3,4,6].* 1) College of Nursing, Michigan State Univ, East Lansing, MI; 2) Microbiology and Molecular Genetics, Michigan State Univ, East Lansing, MI; 3) Pediatrics and Human Development, Michigan State Univ, East Lansing, MI; 4) Comparative Medicine and Integrative Biology, Michigan State Univ, East Lansing, MI; 5) Epidemiology, Michigan State Univ, East Lansing, MI; 6) Genetics PhD Program, Michigan State Univ, East Lansing, MI; 7) Communicative Sciences and Disorders, Michigan State Univ, East Lansing, MI.

Common health conditions result from the combined effects of genetic and environmental factors. The contributing genetic factors may be discovered more easily in founder populations due to their relative genetic homogeneity. The purpose of this report is to describe an academic-community research partnership, using community-based participatory methods, between our institution and a German Catholic community in rural mid-Michigan. Our long-term goal is to foster gene discovery in a way that promotes wellness for community partners and that fosters scholarship for academic partners. Previously, we identified individuals in this community with an autosomal recessive form of hearing loss and found known and novel causative mutations in the *GJB2* gene. Since the community was isolated by language and religion, we hypothesized that they were highly related. We built a genealogical database (N= 28,256 records) that includes the descendants and ancestors of persons who are deaf from the region, including the community founders, to estimate kinship. We used Pedhunter to cluster individuals into 310 discreet family units. However, 27,747 individuals (98%) were assigned to a single remarkable pedigree. Kinship was estimated using F(ped) and F(isonymy). We observed that, in the contemporary generation, 44% of offspring occurred from parents who share a common relative. The F(ped) value was 0.0043, and the matings within the community share a common ancestor 8 generations past. The F(isonomy) value was two-fold higher than F(ped), as has been observed in other studies. This degree of kinship is comparable to other isolated populations in North America. Given this evidence for high kinship, we are extending the research partnership beyond hearing loss to examine genetic and environmental factors involved in Alzheimer disease, age-related hearing loss, and other common chronic conditions. A community-wide health needs assessment and recruitment into a research participant registry is currently underway. The infrastructure, processes and implementation phases of this community-based, translational genomics project will be further described.

1534/T

Relative Risk Score of Genetic Markers for Ancestry Inference. *R. Siburian[1], J. Fagerness[1,3], S. Haddad[1], A. Kirby[1], J. Lerner-Ellis[1], D. Pauls[1,2,3], J. Smoller[1,2,3].* 1) PNGU, Massachusetts General Hospital, Cambridge, MA; 2) Harvard Medical School, Boston, MA; 3) Broad Institute of Massachusetts Institute of Technology and Harvard University, Cambridge, MA.

Ancestry informative marker (AIMS) panels are used to differentiate and cluster individuals in a dataset and to detect population structure caused by genetic drift and natural selection. In genetic association studies, understanding the population structure and assigning individuals in a genetic dataset to their subpopulations is important in the prevention of false-positive and false-negative associations caused by population stratification. Many statistical methods, each using their own inferential algorithms, have been used to ascertain markers for ancestral informativeness. However, these inferential algorithms may be computationally intensive to calculate and lack predictive measurements. Using Principal Component Analysis (PCA) on the HapMap Phase 3 dataset, we determined that the 11 populations grouped into 4 clusters: a cluster containing 4 African populations, a cluster containing 3 Asian populations, a cluster containing 2 European populations and an admixed cluster consisting of an Indian and Mexican population. These 4 clusters were defined as “continental blocks.” We developed a method of selection for the most informative markers by calculating a SNP’s relative risk (RR) for a specific continental block. RR scores were computed by converting the odds ratio from Fisher’s exact-test into a relative risk score. We ran PCA in the HapMap dataset using our top 100 unlinked RR markers and found that it clustered the 11 populations similarly to the PCA data using all markers. We developed a method for ancestry prediction by using a marker’s relative risk score in an allelic scoring system; this provided us a quantitative measurement of genetic load for a known continental population. To validate our panel’s ability to detect population structure, predict ancestry and prevent bias, we ran PCA on an independent dataset, the Human Genome Diversity Project (HGDP). We found that our panel clustered the individuals in the HGDP dataset very well in comparison to the data’s complete marker set and provided an accurate predictive score for continental ancestry. Our results suggests the relative risk method can select a small set of markers for ancestral informativeness to detect for population sub-structure and also provide an intuitive method for ancestry prediction.

1535/T

Global Distribution and Haplotype-Directed Resequencing of a High-Activity ACN9 Allele. *W.C. Speed, E. Straka, J.R. Kidd, K.K. Kidd.* Genetics, Yale University School of Medicine, New Haven, CT.

ACN9 has been implicated in predisposition to alcohol dependence (PMID: 18163977). While functionally uncharacterized in humans, a yeast Acn9 phenotype implicates a role for the ACN9 protein in acetate metabolism, downstream of ADH and ALDH2 in the alcohol metabolic pathway. Using public data on ACN9 expression for the HapMap samples (PMID: 17873874), 12 SNPs at ACN9 showed significant cis associations with higher expression at the population level. We have selected 11 SNPs distributed across 90.7kb encompassing ACN9, including 5 of the SNPs implicated in alcohol dependence and 4 of the SNPs associated with higher mRNA levels. All 11 SNPs were genotyped on 2465 individuals of a global set of 47 populations. Only one haplotype, very divergent from the ancestral, is associated with high expression levels and reaches high frequencies in SE Asia and the Americas. To find cryptic variation which might be responsible for the higher expression, we selected individuals for next-generation sequencing based on their haplotypic phenotype. 8.2kb PCR amplicons upstream of and including exon 1 were resequenced at greater than 30x coverage for four haplotypes: 20 chromosomes carrying the high expression haplotype (found at 1.6% in Europe, but 37.9% in the Americas); 10 chromosomes of the ancestral haplotype (found at 17% in Africa and negligible levels elsewhere); and 10 chromosomes each of the two remaining globally common haplotypes. These 4 haplotypes represent 90% of the global haplotype variation. All 5 SNPs in this region with reported frequencies in dbSNP were seen; 7 SNPs in dbSNP which had no frequency information were confirmed. 16 novel SNPs were identified, as well as 6 insertion/deletion polymorphisms; our targeted resequencing strategy doubled the known variation at this gene promoter. Two of the SNPs located in the 5’ UTR were chosen for genotyping on the complete population set. A variant allele 204bp upstream of the ATG was seen almost exclusively in North and South American populations, where it reaches an allele frequency of up to 30%. The haplotype carrying this allele has average frequencies of 16.3% in North American and 6.2% in South American populations; in all other regions the haplotype frequency falls below 1%. These data suggest the hypothesis that selection involving this allele may be responsible for the high frequency in Native Americans. Funded in part by USPHS AA009379 (KKK).

1536/T

Ethnic differences in allele distribution of the Androgen Receptor (*AR*) $(CAG)_n$ repeat in the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) cohort. *M. Urbanek[1], L.P. Lowe[2], H. Lee[1], A.R. Dyer[2], M.G. Hayes[1], B.E. Metzger[1], W.L. Lowe[1], C. Ackerman[1], HAPO Study Cooperative Research Group.* 1) Dept Med, Div Endocrinol, Northwestern Univ, Chicago, IL; 2) Department of Preventive Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL.

The androgen receptor (AR) is important in reproductive organ development and homeostasis of the pancreas, liver and skeletal muscle in adulthood. The *AR* gene contains a highly polymorphic $(CAG)_n$ repeat in exon 1 that codes for a glutamine tract in the protein’s N-terminal transactivation domain. Expansion of the *AR* $(CAG)_n$ repeat (>38 repeats) beyond the normal range causes spinal and bulbar muscular atrophy (SBMA), also known as Kennedy disease, which is an X-linked form of spinal muscular atrophy. Features of SBMA include gynecomastia, infertility, androgen insensitivity, and increased incidence of type 2 diabetes. The *AR* $(CAG)_n$ repeat within the normal range (10-36 repeats) is associated with body fat, insulin levels, and leptin in healthy men, and with obesity and leptin in diabetic men. The $(CAG)_n$ repeat has been suggested to regulate AR activity, with longer alleles conferring reduced receptor activity. Therefore, the evaluation of the allelic distribution of this polymorphism in populations of various ethnic origins is crucial in understanding the interindividual variability in AR activity. We evaluated the ethnic variation of this polymorphism by genotyping 4424 Caucasian mothers and 3347 offspring of Northern European ancestry, 1957 Thai mothers and 2089 offspring, 1208 Afro-Caribbean mothers and 1209 offspring; and 774 Hispanic mothers and 762 offspring from the multi-ethnic Hyperglycemia and Adverse Pregnancy Outcome cohort.

Allele distributions of maternal genotypes were compared using Pearson’s chi-squared and Monte Carlo tests. The distribution of $(CAG)_n$ alleles between all four ethnicities is significantly different ($P<0.0001$). These highly significant differences were confirmed using the Monte Carlo test ($P<0.001$). Separate chi-squared analyses were used for pairwise comparisons of ethnicities. These analyses confirmed that there are significant differences between each pair of ethnicities tested ($P<10^{-28}$), even after adjustment for multiple testing. The Afro-Caribbean group had the shortest repeat lengths, and the repeat length increases from Afro-Caribbeans, to Caucasians, to Hispanics, and then Thais. Using this large dataset, we found significant interethnic differences in the allele frequencies of the *AR* exon 1 $(CAG)_n$ polymorphism. Our results suggest that there may be underlying ethnic differences in androgenic pathway activity and androgen sensitivity.

1537/T

Constructing haplotype trees from genome-wide SNP genotypes. *S. Vattathil[1,2], J. Novembre[3], P. Scheet[2,1].* 1) Graduate School of Biomedical Sciences, Univ of Texas at Houston, Houston, TX; 2) Dept of Epidemiology, Univ of Texas M.D. Anderson Cancer Center, Houston, TX; 3) Dept of Ecology and Evolutionary Biology, Univ of California - Los Angeles, Los Angeles, CA.

Multiple approaches exist for modeling population haplotype variation from unphased genotypes, often for the purposes of prediction, e.g. imputing missing genotypes or reconstructing haplotypes. In certain settings, however, inference from such models may also be useful. For example, the topology of the genealogy for the haplotypes in a sample provides a natural way to group the haplotypes. Although heuristic methods exist for estimating the genealogy that describes a non-recombining haplotype segment, they are computationally intractable for whole-genome data from a large number of samples. Here we propose an algorithm for constructing local genealogies -- or trees -- for haplotypes sampled in the form of unphased genotype data. Our method is fast, since it leaves the most recent branches of the tree unresolved, and may be applied pointwise to data from whole-genome scans of thousands of individuals.

Our tree-building algorithm is applied to the clusters of a statistical model for haplotype variation (P. Scheet & M. Stephens, AJHG 78:629-644, 2006), thus generating trees of consensus haplotypes. The consensus haplotypes summarize the variation in the sample, and can be interpreted as ancestral haplotypes from which the sample-level haplotypes descended. We use the summary haplotypes as the external nodes for the genealogy and define relationships among the upper, or earlier, branches of the tree, but represent the lower branches by star-shaped genealogies. We construct trees by using PHYLIP (J. Felsenstein, 2004) to apply a neighbor-joining algorithm. We build a tree at every marker in the dataset, using information from flanking loci to calculate pairwise differences between segments. Since a tree is most interpretable for haplotype segments containing markers in tight linkage disequilibrium, which have experienced little recombination, the flanking loci for each target marker are chosen by sampling adjacent loci using a parameter analogous to a population recombination rate. We apply the method to data collected for a GWA study for lung cancer risk to explore features of the model and interrogate association signals. Our method is reminiscent of that of Minichiello & Durbin (AJHG, 79:910-922, 2006), who applied their method to disease mapping. Our approach can augment methods that utilize haplotypes for inference of population genetic phenomena.

1538/T

A general model for admixture histories in hybrid populations. *P. Verdu, N.A. Rosenberg.* Human Genetics, University of Michigan, Ann Arbor, MI.

Admixed human populations have previously been used for inferring human migrations, detecting natural selection, and finding disease genes. However, these applications often use a simple statistical model of admixture rather than a modeling perspective that incorporates the history of the admixture process. We have developed a general new model of admixture that mechanistically accounts for complex historical admixture processes. We consider M source populations contributing to the ancestry of a hybrid population, potentially with variable contributions across generations. For a random individual in the hybrid population at generation g, we study the fraction of genetic ancestry originating from one of the source populations by computing its moments as functions of time and of introgression parameters. We show how very different admixture processes can produce identical mean admixture proportions but different variances. In a case with two source populations, we also show that when introgression parameters for each source population are constant over time, the long-term limit of the expectation of admixture proportions depends only on the ratio of the introgression parameters. Further, in this constant admixture process, we show that the variance of the admixture proportion can reach a maximum before decreasing to its long-term limit. Our approach will facilitate the inference of admixture mechanisms, illustrating how higher moments of the distribution of admixture proportions can be informative about the admixture processes contributing to the genetic diversity of hybrid populations.

1539/T

Genetic distinctiveness of Spanish Basque population with other surrounding Spanish populations revealed by genome wide high density SNP genotype data. *W. Wang[1,2], L. Rodriguez[4], B. Henn[3], J. Bertranpetit[4], D. Comas[4], C. Bustamante[1,3].* 1) Department of Biological Statistics and Computation Biology, Cornell University, Ithaca, NY; 2) Life Sciences Core Laboratories Center, Cornell University, Ithaca, NY; 3) Department of Genetics, Stanford University, Palo Alto, CA; 4) Institute of Evolutionary Biology (UPF-CSIC), CEXS-UPF-PRBB, 08003 Barcelona, Catalonia, Spain.

Basque is generally considered a culture isolate, while their genetic distinctiveness with surrounding populations is controversial. Varying extent of genetic differentiation of Basques has been reported in a number of studies using different types of markers, which include mtDNA and Y chromosome markers, small sets of candidate SNP, genome-wide SNP, STR and other classical genetic markers. In this study, we investigated the genome wide genetic differentiation using high density SNP markers in 4 populations within Spain: Basques, southern Spain, northwestern Spain, and Canary Islands. Principle component analysis revealed that Basque could be effectively separated from the other 3 Spanish populations, and it was more similar to the HapMap CEU population of western European origin. The southern and northwestern Spanish populations were indistinguishable from each other and from the HapMap TSI population of southern European origin. Canary Islands population demonstrated more similarity to African population than the other 3 Spanish populations. The overall homozygosity rate of Basque (75.9%) was slightly higher than those of the other 3 Spanish populations (74.8% to 75.2%), which were similar to CEU and TSI (75.4%). However, the total length of extended runs of homozygosity in Basque was significantly larger than those of the other 3 Spanish populations, as well as CEU and TSI. This suggests the Basque are a more genetically isolated population than the others, presumably due to a combination of geographic factors and cultural practices. Taken together, the present study using genome wide high density SNP genotypes suggests that Basque are genetically distinct from neighboring Spanish populations, as well as from other western and southern European populations, although the magnitude of differentiation is subtle.

1540/T

Admixture in New World populations: an analysis of Y-chromosome, mtDNA, and genome-wide microarray data. *W.S. Watkins[1], J. Xing[1], D.J. Witherspoon[1], Y. Zhang[1], S.R. Woodward[2], L.B. Jorde[1].* 1) Department of Human Genetics, University of Utah, Salt Lake City, UT; 2) Sorenson Molecular Genealogy Foundation, Salt Lake City Utah.

The first major interaction between Native Americans and Europeans is documented historically and occurred less than 550 years ago. This recent time frame provides an excellent opportunity to investigate the effects of admixture between two populations that were previously separated for hundreds of generations. To characterize European admixture in Native American populations, we sampled and analyzed a group of isolated Totonac agriculturists from tropical Mexico near Veracruz and a group of native Bolivians predominantly from the mountainous region near La Paz, Boliva. Mitochondrial sequencing of HVS1 showed that all samples had pre-Columbian mtDNA haplogroups (A, B, C, and D). Using a panel of 48 STRs or 12 Y-chromosome SNPs, Totonac Y-chromosomes lineages were all assigned to the pre-Columbian haplogroup Q1a3a, and Bolivian Y-chromosome lineages were assigned to haplogroups Q1a3a, R1, and J2. Haplogroups R1 and J2 are common in European populations. Principal components analysis (PCA) using >800K autosomal SNPs typed in 24 Totonacs and 23 Bolivians showed that all Totonacs and 14 Bolivians clustered distinctly from Eurasian individuals. Nine Bolivians, however, were positioned between the New World and European PCA clusters. Admixture analysis showed that these nine samples had 21 - 33% European admixture using a European reference population. All three observed Y-chromosome haplogroups, including the well-studied pre-Columbian haplogroup Q1a3a, occurred in the admixed individuals. Two of the nine admixed individuals had pre-Columbian mtDNA and Y-chromosome haplogroups but 21-23% European ancestry. This result demonstrates that Y-chromosome and mtDNA haplogroups are only partial indicators of an individual's complete ancestry.

1541/T

Inference of human expansion in Eurasia and genetic diversity in India. *J. Xing[1], W.S. Watkins[1], Y. Hu[2], C.D. Huff[1], A. Sabo[2], D.M. Muzny[2], R.A. Gibbs[2], L.B. Jorde[1], F. Yu[2].* 1) Dept of Human Genetics, Eccles Institute of Human Genetics, University of Utah, Salt Lake City, UT; 2) Human Genome Sequencing Center, Dept of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Genetic studies of populations from the Indian subcontinent are of great interest for many reasons, including India's large population size, its complex demographic history, and its unique social structure. Despite recent large-scale efforts in discovering human genetic variation, India's vast reservoir of genetic diversity remains largely unexplored. To address this issue and to study human migration history in Eurasia, we resequenced one of the 100 kb ENCODE regions in 92 samples collected from four groups - representing three castes and one tribal group - from the state of Andhra Pradesh in south India. By comparing the four south Indian populations with eight HapMap populations that are sequenced for the same region, we found that more than 15% of the total SNPs in the twelve populations in this region are Indian-specific (including HapMap GIH), and 30% of all SNPs in the south Indian populations are not seen in any HapMap population. For this 100 kb region, several Indian population samples, such as middle-caste Yadava, and lower-caste Mala/Madiga, have nucleotide diversity as high as HapMap African populations. In contrast to many other Eurasian populations, the diversity levels in the Indian samples are not correlated with their geographic distances from eastern Africa. Using the unbiased allele-frequency spectrum from twelve Old World populations, we were also able to investigate the divergence and expansion of human populations in Eurasia. The divergence time estimates among continental groups suggest that all Eurasian populations in this study diverged from Africans during the same time frame (~100-120 thousand years ago). The divergence times among the individual Eurasian populations were more than 40 thousand years later than their divergence with Africans, supporting the long-term existence of an ancestral Eurasian founding population after the out-of-Africa diaspora.

1542/T

Selecting genetic marker panels to detect population stratification using a multidimensional principal components and hierarchical clustering approaches. *K. Cho[1,2], J.R. Kidd[1], K.K. Kidd[1].* 1) Department of Genetics, Yale School of Medicine, New Haven, CT; 2) Division of Biostatistics, Yale School of Public Health, New Haven, CT.

Identifying population stratification is essential in case-control studies designed to elucidate the genetics of complex human diseases. Self-reported ethnicity cannot fully capture the underlying genetic population stratification and can confound results, even involving European populations, which are very similar overall. Several panels of ancestry informative markers (AIMs) have been proposed to address this issue. Selection of AIMs depends on the populations and the biostatistical methods used. Many such studies have used only a few very distantly related populations. In contrast, we are studying 34 Eurasian populations, excluding African and Native American populations. We investigate general dimension reduction algorithms using correlations of markers to multiple principal components and the heatmap hierarchical clustering approach and apply these algorithms to select AIMs from a pool of 104 single nucleotide polymorphisms (SNPs) with a globally high Fst genotyped in this geographically restricted set of populations. Using multidimensional principal components and hierarchically evaluating the dendrogram from the heatmap, we examine several subsets of SNPs to capture a wide range of ancestry information minimizing redundancy. We then use the STRUCTURE program to evaluate the performance of panels and derived subsets. From our set of SNPs with varying levels of Fst for the Eurasian populations, we have selected three subsets of 50 SNPs using the two approaches described and by selecting the top 50 highest Fst SNPs. These three panels contain 10 overlapping SNPs and have been compared to the initial panel of SNPs. Overall among the three panels, the multidimensional principal components SNPs panel shows the best clustering using K=2 to 6, followed by the panel using hierarchical clustering. Further investigation of a general approach combining these two methods may help identify an AIMs panel for the underlying populations. These dimension reduction algorithms are useful in selection of AIMs panels with the minimum number of SNPs that retain most information from any given set of SNPs from any platforms already typed. Such SNP panels have a wide range of applications both in genetic studies and forensic studies.

1543/T

Using EuroAIMs to measure admixture proportions in atypical European populations: the case of Canary Islanders. *C. Flores[1,2], M. Pino-Yanes[1,2], A. Corrales[1,2], A. Hernandez[3], S. Basaldua[1], L. Guerra[4], J. Villar[2,5,6].* 1) Research Unit, Hospital Universitario N.S. de Candelaria, Tenerife, Spain; 2) CIBER de Enfermedades Respiratorias, Instituto de Salud Carlos III, Madrid, Spain; 3) Instituto Nacional de Toxicologia y Ciencias Forenses, Delegación de Canarias, Tenerife, Spain; 4) Hematology Service, Hospital Universitario Dr. Negrin, Las Palmas de Gran Canaria, Spain; 5) Multidisciplinary Organ Dysfunction Evaluation Research Network, Research Unit, Hospital Universitario Dr. Negrin, Las Palmas de Gran Canaria, Spain; 6) Keenan Research Center, St. Michael's Hospital, Toronto, ON, Canada.

Using ancestry informative markers (AIMs) allows reducing the number of makers needed for population stratification adjustments in association studies. As few as 100 AIMs are sufficient to adjust for the largest European axis of differentiation (i.e. EuroAIMs). However, their use for ancestry inference and adjustment in association studies in atypical European populations such as the Canary Islanders, a recently African-admixed population from Spain, needs to be addressed. We aimed to explore whether EuroAIMs were suitable both for the inference of Spanish and Northwest African admixture proportions and for ancestry adjustments in association studies including samples from Canary Islanders. We analyzed samples from Canary Islanders, mainland Spanish (IBE) and Northwest Africans (NWA) for 93 EuroAIMs and compared the data with CEU and YRI from HapMap, Basques and Mozabite from HGDP, as well as from previously analyzed European samples. The major genetic difference was observed between NWA and all European populations, preserving the northwest-to-southeast differentiation of European populations in the second axis. Analyses revealed that Canary Islanders were intermediate between IBE and NWA, and that direct sub-Saharan African influences were negligible. Assessment of individual admixtures without prior population information clearly identified two subpopulations corresponding to NWA and IBE, while Canary Islanders were admixed with an average of 17.4% Northwest African contribution varying largely among individuals (range 0-95.7%). As few as 23 EuroAIMs correctly estimated population membership to IBE and NWA, while 69 EuroAIMs were required to accurately estimate individual admixture proportions in Canary Islanders. Ancestry estimates based on a subset of 69 EuroAIMs also controlled significant allele frequency differences between IBE and Canary Islanders. These data suggest that a handful of EuroAIMs would be useful to control false-positives in association studies performed in Spanish populations. Supported by FUNCIS 23/07 and grants from the Spanish Ministry of Science and Innovation PI081383 and EMER07/001 to CF.

1544/T

Effects of incompatibility selection on genomic patterns of neutral variation. *D. Fusco[1], M.K. Uyenoyama[2].* 1) Computational Biology and Bioinformatics Program, Duke University, Durham, NC; 2) Dept of Biological Sciences, Duke University, Durham, NC.

Geography has emerged as a major determinant of the pattern of genetic variation in global samples of human genomes. Extreme values of Fst have been interpreted as prima facie evidence of strong directional selection (selective sweeps) confined to certain geographical regions. We present a theoretical analysis of a distinct form of selection under which the well-functioning wild-type allele of a given deme may reduce fitness upon placement on the genomic background of a different deme. We call this process incompatibility selection - local selective neutrality balanced by negative selection in other demes. We address the consequences of incompatibility selection on patterns of variation at globally neutral sites (marker loci) throughout the genome. These patterns differ from expectations under recent selective sweeps. We show that genealogical histories at marker loci can be modeled as a neutral process with an appropriately scaled backward migration rate. We examine the effects of sex-specific selection and sex-specific rates of crossing-over on the backward migration rate at neutral marker loci. Under sex-specificity of this kind, the barrier to introgression at markers departs in general from multiplicativity even in the absence of multiplicative epistasis among incompatibility loci. This departure reflects linkage disequilibrium (LD) between incompatibility loci conditional on transmission of the marker allele. Positive LD reduces and negative LD increases the barrier relative to the multiplicative expectation. Negative LD arises, for example, between an incompatibility allele that induces a greater reduction in the fitness of male than female carriers and an incompatibility allele at another locus that induces greater harm to female than male carriers. LD of this kind arises even between unlinked incompatibility loci, implying that the presence of incompatibility loci anywhere in the genome can affect introgression rates at neutral marker loci. Results from forward-in-time simulations of sex-specific selection, recombination, and migration confirm that a neutral process with our expression for the backward migration rate provides a useful approximation of the full complex process. In particular, values of Fst and other summary statistics observed in the simulations conform to expectation under our neutral approximation. We also extracted from the simulations the distribution of waiting times between ancestral migration events along lineages.

1545/T

CoAIMs: A Cost-Effective Panel of Ancestry Informative Markers for Determining Continental Origins. *E.R. Londin[1], M.A. Keller[1], C. Maista[1], G. Smith[1], L.A. Mamounas[2], R. Zhang[2], S.J. Madore[1], K. Gwinn[2], R.A. Corriveau[1].* 1) NINDS Repository, Coriell Inst Med Res, Camden, NJ; 2) National Institute for Neurological Disorders and Stroke, Bethesda, MD.

Genetic ancestry is known to impact outcomes of genotype-phenotype studies that are designed to identify risk for common diseases in human populations. Failure to control for population stratification due to genetic ancestry is a significant confounder of disease association studies. Self-identified race is the most common method used to track and control for population stratification; however, social constructs of race are not necessarily informative for genetic applications. The use of ancestry informative markers (AIMs) is a more accurate method for determining genetic ancestry for the purposes of population stratification. Here we use a panel of 36 microsatellite (MSAT) AIMs to determine continental admixture proportions in the context of a biorepository collection. This panel, named CoAIMs, consists of MSAT AIMs chosen based upon their measure of genetic variance (Fst), allele frequencies and their suitability for efficient genotyping. Genotype analysis with a Bayesian clustering method (STRUCTURE) is able to discern continental origins including Europe/Middle East (Caucasians), East Asia, Africa, Native America, and Oceania in reference populations. In addition to determining continental ancestry for such individuals without significant admixture, we applied CoAIMs to ascertain admixture proportions of a large collection of individuals of self-declared race. CoAIMs was used to efficiently and effectively determine continental admixture proportions in a sample set from the NINDS Human Genetics DNA and Cell Line Repository. Individuals of self-declared Caucasian (N=92), African-American (N=200), and Hispanic (N=200) race were analyzed. African American individuals displayed admixture of both African and European ancestry, with 2/200 (1%) of the samples having nearly 100% Caucasian Ancestry, suggesting discordance between self-declared and genetic race. Caucasian and Native American represented the highest ancestral proportions in self-reported Hispanic populations. The determination of genetic ancestry in biorepository collections can increase the utility of these samples for gene discovery. The CoAIMs panel used here has potential for broad applicability as a cost effective tool for determining admixture proportions.

1546/T

The genetic structure of South Asian populations as revealed by 650 000 SNPs. *M. Metspalu[1], G. Chaubey[1], B. Yunusbayev[1,2], I. Gallego Romero[4], M. Karmin[1], C. Basu Mallick[1], E. Metspalu[1], K. Thangaraj[3], L. Singh[3], S. Shanmugalakshmi[6], K. Balakrishnan[6], R. Pitchappan[5], T. Kivisild[4,1], R. Villems[1].* 1) Dept. of Evolutionary Biology, Estonian Biocentre and Tartu University, Tartu, Estonia; 2) Institute of Biochemistry and Genetics, Ufa Research Center, Russian Academy of Sciences, Ufa 450054, Russia; 3) Centre for Cellular and Molecular Biology, Hyderabad, India; 4) Leverhulme Centre of Human Evolutionary Studies, The Henry Wellcome Building, University of Cambridge, Fitzwilliam Street, Cambridge, CB2 1QH, UK; 5) Department of Immunology, School of Biological Sciences, Madurai Kamaraj University, India; 6) School of Biotechnology, Bharathidasan University, Trichirappalli, India.

The onset of the era of analyses of dense marker sets covering the whole genome has revolutionised the field of (human) population genetics. Driven largely by the needs of biomedical research the new data is helping to unveil our demographic past outlined by the study of mtDNA and Y-chromosome variation during the past ca. 20 years. Here we have analysed (Illumina 650K SNPs) over 320 new samples from South and Central Asia and the Caucasus together with the publicly available databases (HGDP panel and our published dataset of ca. 600 Eurasian samples) and illustrate the power of full genome analyses by addressing two specific questions: i) the nature of genetic continuity and discontinuity between South Asia, Middle East and Central Asia, and ii) genetic origins of the Munda speakers of India. We use principal component and structure-like analyses to reveal the structure in the genome wide SNP data. The most striking feature of the genetic structure of South Asian populations is the clear separation of the Indus valley and southern India populations. The genetic component prevalent in the latter region is marginal in the former and absent outside South Asia. The component ubiquitous to Indus valley is, in contrast, also present (ca. 30 - 40%) among Indo-European speakers of Ganges valley and Dravidic speakers in southern India. Furthermore this component can also be found in Central Asia and the Caucasus as well as in Middle East. We explore possibilities to identify the source region for this genetic component. Alternative models put the origins of Munda languages speakers either in South Asia (the Munda speakers sport exclusively autochthonous South Asian mtDNA variants) or to Southeast Asia where the other Austro Asiatic languages are spread. Y-chromosome variation supports the latter model through sharing of hg O2a in both regions. We show that in addition to the dominant ancestry component shared between the Indian Dravidic and Munda speakers the latter retain (up to 30%) an ancestry component otherwise prevalent in East Asia. There is no widespread sign of South Asian ancestry component in Southeast Asia. This provides genomic support to the model by which Indian Austro-Asiatic populations derive from dispersal from Southeast/East Asia followed by an extensive admixture with local Indian populations.

1547/T

The History in our Genes: the Complex Structure of the South African Coloured Population. *M. Möller[1], L. Quintana-Murci[2,3], E. de Wit[1], W. Delport[4,5], C. Harmant[2,3], C.E. Rugamika[4], H. Quach[2,3], A. Meintjes[4], O. Balanovsky[6], V. Zaporozhchenko[6], C. Bormans[7], P.D. van Helden[1], C. Seoighe[8], D.M. Behar[2,9], E.G. Hoal[1].* 1) Molecular Biology and Human Genetics, MRC Centre for Molecular and Cellular Biology, DST/NRF Centre of Excellence for Biomedical TB Research, Stellenbosch University, Tygerberg, Western Cape, South Africa; 2) Institut Pasteur, Human Evolutionary Genetics, Department of Genomes and Genetics, Paris, France; 3) Centre National de la Récherche Scièntifique, Paris, France; 4) Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Cape Town, Western Cape, South Africa; 5) Department of Pathology, Antiviral Research Center,University of California, San Diego, USA; 6) Research Centre for Medical Genetics, Russian Academy of Medical Sciences, Moscow, Russia; 7) Genomics Research Center, Family Tree DNA, Houston, Texas, USA; 8) School of Mathematics, Statistics and Applied Mathematics, National University of Ireland, Galway, Ireland; 9) Molecular Medicine Laboratory, Rambam Health Care Campus, Haifa, Israel.

The study of recently admixed populations provides unique tools for understanding recent population dynamics, socio-cultural factors associated with the founding of emerging populations, and the genetic basis of disease by means of admixture mapping. The geographical position and complex history of South Africa has led to the establishment of the unique admixed population known as the South African Coloured. We performed a Genome-Wide Analysis of the genetic make-up of this population. We genotyped 959 self-identified individuals from the Western Cape area, using the Affymetrix 500k genotyping platform. This resulted in nearly 75 000 autosomal SNPs that could be compared with populations represented in the International HapMap Project and the Human Genome Diversity Project. Analysis in STRUCTURE revealed that the major ancestral components of this population are predominantly Khoesan (32-43%), Bantu-speaking Africans (20-36%), European (21-28%), and a smaller Asian contribution (9-11%), depending on the model used. However, the autosomal data provides little evidence about the mode in which this admixed population was founded. We went on to show, through detailed phylogeographic analyses of mitochondrial DNA and Y-chromosome variation in a large sample of South African Coloured individuals, that this population derives from at least five different parental populations (Khoisan, Bantus, Europeans, Indians and Southeast Asians), who have differently contributed to the foundation of the South African Coloured. Our analyses reveal extraordinarily unbalanced gender-specific contributions of the various population genetic components, the most striking being the massive maternal contribution of Khoisan peoples (more than 60%) and the almost negligible maternal contribution of Europeans with respect to their paternal counterparts. The overall picture of gender-biased admixture depicted in this study indicates that the modern South African Coloured population results to a large degree from the early encounter of European and African males with autochthonous Khoisan females of the Western Cape of Good Hope hundreds of years ago.

1548/T

The geographic structure of rare variant diversity. *J. Novembre[1], D. Wegmann[1], S. Gopalakrishnan[2], M. Zawistowski[2], P. St. Jean[3], L. Li[3], M.G. Ehm[3], J. Li[3], Y. Li[3], G. Abecasis[2], J.C. Whittaker[3], S.L. Chissoe[3], V.E. Mooser[3], M.R. Nelson[3], S. Zöllner[2].* 1) Dept Eco & Evo Biol, Univ California, Los Angeles, Los Angeles, CA; 2) Biostatistics Department, School of Public Health, University of Michigan; 3) GlaxoSmithKline, Research Triangle Park, North Carolina 27709, USA.

The challenges of population stratification for common variant association tests are well understood, but it is less clear how population stratification affects rare variant mapping techniques and how often rare variants discovered in one population are shared in closely related populations. To investigate these questions we analyze patterns of rare variant diversity amongst 15,000 subjects who have been re-sequenced with average depth >20x across 202 genes as part of the GSK QPOC DeepSeq project (see abstract by Nelson et al). The scale of the study allows us to document rare variants in multiple populations. Specifically, the sample includes >275 individuals from 4 globally distributed populations (African-American, Chinese, European, and Indian). Within Europe, the sample contains >500 individuals from each of more than 6 geographically defined populations. We quantify whether rare variant diversity differs among populations and the extent to which rare variants at different frequencies and functional categories are structured at global and local scales. The results underscore the importance of considering population stratification when performing rare variant mapping techniques.

1549/T

Autosomal African admixture in Yemeni populations. *R.L. Raaum[1], A.M. Al-Meeri[2], C.J. Mulligan[3].* 1) Anthropology, Lehman College & The Graduate Center, CUNY, New York, NY; 2) Biochemistry, Faculty of Medicine, Sana'a University, Sana'a, Yemen; 3) Anthropology, University of Florida, Gainesville, FL.

Approximately 30% of mtDNA lineages in South Arabian samples are African L haplotypes, whose origin has usually been attributed to migration and assimilation of African females into the Arabian population over approximately the last 2,500 years. Few In contrast, few Y chromosome lineages of clear recent sub-Saharan African origin have been found in Southern Arabian populations. This bias in maternal and paternal lineages is in accord with historical accounts of the female bias in the Middle Eastern slave trade. In order to evaluate autosomal African ancestry, we collected high-resolution SNP genotype data from a geographically representative set of 62 Yemenis selected from a collection of 552 samples acquired in the Spring of 2007. The ancestry of chromosomal segments in the Yemeni population was estimated using a haplotype-based local ancestry estimation method, HAPMIX. The HAPMIX method is based on a two way admixture model that requires two phased reference populations; we used the HapMap Yoruba in Ibadan, Nigeria (YRI), Luhya in Webuye, Kenya (LWK), Maasai in Kinyawa, Kenya (MKK), and CEPH US residents with ancestry from northern and western Europe (CEU) samples. The three African reference populations include two Bantu-speaking groups (YRI and LWK) and one Nilotic-speaking group (MKK). We estimated local ancestry in the Yemeni sample with all three European-African reference population combinations (CEU-YRI, CEU-LWK, CEU-MKK). The correlations among African ancestry calculated using all three reference population combinations are high ($r > 0.98$ in all pairwise correlations). Furthermore, there is no significant difference between the average proportion of African ancestry in Yemenis calculated using either of the two Bantu-speaking reference populations: CEU-YRI (mean 0.062, sd 0.044) and CEU-LWK (mean 0.076, sd 0.049) ($p=0.13$, two-tailed Welch two sample t-test). However, the average African ancestry calculated using the Maasai reference population (CEU-MKK, mean 0.148, sd 0.060) is significantly greater from that calculated using either the Yoruba or Luhya reference populations ($p < 0.0001$ in both comparison, two-tailed Welch two sample t-test). These data suggest that the source population for the African ancestry of the Yemeni population is more similar to the contemporary Maasai population than either the Luhya or Yoruba.

1550/T

Admixture in Ashkenazi Jewish cohorts and implications for association studies. *V. Vacic[1], E.E. Kenny[1,2], A. Gusev[1], I. Peter[3], J. Cho[4], G. Atzmon[5], H. Ostrer[6], S.B. Bressman[5], I. Ozelius[3], I. Pe'er[1].* 1) Columbia University, New York, NY; 2) The Rockefeller University, New York, NY; 3) Mount Sinai School of Medicine, New York, NY; 4) Yale University, New Haven, CT; 5) Albert Einstein College of Medcine, Yeshiva University, New York, NY; 6) Langone Medical Center, New York University, New York, NY.

Studies of complex genetic disorders may benefit from focusing on population isolates, such as Ashkenazi Jews (AJ). However, in order to truly exploit the advantages of reduced genetic diversity the self-declared AJ ancestry of study participants should be independently confirmed with available genetic data. We investigate whether the AJ cohorts display genetic heterogeneity, such as e.g. different rate of admixing in cases and controls, which could potentially confound disease association studies. We applied principal component analysis (PCA) to AJ cohorts ascertained in Israel and the US East Coast with the goal of characterizing population structure. As described previously, when compared to the HapMap samples with CEU, YRI and CHB/JPT ancestry, virtually all AJ samples cluster with the CEU. Similar analysis done on CEU and Jewish HapMap samples from Ashkenazi, Sephardic and Middle Eastern Jewish communities revealed that 97.8% of AJ samples cluster along the AJ-CEU axis, with modes at AJ and CEU cluster centers and at approximately quartile distances between them. We postulate that these groups correspond to 100-0, 75-25, 50-50, 25-75, and 0-100% AJ-CEU admixtures. Notably, only 91.7% of self-reported AJ individuals fall into the reference JHapMap panel AJ cluster, with 1.6, 3.3, 0.5 and 0.7% in the admixed modes ordered by decreasing fraction of AJ ancestry. We also observe admixing with the non-AJ Jewish communities: 0.7% of samples fall within the non-AJ clusters and 1.4% at a subgroup approximately halfway between the AJ and non-AJ cluster centers. In our dataset we found that when compared to the sample as a whole or only to controls, individuals with Crohn's disease (CD) show significantly more admixing: 78.1, 3.1, 8.5, 2.0 and 0.9% in the 100, 75, 50, 25 and 0% AJ subgroups respectively. Also, CD samples show more admixing with non-AJ groups (2.8 and 1.0% in the 50-50 and 0-100 AJ-non-AJ subgroups). Isolates typically exhibit a greater amount of cryptic relatedness compared to outbred populations, which motivates an orthogonal method for verifying AJ ancestry based on identity-by-descent (IBD). The high background level of IBD within the Ashkenazi Jewish community can be used to estimate degree of AJ ancestry by averaging the IBD between a sample under study and the AJ individuals in the JHapMap panel. Our preliminary results show that this method recapitulates the high-level results from the PCA analysis and provides better resolution.

1551/T

Balancing ethics and genetics: classifying individuals by their ancestry groups. *R. Zuvich[1,2], E.W. Clayton[3], M. Basford[4], J. Denny[5,6], D.M. Roden[6,7], J.L. Haines[1,2], M.D. Ritchie[1,2].* 1) Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN; 2) Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 3) Center for Biomedical Ethics and Society, Vanderbilt University, Nashville, TN; 4) Office of Personalized Medicine, Vanderbilt University, Nashville, TN; 5) Department of Biomedical Informatics, Vanderbilt University, Nashville, TN; 6) Department of Medicine, Vanderbilt University, Nashville, TN; 7) Department of Pharmacology, Vanderbilt University, Nashville, TN.

Individuals with different genetic ancestry differ in allele frequencies of various single nucleotide polymorphisms (SNPs), linkage disequilibrium patterns, and disease susceptibility, all of which affect analysis of the data and interpretation of the results. Therefore, characterization of an individual's genetic ancestry is imperative to minimize spurious association results. The Vanderbilt DNA Databank (BioVU) is a DNA repository of >85,000 DNA samples (both adult and pediatric), which are de-identified and linked to electronic medical records. In a preliminary study, we genotyped 360 SNPs using the Illumina DNA Test Panel, which contains ancestry informative markers (AIMs) on ~1,500 DNA samples from patients whose race/ethnicity was identified by hospital personnel ("observer-reported"). Using an ancestry proportion threshold of 90%, there was 95.7% concordance in people of European ancestry between the observer-reported race/ethnicity and the ancestry group identified by the AIMs; while people of African ancestry had far less concordance (22%). However, lowering the ancestry proportion threshold to 75% increases African ancestry concordance to 74%. This is due to the expected proportion of admixture in African American individuals. About 20% of samples in BioVU do not have a race/ethnicity noted in the EMR and characterization of these samples using AIMs would be of great utility. We genotyped an additional ~7,900 DNA samples from BioVU using the DNA Test Panel to characterize genetic ancestry and determine whether using a smaller subset of AIMs yields the same classifications as the full set of AIMs. While characterizing genetic ancestry is important for association studies, this process raises many ethical and social implications about "labeling" patients based on genetic markers. Additionally, with admixed populations, such as those in the United States, the utility of AIMs are limited in their ability to explore genetic ancestry and provide no data to describe socially defined categories of race and ethnicity. We suggest that including AIMs in the EMR will be important to interpret and apply the results of genetics research in the clinic, but that it will be critical to declare that these markers of genetic ancestry do not necessarily correspond to a particular race or ethnicity.

1552/T

W. African recessive *LEPRE1* allele (c.1080+1G>T) in Trinidad and Tobago - Does perinatal lethal Osteogenesis Imperfecta (OI) influence the high neonatal death rate? *M.G. Pepin[1], U. Schwarze[1], V. Singh[2], M. Romana[3], A. Jones-LeCointe[2], S. Paray[2], P.H. Byers[1].* 1) Dept Pathology, Univ Washington, Seattle, WA; 2) Mt. Hope Hospital, University of West Indies Medical School, Trinidad and Tobago; 3) Inserm/Université des Antilles et de la Guyane, Centre Hospitalier Universitaire de Pointe-à-Pitre, Guadeloupe.

Almost all African American (AA) infants with *LEPRE1*-related perinatal lethal OI are homozygous for a single mutation (c.1080+1G>T, IVS5+1G>T). Carrier frequency in AA populations is between 1/100 and 1/241 depending upon geographic region. Studies of DNA samples from W. Africa, particularly from tribes originating near Ghana and Nigeria, found carrier frequencies between 2% and 5%. To determine the carrier frequency of the *LEPRE1* W. African allele in Tobago we purified 200 DNA samples and sequenced the exon and surrounding intron sequence of the c.1080+1G>T region of the gene. We reviewed all neonatal death records (n=172) at the Mt. Hope Hospital in St Augustine Trinidad between 2006 and 2009 to assess cause of death and prevalence of neonatal lethal skeletal dysplasias, including OI. Three neonatal deaths with possible skeletal dysplasia were identified although OI was an unlikely cause. The carrier frequency of the W. African allele in Tobago was identical to the reported frequency in the US (0.5%). Our data does not support the hypothesis that *LEPRE1* related neonatal lethal OI contributes to the perinatal mortality rate in Trinidad and Tobago. The expected rate of lethal OI due to homozygous *LEPRE1* mutation based on present birth rates is predicted to be one case every 15-17 years. The *LEPRE1* sequencing data from Tobago samples revealed 11 intron and exon variants in the 440 bp fragment sequenced. Each characterized a unique allele with a frequency in the population studied that ranged from 50% to less than 1%. The *LEPRE1* lethal mutation occurred on the background of the most common allele. Data from slave trade logs of port of departure in Africa and disembarkation in Tobago and the *LEPRE1* allele variation data suggest that the African descendants of Tobago lived in relatively close proximity in W. Africa, between the Gold Coast to the northern regions of Congo consistent with studies of globin allele origins.

1553/T

A functional polymorphism in *CASP3* confers susceptibility to Kawasaki disease. *Y. Onouchi[1], K. Ozaki[1], J.C. Burns[2,22], C. Shimizu[2,22], M. Terai[3], H. Suzuki[4], K. Yasukawa[5], Y. Suzuki[6], K. Sasago[6], T. Saji[7], T. Yoshikawa[8], T. Nagai[9], K. Hamamoto[10], F. Kishi[11], K. Ouchi[12], Y. Sato[13], J.W. Newburger[14,22], A.L. Baker[14,22], S.T. Shulman[15,22], A.H. Rowley[15,22], M. Yashiro[16], Y. Nakamura[16], K. Wakui[17], Y. Fukushima[17], A. Fujino[18], T. Tsunoda[19], T. Kawasaki[20], A. Hata[6], Y. Nakamura[21], T. Tanaka[1].* 1) Lab Cardiovascular Disease, Ctr Genomic Med RIKEN, Yokohama Kanagawa, Japan; 2) Department of Pediatrics, University of California San Diego, School of Medicine, La Jolla, CA, and Rady Children's Hospital San Diego, CA; 3) Department of Pediatrics, Tokyo Women's Medical University Yachiyo Medical Center, Yachiyo, Japan; 4) Department of Pediatrics, Wakayama Medical University, Wakayama, Japan; 5) Department of Pediatrics, Chiba University Graduate School of Medicine, Chiba, Japan; 6) Department of Public Health, Chiba University Graduate School of Medicine, Chiba, Japan; 7) Department of Pediatrics, Toho University School of Medicine, Tokyo, Japan; 8) Department of Pediatrics, Fujita Health University, Toyoake, Japan; 9) Department of Pediatrics, Dokkyo Medical University, Koshigaya Hospital, Koshigaya, Japan; 10) Department of Speech and Hearing Sciences, International University of health and welfare, Fukuoka, Japan; 11) Department of Molecular Genetics, Kawasaki Medical School, Kurashiki, Japan; 12) Department of Pediatrics, Kawasaki Medical School, Kurashiki, Japan; 13) Department of Pediatrics, Fuji Heavy Industry LTD. Health Insurance Society General Ohta Hospital, Ohta, Japan; 14) Department of Cardiology, Boston Children's Hospital, Boston, MA; 15) Department of Pediatrics, Feinberg School of Medicine Northwestern University, Children's Memorial Hospital, Chicago, IL; 16) Department of Public Health, Jichi Medical School, Minamikawachi, Japan; 17) Department of Preventive Medicine, Shinshu University School of Medicine, Matsumoto, Japan; 18) Department of Surgery, National Center for Child Health and Development, Tokyo, Japan; 19) Laboratory for Medical Informatics, Ctr Genomic Medicine, RIKEN, Yokohama, Japan; 20) Japan Kawasaki Disease Research Center, Tokyo, Japan; 21) Laboratory for Molecular Medicine, Human Genome Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan; 22) U. S. KD Genetics Consortium.

Kawasaki disease (KD; OMIM 611775) is an acute systemic vasculitis syndrome of infants and young children. Previously we conducted a genome-wide sibpair study for KD and found ten candidate loci with positive linkage signals. From one candidate region in 19q13.2, we have identified a SNP of *ITPKC* gene which is significantly associated with KD and the risk for IVIG unresponsiveness both in Japanese and European American populations. In an attempt to identify a novel susceptibility gene we performed a positional candidate gene study for 4q35 region. We found that multiple common variants in *caspase-3* (*CASP3*) gene were significantly associated with KD both in Japanese and in European American populations and a G to A substitution of one commonly associated SNP located in the 5' UTR of *CASP3* (rs72689236; $P = 4.2 \times 10^{-8}$ in the Japanese and $P = 3.7 \times 10^{-3}$ in the European Americans) abolished binding of NFATc2 to the DNA sequence surrounding the SNP. Allele specific transcript quantification analysis showed a consistent result that the amount of the transcripts from the A allele of *CASP3* was less than that from the G allele in PBMCs. Interestingly, the GGAA motif of which the first G nucleotide is substituted by the SNP in human is seen in a similar position within the first non-coding exon of rodent *Casp3* genes, suggesting that the enhancer element might be evolutionarily conserved. CASP3 is one of the key molecules of apoptosis and it has been reported that apoptosis of neutrophils and lymphocytes is delayed in the acute phase of KD. Our data indicates that reduced expression of CASP3 might be involved in the pathogenesis of KD possibly through affecting apoptosis of immune cells.

1554/T

Identification of Novel Sequence Variants in Neuroligin Genes Contributing Autism Susceptibility. *D. Ramachandran[1], K.M. Steinberg[1,2], A.C. Shetty[1], V. Patel[1], M.E. Zwick[1].* 1) Human Genetics, Emory University, Atlanta, GA; 2) Graduate Program in Population Biology, Ecology and Evolution, Emory University, Atlanta, GA.

Autism Spectrum Disorders (ASDs) exhibit high heritability and affect four times as many males as females, yet the genetic risk factors remain largely unidentified. Several mutations in the neuroligin pathway have recently been implicated in the pathogenesis of ASD. We explored the hypothesis that other rare genetic variants found in neuroligin genes may contribute to ASD susceptibility. We performed paired end multiplexed sequencing of the genomic regions containing NLGN4X, NLGN3 and NRXN1β using the Illumina GAII platform on 144 male ASD probands from the Autism Genetic Resource Exchange (AGRE) collection. We observed a total of 314 single nucleotide variants (SNVs) and 139 insertion/deletion (indels) from these three loci. Of this, 167 variants are reported in either dbSNP130 or 1000 genomes project Pilot 1 data. We focused our attention on novel, rare variants found at highly conserved sites. In NLGN4X, we discovered one 3' UTR SNV, one 5'UTR SNV, and a novel 2-base 3'UTR indel. In NLGN3X, we found three 3'UTR variants, of which two are present in a single case. In NRXN1β, we observed two exonic heterozygous missense replacements (V193G; E357V) and five rare 3'UTR variants. The functional effects of the missense variants in NRXN1β were predicted to be damaging using SIFT, PolyPhen and Panther. We also identified one heterozygous exonic indel and 25 UTR indels that are highly conserved and not previously reported. All the rare and highly conserved single nucleotide variants and indels have been validated by Sanger sequencing. We will present results from functional assays to directly assess the effects of these rare variants. Our results suggest that rare variants in NLGN pathway genes may influence ASD susceptibility and demonstrates a study paradigm required to fully characterize the contribution of rare genetic variation to complex disorders like ASD.

1555/T

Greater than three-fold variation in mean leukocyte telomere length in young men living in eleven European countries: variation is not obviously related to population genetic structure or ecological variation in infectious exposures. *D.T.A. Eisenberg[1,2], K.D. Salpea[3], C.W. Kuzawa[1,2], M.G. Hayes[1,4,5], S.E. Humphries[3], European Atherosclerosis Research Study II group.* 1) Department of Anthropology, Northwestern University, Evanston, Illinois, 60208, USA; 2) Cells 2 Society: the Center for Social Disparities and Health, Institute for Policy Research, Northwestern University, Evanston, IL 60208; 3) Centre for Cardiovascular Genetics, Department of Medicine, Royal Free and University College Medical School, London, UK; 4) Division of Endocrinology, Metabolism and Molecular Medicine, Department of Medicine, Northwestern University Medical School, Chicago, Illinois, 60611, USA; 5) Center for Genetic Medicine, Northwestern University, Chicago, Illinois, 60611, U.S.A.

Telomeres are repetitive DNA sequences (5'-TTAGGG-3' in vertebrates) found at the ends of chromosomes. These telomere sequences shorten with age in proliferating human tissues, and with cell replications in vitro; and are therefore implicated in senescence. Previous studies suggest that shorter telomeres impair immune and cardiovascular function and result in increased mortality. Although few, prior studies have documented ethnic/population differences in human telomere lengths. In the US, African-American samples have tended to show longer leukocyte telomere lengths (LTLs) than European-Americans. Further, Salpea and colleagues (2008) demonstrated that LTLs vary across regions of Europe by over 25%. The causes of these population differences remain poorly understood. Here we extend the work of Salpea and colleagues by reporting variation in mean leukocyte telomere length (LTL) from 765 individuals from 14 study centers across 11 European countries (N from each study centers ranges from 32-71). Subjects are male students (ages 18-28), half of whom had fathers with myocardial infarction(s) before 55 and the remainder age-matched controls. Controlling for age and case/control status, telomere lengths averaged 10.20 kilobases (interpolated from qPCR measures) across study centers and ranged from 5.10 kilobases in Naples, Italy to 18.64 kilobases in Ghent, Belgium—a greater than three-fold difference across populations. These population level differences in LTLs were not explained by population genetic structure as measured by the eigenvalues from the first two principal components of a previous analysis of the genetic structure of European populations (Novembre and colleagues 2008), nor by national level ecological analysis of an index of infection/economic status (United Nations Infant Mortality Rates). In sum, we show considerable population variation in LTL in Europe that is not obviously a result of broad measures of population structure or infection/economic exposure measured in early life or in adulthood. Studying telomere dynamics in a wider variety of populations, and with greater attention to lifecycle dynamics, will be important to help elucidate the causes and possible consequences of human population variation in telomere length. This work was supported by the British Heart Foundation [KDS (FS/06/053) and SEH (RG2005/014)] and by the EC Concerted Action BMH1 CT92-0206. DTAE is supported by an NSF Graduate Research Fellowship.

1556/T

The VNTR polymorphism of the dopamine D4 receptor (*DRD4*) gene in Oceanic populations. *I. Naka[1], N. Nishida[2], R. Kimura[3], T. Furusawa[4], K. Natsuhara[5], T. Yamauchi[6], M. Nakazawa[7], Y. Ataka[8], T. Ishida[9], T. Inaoka[10], Y. Matsumura[11], R. Ohtsuka[12], N. Tsuchiya[1], J. Ohashi[1].* 1) Doctoral Program in Life System Medical Sciences, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Ibaraki, Japan; 2) Department of Human Genetics, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan; 3) Transdisciplinary Research Organization for Subtropics and Island Studies, University of the Ryukyus, Okinawa, Japan; 4) ASNET Promotion Office, Division for International Relations, The University of Tokyo, Tokyo, Japan; 5) School of Nursing, Fukuoka Prefectural University, Fukuoka, Japan; 6) Department of Health Sciences, Hokkaido University School of Medicine, Hokkaido, Japan; 7) Department of Public Health, Graduate School of Medicine, Gunma University, Gunma, Japan; 8) School of Policy Studies, Kwansei Gakuin University, Hyogo, Japan; 9) Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Tokyo, Japan; 10) Department of Environmental Sociology, Faculty of Agriculture, Saga University, Saga, Japan; 11) Faculty of Healthcare, Kiryu University, Gunma, Japan; 12) Japan Wildlife Research Center, Tokyo, Japan.

The dopamine D4 receptor (*DRD4*) gene contains a 48-base pair variable number of tandem repeat (VNTR) in exon 3, encoding the third intracellular loop of this D2-like receptor. The lengths of VNTR vary from 2 to 11 repeats (2R to 11R), and alleles with 2R, 4R, and 7R are commonly observed in human populations. The *DRD4* VNTR has been suggested to be associated with behavioral and psychiatric phenotypes including novelty seeing, drinking behavior, and attention deficit/hyperactivity disorder, although there are conflicting reports. Further, the 7R allele has been suggested to be subjected to positive selection since the linkage disequilibrium from 7R is more extended than those from the other common VNTR alleles (i.e., 2R and 4R). In this study, the VNTR polymorphism of *DRD4* was investigated in 7 Oceanic populations (4 Melanesians, 1 Micronesian, and 2 Polynesians). In the studied populations, only alleles with 2 to 7 repeats were observed. The 7R allele was frequent in non-Austronesian speaking Melanesian population (Gidra), whereas rare in Micronesian (Rawaki) and Polynesian (Ha'apai and Nuku'alofa of Tonga) populations. Interestingly, the 5R allele was frequently found in Micronesian population (Rawaki), but nearly absent in the other Oceanic populations. These observations are considered to reflect their evolutionary history. Our results will provide useful information for association studies of the *DRD4* gene in Oceanic populations.

1557/F

Molecular Confirmation of Hemophilia A Through RNA Analysis. *H. Best, T. Lewis, P. Bayrak-Toydemir.* ARUP Institute for Clinical and Experimental Patho, Salt Lake City, UT.

BACKGROUND: Hemophilia A is a hereditary bleeding disorder caused by mutations in the F8 gene (Xq28). A subset of patients, 5-10%, with mild or moderate hemophilia has no detectable mutation in the coding regions of the F8 gene. For these patients it is feasible that intronic mutations affecting splicing are responsible for the disorder. Here we describe a 9 year old male with a clinical diagnosis of hemophilia A based on Factor VIII activity levels reported to be between 5-12% (consistent with mild to moderate disease) that was referred for F8 gene sequencing. Previous testing for F8 gene inversions was negative and DNA sequencing revealed no mutations within the coding regions of the gene. METHODS AND RESULTS: The proband's genomic DNA was sequenced for the F8 gene and a c.602-9_602-7delCTT variant was detected. Based on splice prediction software, this variant was predicted to be a splice site mutation. To verify that the variant led to alternative splicing, RNA was collected from the patient and cDNA synthesized using the protocol described in El-Maarri et al., 2005. PCR across exons 4-8 yielded an abnormal sized PCR product of 542 bp rather than the expected 611 bp product. Sequencing of the cDNA amplicon product showed a read through straight from exon 4 to exon 6, with exon 5 missing. Exon 5 is 69 bp in length accounting for the size difference observed in the PCR product. The same variant, c.602-9_602-7delCTT, was observed by genomic sequencing in the proband's affected half-brother whose Factor VIII activity levels were reported to be between 3-13%. cDNA analysis of the sibling showed the same aberrant band pattern for the amplicon spanning exons 4-8. CONCLUSIONS: Analysis of cDNA is a useful way of investigating predicted splicing mutations in hemophilia A patients. Our analysis of a proband and an affected sibling with presumed splice site mutations demonstrated that the mRNA transcript produced skips exon 5 of the F8 gene with no full-length wild type transcript detected.

1558/F

Evaluating the alternative splicing of interleukin 7 receptor alpha chain (IL7Rα) and its role in multiple sclerosis. *S. Gregory[1, 2], I. Evsyukova[3,4], M. Garcia-Blanco[2, 4, 5].* 1) Duke Center for Human Genetics; 2) Duke University Department of Medicine; 3) Duke University Department of Biochemistry; 4) Duke University Center for RNA Biology; 5) Department of Molecular Genetics and Microbiology, Duke University.

Multiple sclerosis (MS) is a debilitating neurodegenerative disorder of the central nervous system that is thought to be mediated by T-cell autoimmunity. The pathophysiology of MS, which affects 400,000 individuals in the US, causes chronic inflammation, myelin loss and progressive neurological dysfunction. Recently we identified genetic association (p-val 2.9x10-7) of a non-synonymous SNP, rs6897932 (C®T, Thr244Ile), within exon 6 of the interleukin 7 receptor gene (IL7Rα) with MS. IL7Rα is an attractive candidate in the etiology of the disease because it facilitates interleukin 7 (IL7) signaling at specific stages during T cell development in the thymus, and also during naïve and memory T cells maintenance in the periphery.

Functional analysis of the associated SNP revealed that the "C" risk allele results in increased skipping of exon 6. Previous studies have shown that alternative splicing of exon 6 leads to in vitro production of either membrane-bound (exon 6 included) or soluble (exon 6 skipped) isoforms of IL7Rα. To address our hypothesis that increased skipping of exon 6 alters the ratio of the membrane-bound to soluble isoforms of IL7Rα and negatively affects IL7 signaling, we sought to identify the cis-acting motifs and transacting factors that regulate IL7Rα splicing.

Our data show that the "C" risk allele, which results in a two fold increase in the soluble isoform of IL7Rα, augments a weak exon splice silencer (ESS). Mutation analysis also reveals that exon 6 contains two exon splice enhancers (ESE) and that weak 5' and 3' splice accepter and splice donor sequences surround exon 6 in the human most likely account for the fact that exon 6 is not alternatively spliced in the mouse. We have successfully recapitulated the ESE and ESS sequence motifs within a splicing vector in a heterologous context and in vitro. Together with RNA affinity chromatography, these constructs will facilitate the identification of the trans-acting protein factors regulating exon 6 splicing. In addition to helping define the complex mechanisms of IL7Rα splicing, identifying the proteins that regulate IL7Rα will provide additional candidates genes that contribute to the genetic risk of developing MS.

1559/F

FLJ Human cDNA Database and tissue specific expressed alternative splicing variants. *T. Isogai, A. Wakamatsu.* Grad Sch Pharmaceutical Sci, Univ Tokyo, Tokyo, Japan.

Human gene number was predicted to be about 20 thousand. But the number of the mRNA was predicted to be several times of gene number. Those varieties were thought to be caused by alternative splicing. In our NEDO human cDNA project, we sequenced 55,402 of human full-length cDNAs (FLJ cDNAs) covered 14,959 genome loci, and also obtained about 1.5 million of 5'-end sequences (5'-EST), about 500 base length, of full-length cDNAs covered 17,287 genome loci from about 100 kinds of cDNA libraries consist of human tissues and cells constructed by oligo-capping method. The majority of the insert size of those was over 2 kb and the full-length rate of 5'-end of those was 90%. Then we constructed FLJ Human cDNA Database ver. 3.0, http://flj.lifesciencedb.jp/. In those 11,769 of full-length sequenced FLJ cDNAs covered 7,025 genome loci, which were obtained as new ORFs at that time by mainly focusing on variations of splicing and transcription start site (TSS) from our cDNA resources described above, were included. Those ORFs were consisted in 3,403 cDNAs, 3,105 genome loci, of alternative N-terminus variants by different TSSs, 8,277 cDNAs, 3,815 genome loci, of alternative splicing variants except for TSSs, and 89 cDNAs of new genome loci. In the selection process we used ATGpr, http://atgpr.dbcls.jp/, the program for identifying the initiation codons and predicting the coding potential in cDNA sequences, for effective cDNA selection from protein coding mRNAs. Elimination efficiency of cDNAs from non-coding RNAs was increased to three fold using ATGpr. In addition, we found some alternative splicing variations which showed specific expression patterns from 11,769 cDNAs. We analyzed gene expression profiles with the about 1.5 million of 5'-ESTs by oligo-capping method described above. Then we selected variant cDNAs shown both characters, a specific expression profile of exons and a variation of motifs and/or domains in coding regions. Obtained cDNAs of each cluster were further analyzed expression patterns of each exon by realtime PCR. As a result, we obtained cDNAs shown tumor tissue specific expressions (FLJ57068, FLJ57884, FLJ52319 etc.), synovial and osteoclast specific expressions (FLJ55256, FLJ55661 etc.) and so on. In conclusion combination of high full-length rate cDNA resources, about 500 base of 5'-ESTs of those and ATGpr was effective for cloning of protein coding alternative splicing variant mRNAs.

1560/F

Evolutionary acquired alternative splicing in the 5'-UTR of retinal dystrophin transcript is a default pathway with weaker translational activity than non-spliced retina specific form. *I. Kubokawa, M. Yagi, H. Awano, M. Ota, A. Nishida, E.K. Dwianingsih, R.G. Malueka, Y. Takeshima, M. Matsuo.* Pediatrics, Graduate School of Medicine Kobe University, Kobe, Japan.

Background The dystrophin gene, which mutations cause Duchnne or Becker muscular dystrophy (DMD/BMD), has been reported to have a retina specific promoter in intron 29. The R-dystrophin transcript has retina-specific exon 1(R1) joining to exon 30 of the dystrophin gene and has been claimed responsible for ophthalmological problems observed in DMD/BMD. However, the structure of the 5'- untranslated region (5'-UTR) of R-dystrophin is not well elucidated. **Materials and Methods** Retina-promoter derived transcript spanning exon R1 to exon 32 of the dystrophin gene was RT-PCR amplified from total RNA of human retina and other tissues. The translational activity of 5`-UTR was analyzed by the dual-luciferase reporter assay system. **Results and Discussion** RT-PCR amplification of the R-dytrophin transcript in human retina using a conventional primer set revealed a clear product consisting of exon R1 and exon 30, 31 and 32. In contrast, three amplified products were obtained using outer forward primer. Two main products were found the longer form with exons R1, 30, 31 and 32, and the shorter form with deletion of 98bp from exon R1. However, they encoded the identical open reading frame. The deleted 98bp sequence within exon R1 was disclosed a cryptic intron that was evolutionally acquired. The shorter form without the cryptic intron was ubiquitously expressed with wide expression ranges, while the longer form was retina specific expressed. Splicing of the cryptic intron was considered a default splicing pathway. To clarify the physiological difference between two main variants, their 5'-UTRs were examined for translational activity. Remarkably, the shorter ubiquitously expressed form showed lower translational activity than the longer one. The lower activity was supposed due to the removal of internal ribosome entry sites by the cryptic intron splicing. This splicing seemed a molecular switch that regulates translation of the R-dystrophin transcript. Here, an evolutionary acquired alternative splicing was first identified in the 5'- UTR of the retinal dystrophin transcript and considered a default splicing pathway with weaker translational activity than non-spliced retina specific form.

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Novel FADS1 transcripts generated by alternative polyadenylation, alternative transcription initiation and alternative splicing. *W.J. Park, K.S.D. Kothapalli, J.T. Brenna.* Division of Nutritional Sciences, Cornell University, Ithaca, New York.

Omega-3 (ω3 or n-3) and omega-6 (ω6 or n-6) long chain polyunsaturated fatty acids (LCPUFA) are nutrients and bioactive metabolites associated with most of the human diseases, specifically cardiovascular (CVD), neurological, metabolic syndrome, and are also critical for growth and development. The nonheme, iron-containing, oxygen-dependent fatty acid desaturase enzymes (coded by FADS1 and FADS2, and hypothetically by FADS3) introduce double bonds between two carbon atoms and are of key importance in LCPUFA biosynthesis. Twenty carbon PUFA are the direct precursors for the biosynthesis of physiologically active eicosanoids. FADS1 protein products catalyze the desaturation of 20 carbon PUFA dihomo-gamma-linolenic acid (20:3n-6) to arachidonic acid (20:4n-6) and eicosatetraenoic acid (20:4n-3) to eicosapentaenoic acid (20:5n-3). It is presently assumed that both n-3 and n-6 families of fatty acids, share and compete for the same enzymes, and only a single transcript has been identified for FADS1. The enormous diversity that can be generated at the RNA level is gaining importance as a significant mechanism in the regulation of gene expression and in expanding the diversity of proteome. Recently, we have shown for the first time the existence of alternative transcripts (ATs) for FADS2 and FADS3 genes in a primate (neonate baboon) generated by alternative splicing events. We have also shown the expression of these transcripts in baboon tissues, their conservation in several vertebrate species and reciprocal changes in expression in response to human neuronal cell differentiation. To investigate whether FADS1 is also subjected to alternative splicing, we performed both 5' and 3' rapid amplification of cDNA ends (RACE) using gene specific primers for FADS1. Multiple products were detected on 2% agarose gels. Cloning and sequencing of these products show at least seven alternative transcripts for FADS1. The identified transcripts are generated by alternative selection of Poly A sites, different 5' UTRs, and by internal exon deletions resulting from alternative splicing. These data show, for the first time, that FADS1 produces several mRNA isoforms, and thus that all three FADS genes do so. Our results indicate that these ATs might play critical roles either as mediators of LCPUFA biosynthesis and/or regulation. Thus, consideration of ATs represents possible conceptual change for FADS and in general for diet-gene interaction studies.

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A Study of PMP22 Localization in Mouse Cochlea Throughout Postnatal Development of the Inner Ear. *T. Carver, M. Kovach.* Biol & Enviro Sci/ 2653, Univ TN at Chattanooga, Chattanooga, TN.

Peripheral myelin protein-22, referred to as PMP22, is a 22 kD glycoprotein associated with the Gas (Growth arrest specific) gene family. PMP22 is largely expressed in Schwann cells and appears to play a role in myelination of neural tissues. However, it has also been found expressed at lower levels in non-neural tissues such as lungs and intestines throughout development. The non-neural pattern of PMP22 gene expression is more akin to the expression pattern characteristic of Gas genes, which are induced during periods of growth arrest, but down regulated at terminal differentiation and cell senescence. Thus, in non-neural tissues, PMP22 may be involved more with cell growth regulation. This dual expression pattern is consistent with the description of both neural and cochlear components to hearing loss in patients with the disorder Charcot-Marie-Tooth (CMT). CMT1E is an autosomal dominant, genetic variant of CMT disease in which patients present with profound sensori-neural deafness in addition to progressive peripheral neuropathy. Since its initial description, several other independent cases of CMT1E have been reported, the majority of which are due to point mutations in the PMP22 gene. This study was designed to gain a better understanding of PMP22 expression in the cochlea throughout development of the inner ear and its role in deafness. The Trembler-J (TrJ) mouse was chosen as a model for PMP22-associated auditory dysfunction with B6 (Wt) being the control. Cochlea were extracted from the mice at various postnatal developmental time points (Days: 1, 7, 14, 21 and 42) and prepared for examination by Immunohistochemistry (IHC) with anti-PMP22 antibody. IHC reveals the presence of PMP22 protein in non-neural tissues of the cochlear chamber in addition to high levels of protein localization in the cochlear nerve. In the neural tissue of Wt, the level of PMP22 increases through D21, while TrJ shows little PMP22 presence. However, this pattern is reversed in the non-neural cells in the organ of corti. At D7, PMP22 is detected in the inner and outer hair cells of both Wt and TrJ. By D21, PMP22 becomes more intense and widespread in TrJ including the inner hair cell, Claudius', Hensen's and Deiters' cells. In comparison, Wt becomes less intense and more localized to the Hensen's and Deiters' cells. This divergence in protein localization patterns between Wt and TrJ mirrors expression levels of non-neural PMP22 transcripts as determined by qRT-PCR.

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Regulation of expression of chitinase genes in mouse tissues. *F. Oyama, K. Tsuda, M. Ohno, M. Sakaguchi, Y. Sugahara.* Dept Applied Chemistry, Kogakuin Univ, Hachioji, Tokyo, Japan.

Chitin, an integral component of the fungal cell walls, the exoskeletons of crustaceans and insects, and the microfilarial sheaths of parasites, is the second abundant polysaccharide in nature. Although mammals do not have chitin and chitin synthase, two major chitinases, chitotriosidase and acidic mammalian chitinase (AMCase), have been identified in both human and mouse. Marked elevation of plasma chitotriosidase activity was reported in Gaucher disease, an autosomal recessive lysosomal storage disorder. In addition, significant increases in AMCase mRNA and protein were detected in an induced asthma mouse model and patients. Little is known, however, about the regulation and physiological functions of the chitinases in mammals. We studied expression of both chitinases in mouse tissues by quantitative RT-PCR. Clear tissue specificities were observed in the expression patterns in both chitinases mRNA levels. High levels of chitotriosidase mRNA were identified in testis, stomach, spleen and lung. AMCase mRNA was predominantly expressed in stomach and moderately in testis, spleen and lung. These results indicate that expression of chitinases is regulated in a tissue-type specific manner in mouse tissues.

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Development of bioinformatics strategies to predict the functionnal impact of polymorphisms in regulatory regions. *P. Beaulieu[1], M. Ouimet[1], V. Gagné[1], T. Pastinen[3], D. Sinnett[1,2].* 1) Centre de recherche, Hopital Ste-Justine, Montréal, QC, Canada; 2) Departement de Pédiatrie, Université de Montréal, Montreal, QC, Canada; 3) McGill University and Genome Quebec Innovation Center, Montreal, QC, Canada.

Previous efforts to identify functional disease-causing DNA variants were essentially oriented towards the coding regions of candidate genes since these variants have a direct impact on the structure and function of the affected proteins. However, abnormal expression of finely regulated genes can also lead to disequilibria in different metabolic pathways and/or biological processes. Thus, investigation of the functional impact of polymorphisms as well as the determination of the importance of evolutionary conservation in the regulatory regions of candidate genes should improve our knowledge of complex disease aetiologies. As part of an ongoing regulatory genomics initiative, we have integrated several layers of information such as gene structure, SNP content, and probability of transcription factors binding to DNA according to genomic patterns together with experimental results from electrophoretic mobility shift assays (EMSA) and several types of in vitro/vivo promoter activity assays. This information is integrated in web-based environment that can be used to combine and analyse regulatory genomics data (www.regulatorygenomics.org). Here we present a detailed computational analysis of regulatory regions and attempt to improve their functional annotation. This allows us to establish criterias and parameters usable for better predictions of active regulatory regions as well as improved prediction of regulatory SNPs with impact on gene expression. Such functional annotation provide a more effective selection of regulatory targets and thereby facilitates our efforts understand human complex diseases.

1565/F

Rheumatoid arthritis (RA) associated-gene, CD244 expressed in RA synovial tissue. *A. Suzuki[1], R. Yamada[1,2], Y. Kochi[1], Y. Okada[1,3], T. Sawada[4], K. Matsuda[5], Y. Kamatani[5], M. Mori[3], K. Shimane[1,3], A. Takahashi[1], Y. Nakamura[1,5], K. Yamamoto[1,3].* 1) Center for Genomic medicine, Kanagawa, RIKEN, Yokohama City, Japan; 2) Center for Genomic Medicine, Kyoto University, Tokyo, Japan; 3) Department of Allergy and Rheumatology, Graduate School of Medicine, University of Tokyo, Japan; 4) Tokyo Medical University, Tokyo, Japan; 5) Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

RA is well-known as an autoimmune disease and is a chronic inflammatory disorder characterized by the destruction of multiple joints. Many genome wide association studies were performed and multiple RA-susceptibility loci and autoimmune-susceptibility loci have been identified. These studies suggested that multiple genes and its functions were related with disease causing and development. These studies also indicated an important factor regarding genetic factors of RA and autoimmune diseases; some of the RA-susceptible polymorphisms also increase the risks of other autoimmune diseases as reported for e.g., STAT4 (ref1) and FCRL3 (ref2) with RA and SLE. One of the mechanisms of the inflammation in autoimmune diseases associated with signal transduction via signaling lymphocytic activation molecule (SLAM). It was reported that SLAM family gene, e.g., Ly108 is also associated with systemic lupus erythematosus (SLE). We studied whether variants of the SLAM family gene in the chromosome 1q region are associated with susceptibility to RA. The association peak in the block was observed at two functional SNPs (rs3766379 and rs6682654) in CD244 in two independent RA cohorts from Japan (P= 3.23 x 10-8 and P=7.45 x 10-8). We found a Japanese cohort of systemic lupus erythematosus (SLE) that had the similar genotype distribution with RA cohorts. These disease-associated SNPs, rs3766379 and rs6682654 also have been shown as functional SNPs(ref3). Furthermore, we indicated that CD244 also expressed in synovial tissues of CIA mice and RA patients. We supposed that up-regulation of CD244 affect on RA and other autoimmune diseases, including SLE. Thus, CD244 is a genetic risk factor for RA and may have a role for autoimmunity in RA. 1) Remmers, E.F. et al. N Engl J Med 357, 977-86 (2007). 2) Kochi, Y. et al. Nat Genet 37, 478-85 (2005). 3) Suzuki, A. et al. Nat Genet 40, 1224-1229 (2008).

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Neto2 is a novel regulator of the potassium chloride co-transporter KCC2 and GABA-mediated synaptic inhibition. *E.A. Ivakine[1], B.A. Acton[2], M.A. Woodin[2], R.R. McInnes[1,3].* 1) Program in Stem Cell and Developmental Biology, Hospital for Sick Children Research Institute, Toronto, M5G1L7,; 2) Department of Cell & Systems Biology, University of Toronto, 25 Harbord Street, Toronto, M5S 3G5; 3) The Lady Davis Institute, Jewish General Hospital, McGill University, Montreal, H3T1E2.

Neto1 and Neto2 are two related neuronal single pass transmembrane proteins, expressed highly in the developing and adult brain. Recently, we and others reported that the Neto proteins are important regulators of synaptic strength and excitatory neurotransmission (Ng et al. PLoS Biol. 2009; 7(2):e41; Zhang et al. Neuron. 2009; 12;61(3):385-96). The objective of the current study was to define the role of Neto2 in GABA-mediated synaptic inhibition. Using a GST pulldown approach followed by LC-MS/MS we identified KCC2 as a putative Neto2 interactor. KCC2 is a K^+-Cl^- cotransporter required for synaptic inhibition in the mature CNS, where it establishes a Cl^- ion gradient. We validated the Neto2:KCC2 interaction by co-immunoprecipitating them from both hippocampal protein lysates and HEK-293 cells. Using a series of Neto2 deletion constructs we determined that the Neto2:KCC2 interaction was mediated by the Neto2 ectodomain. To define the functional consequences of this interaction, we measured the strength of synaptic inhibition, as determined by the Cl^- equilibrium potential (E_{Cl} = membrane potential where net flow of chloride ions is zero). We found that in *Neto2-/-* neurons E_{Cl} was significantly depolarized vs. wild type neurons (-53.74 ± 2.43 mV vs. -75.22 ± 2.05 mV; $p < 0.001$), demonstrating a highly significant increase in the intracellular Cl^- concentration, and suggesting a decrease in KCC2 activity. This depolarized E_{Cl} can be completely rescued by reintroducing a full length *Neto2* into *Neto2-/-* neurons; E_{Cl} = -73.12 ± 3.07 mV ($p < 0.001$). Importantly, shRNA-mediated knock-down of Neto2 in mature wild type cultured neurons also causes a depolarization of E_{Cl} (-54.43 ± 2.12 mV; $p = 0.016$), indicating that maturation of *Neto2-/-* and *wt* neurons occurs at similar rate. To define the consequences of *Neto2* deficiency on KCC2 function, we first analyzed KCC2 levels in wild type and *Neto2*-deficient mice: the lack of Neto2 had no effect on KCC2 abundance, either on the cell surface or in total hippocampal lysates. We conclude that Neto2 is a novel regulator of neuronal Cl^- homeostasis through its interaction with KCC2, thereby profoundly influencing GABA-mediated synaptic inhibition. Through its ectodomain Neto2 directly binds to KCC2 and regulates KCC2 activity without affecting either total or cell surface levels of the transporter.

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Neuron-specific enhanced expression of TAF1 and its isoform. *S. Makino[1], G. Tamiya[2], I. Tooyama[1].* 1) Molecular Neuroscience Research Center, Shiga University of Medical Science; 2) Advanced Molecular Epidemiology Research Institute, Yamagata University School of Medicine.

We previously found a neuron-specific isoform of the *TAF1* (TATA box binding protein-associated factor 1), which is the disease causative gene of X-linked recessive dystonia-parkinsonism showing severe neurodegeneration in striatum (XDP/DYT3; MIM314250). The *TAF1* gene encodes the largest component of the TFIID complex involved in RNA polymerase II-mediated expression of many genes related cell division. The neuron-specific isoform of the *TAF1* gene, named *N-TAF1*, may have an essential role in neuronal survival in the striatum through transcriptional regulation of many neuron-specific genes. To investigate the detailed function of the neuron-specific isoform of the *TAF1* gene, we carried out over-expression of *N-TAF1* in several mice cell lines. First, we established the stable N2a-TetR cells with an inducible mouse N-TAF1/TAF1 expression vector. The doxycycline treatment of N-TAF1/TAF1 inducible cells allowed a substantial expression of its products. We subsequently performed MTS assay, which could easily be interpreted as cellular mitochondria activities, to examine if the over-expression of N-TAF1/TAF1 affect cell proliferation in Neuro-2a. The MTS assay showed that the N-TAF1 induction for 7 days significantly inhibited N2a proliferation whereas the TAF1 induction elevated cell proliferation. Second, we compared the intracellular localizations of GFP-tagged N-TAF1 and TAF1 protein during the cell cycle and mitosis between mice neuroblastoma, hepatoma and renal carcinoma cell lines. These experiments would reveal the neuron-specific role of *N-TAF1* gene and shed light on the molecular pathogenesis of disease caused by transcriptional dysregulation as well as XDP.

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Targeted Integration of Fluorescent Reporter Genes Utilizing Zinc Finger Nucleases. *D. Vassar, N. Zenser, H. Zhang, F. Zhang, D. Malkov, G. Davis.* Sigma-Aldrich Biotechnology, 2909 Laclede Avenue, St. Louis, MO 63139.

Zinc finger nucleases (ZFNs) are synthetic proteins engineered to bind DNA at a sequence-specific location and create a double strand break. The cell's natural machinery repairs the break in one of two ways: non-homologous end joining or homologous recombination. We relied on the homologous recombination pathway to insert a transgene into a desired location. ZFNs were customized/designed to cut near the near the desired site of integration for the three organelle specific genes. These ZFNs were nucleofected along with a donor construct containing a fluorescent reporter gene flanked by sequences homologous to the target integration site into U2OS cells. Integration resulted in endogenous expression of fluorescent fusion proteins that labeled a particular organelle. Three gene loci were tagged: TUBA1B (α-tubulin 1b, microtubule), ACTB (β-actin, actin stress fibers), LMNB1 (lamin B1, nucleus). Fluorescent proteins were used as reporters. The fluorescent reporters were stably integrated into the genome of the cells. Preservation of protein function and gene regulation was observed. Additionally, due to the specificity of the targeted integration process, multiple reporter genes can be integrated into a cell's genome for further study of cellular processes.

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THE ATM PROTEIN DISPLAYS DISTINCT SPATIAL AND TEMPORAL DYNAMICS AT SITES OF INDUCED DNA DAMAGE. *P.S Bradshaw[1, 2], M.S Meyn[1, 2].* 1) Program in Genetics and Genome Biology, Sick Kids Research Institute, Toronto, ON, Canada; 2) Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada.

Human cells use complex signaling networks to detect and respond to DNA damage in genomic DNA. ATM, the protein kinase inactivated in the cancer syndrome Ataxia-Telangiectasia, controls the major human DNA Double Strand Break (DSB) response pathway. Current models assume that ATM is a critical component of the megabase-sized chromatin complexes that develop around induced DSBs and serve as a platform for DSB repair. To better understand the behavior of ATM at DNA breaks we use UVA light and Hoechst dye to introduce spatially-defined DNA breaks within nuclear chromatin in human fibroblasts. Photo-induction of DNA damage, equivalent to ~10 Gy γ-radiation, causes rapid recruitment of endogenous ATM to damage sites. This recruitment occurs after MRN complex recruitment and prior to DNA damage response factors γH2AX, 53BP1 and MDC1. ATM accumulation peaks 10-30 minutes post damage, then declines with kinetics paralleling those of DSB rejoining. We find the induced foci of many DSB response proteins enlarge and diffuse with time. Mre11 and Nbs1, for example, display both MDC1-dependent diffuse staining and localized MDC1-independent foci 1 hr post DNA break induction. The latter co-localize with foci of the homologous recombination (HR) proteins RPA and Rad51. In contrast, ATM forms localized, non-diffusible foci that are MRN-dependent, MDC1-independent and spatially separate from HR foci. The non-diffusible nature of DNA damage-associated foci of endogenous ATM is confirmed by FRAP analysis of GFP-tagged ATM. Further, mutating the ATM serine 1981 phosphorylation site to alanine impairs but does not completely block accumulation of GFP-ATM. Over-expression of the telomeric protein TRF2, an ATM kinase inhibitor whose binding spans the S1981 residue, also attenuates GFP-ATM accumulation DNA damage sites. Our results demonstrate the dynamic nature of protein complexes formed at DNA damage sites and document three types of complexes: chromatin-associated megabase complexes; discrete foci of HR proteins; and discrete foci of ATM. The spatial and temporal dynamics of ATM foci suggest ATM is not a major component of chromatin-associated megabase complexes and does not directly facilitate HR-mediated repair. Rather, our findings are consistent with ATM rapidly accumulating at localized sites of damage in an MRN-dependent manner, then being displaced as DNA breaks are either rejoined or processed for recombinational repair.

1570/F

VSXl mutational analysis in Indian patients affected by keratoconus: identification of a novel mutation. *I. Goyal[1], M. Kumar[1], D. Pathak[1], M. Tanwar[1], N. Sharma[2], J.S. Titiyal[2], R. Dada[1].* 1) Laboratory for Molecular Reproduction and Genetics, Department of Anatomy, All India Institute of Medical Sciences, New Delhi; 2) Dr R.P. Centre for Ophthalmic Sciences, All India Institute of Medical Sciences, New Delhi.

Keratoconus is the most common of noninflammatory ectatic disorders of cornea, with an estimated incidence between 1 in 500 and 1 in 2000 persons. Although most cases of keratoconus are sporadic, 5% to 10% of patients have a positive family history. The pathogenesis of keratoconus is unknown. The only identified molecular cause for keratoconus involves mutations in VSXl gene. This study was aimed to analyze keratoconus patients for mutations in VSXl gene. All participants had a comprehensive eye examination. DNA was extracted from whole blood samples (5 ml) of all Keratoconus patients and controls using phenol chloroform method. The coding regions of VSXl and exon-intron junctions were amplified using five sets of oligonucleotide primers. Sequencing analysis showed 4 mutations in VSXl gene. R217H, P237P, A182A changes in amino acids were already reported in the literature. We found a novel C>T intronic polymorphism between exon 2-3. As this novel variation is in proximity to the intron-exon junction, it possibly interference with splicing mechanism. Various studies have shown that role of VSXl gene in the pathogenesis of corneal disease is controversial. The lack of evidence to support that VSXl mutations are pathogenic, failure to demonstrate corneal expression of VSXl gene and identification of presumed pathogenic mutations in VSXl gene in less than 5% of keratoconus patients suggested that VSXl gene does not play a major role in the pathogenesis of keratoconus. In conclusion, our study reports a new VSXl mutation and shows no significant correlation between VSXl and keratoconus.

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Effects of TNF-α and IL-1β on the activation of genes related to inflammatory, immune responses and cell death in immortalized human keratinocytes HaCat. *S. Nistico[1], N. Paolillo[2], D. Minella[3], S. Piccirilli[4], G. Emiliano[3], M. Biancolella[5], S. Chimenti[1], G. Novelli[3], G. Nistico[4].* 1) . Department of Dermatology, School of Medicine, University of Rome Tor Vergata, Italy; 2) IRCCS "C. Mondino Institute of Neurology" Foundation, Pavia, Italy; 3) Department of Biopathology and Diagnostic Imaging, School of Medicine, Tor Vergata University, Rome, Italy; 4) Center of Pharmaceutical Biotechnology, University of Rome Tor Vergata, Italy; 5) Department of Preventive Medicine University of Southern California,Los Angeles, CA, USA.

The epidermis is implicated in allergic and severe skin adverse reactions to drugs such as toxic epidermal necrolysis (TEN; OMIM #608579) and Stevens-Johnson syndrome (SJS; OMIM #608579) both characterized morphologically by the rapid onset of keratinocyte cell death by apoptosis. To date, the precise sequence of molecular and cellular events that lead to the development of SJS and TEN is only partially understood. A large body of evidence exists in the literature involving some cytokines of the tumor necrosis factor (TNF) family and interleukins (ILs) in the pathogenesis of cell death in epidermal keratinocytes.By controlling gene expression, TNF-α orchestrates the cutaneous response to environmental damage and inflammation. Interleukin 1 (IL-1) often referred to as prototypic inflammatory cytokine is a family of three closely related proteins that are the products of separate genes.In particular it has been reported that IL-1 treatment causes profound morphological changes of keratinocytes. Several reports of microarrays analysis investigated the transcriptional changes in cells induced by treatment with TNF-α and IL-1β.Important changes in gene expression profiles of human epidermal keratinocytes were reported with TNF-α as well as with the treatment with IL-1α showing that both cytokines are able to activate many genes involved in the inflammatory and immune responses as well as in cell death. To our aims, we applied microarray technology to characterize the modification of gene expression in HaCat cells treated with TNF-α, and IL-1β given alone or in combination. We focused our attention on gene expression changes evoked by both cytokines specifically analyzing up or down-regulation of genes related to inflammatory, immune response, cell death as well as cell differentiation, growth and repair mechanisms, in order to disclose specific genomic markers of pathological responses to inflammatory injuries. Significant differences in the percentage and quality of genes dysregulated by TNF-α and IL-1β were shown. Both cytokines activated a series of genes involved in inflammatory, immune response as well as in cell death. In our experimental conditions, TNF-α, in contrast to IL-1β, did not induce a significant level of apoptosis in keratinocytes. Given together both cytokines produced a synergistic transcriptional response which was due to the activation of several specific genes occurring after application of each cytokine.

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Elevated levels of oxdatively damaged DNA in patients with heterozygous defects in the selenocysteine insertion sequence-binding protein 2 gene (SECISBP2). *M. Karbaschi[1], E. Schoenmakers[2], M.D. Evand[1], K. Chatterjee[2], M.S Cooke[1].* 1) University of Leicester, Leicester, United Kingdom; 2) Institute of Metabolic Science, Cambridge, United Kingdom.

Selenium (Se) is a vital dietary element for eukaryote and prokaryotic cells. In humans, its biological role is mediated principally by incorporation of selenocysteine, into selenium containing proteins. About 30 different selenoproteins have so far been observed in human cells and tissues, performing a variety of different functions including removal of cellular reactive oxygen species, reduction of oxidised methionines in proteins, metabolism of thyroid hormones, transport and delivery of selenium to peripheral tissues, protein folding and ER stress, plus proteins whose precise function is unknown. One of the best-known functions of selenoproteins is displayed by the family of selenium-dependent glutathione peroxidases which reduces hydrogen peroxide, and damaging lipid and phospholipid hydroperoxides to harmless products such as water and alcohols. This function helps to reduce the risk of induction of further oxdatively generated damage to biomolecules and accordingly reduces risk of a variety of damage-related pathological conditions. In this study, we examined the baseline levels of oxdatively damaged DNA in two individuals, one adult and one child with compound heterozygous defects in the selenocysteine insertion sequence-binding protein 2 (SECISBP2) gene. As part of a complex phenotype, using hOGG1-modified comet assay, we noted a significantly elevated baseline levels of 8-oxoguanine and alkali labile sites in dermal fibroblasts versus age/gender match controls in the presence and absence of exogenous H_2O_2 (50 μM, 30 min). Using the same assay, we demonstrated that whilst DNA repair processes were unaffected, antioxidant defences were impaired, the likely source of the elevated baseline levels. Similarly, there was a significant increase in the level of 8-oxoguanine following exposure to 10 J/cm^2 UVA in affected adult, accounting for the pronounced photosensitivity seen in this subject. These results demonstrate the central role of selenoprotein in cellular antioxidant defence.

1573/F

CHD7 promotes transcription of ribosomal RNA: evidence for mechanistic overlap between CHARGE syndrome and disorders of ribosome biogenesis. *G.E. Zentner[1], E.A. Hurd[2], D.M. Martin[2,3], P.C. Scacheri[1,4].* 1) Department of Genetics, Case Western Reserve University, Cleveland, OH; 2) Department of Pediatrics, University of Michigan, Ann Arbor, MI; 3) Department of Human Genetics, University of Michigan, Ann Arbor, MI; 4) Case Comprehensive Cancer Center, Case Western Reserve University, Cleveland, OH.

De novo mutation of the *CHD7* gene, encoding a member of the chromodomain helicase DNA binding protein family, is responsible for approximately two-thirds of cases of CHARGE syndrome, a developmental disorder characterized by a complex constellation of birth defects. Recent studies have defined functions for CHD7 in enhancer-mediated transcriptional regulation and neural crest cell development. Despite these advances, very few genes have been found to be dysregulated by CHD7 haploinsufficiency, and it is not clear if their dysregulation contributes to the phenotypes of CHARGE syndrome. Here we report results of immunofluorescence, subcellular fractionation, and ChIP-seq experiments which show that CHD7 localizes to the nucleolus and associates with rDNA. Consistent with a role for CHD7 as an activator of rDNA transcription, transient overexpression of CHD7 increases the levels of 45S pre-rRNA, the primary transcript of rDNA that is ultimately processed into the mature 18S, 5.8S, and 28S rRNA species. Likewise, siRNA-mediated depletion of CHD7 results in hypermethylation of the rDNA promoter and a concomitant reduction in levels of 45S pre-rRNA. Depletion of CHD7 also reduces cell proliferation, a process linked to rRNA synthesis which is defective in the olfactory epithelium of Chd7 mutant mouse embryos. Consistent with this embryonic olfactory proliferation defect and further supporting a role for CHD7 in rRNA transcription, CHARGE-relevant tissues from Chd7 mutant mouse embryos show reduced levels of 45S pre-rRNA. These results raise the possibility that the pathogenesis of CHARGE syndrome shares common mechanisms with well-defined "ribopathies" such as Treacher Collins syndrome and Diamond-Blackfan anemia, which are caused by deficiencies in ribosome biogenesis.

1574/F

Tandem repeat sequences as causative cis eQTLs for protein-coding gene expression variation: the case of CSTB. *C. Borel[1], E. Migliavacca[1], E.T. Dermitzakis[1], M. Gagnebin[1], A.J. Sharp[1,2], S.E. Antonarakis[1].* 1) Dept Genetic Medicine, Univ Geneva Medical Sch, Geneva, Switzerland; 2) Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York.

Individual variation of gene expression contributes to phenotypic variability. Thus the understanding of the genetic control of gene expression is important to decipher the etiology of genetic traits and disorders. Genetic analysis in cells and tissues from different individuals has revealed both cis and trans eQTLs for many genes; cis eQTL effect is relatively strong since they have been discovered and replicated with relatively small sample sizes. Recent studies have revealed that the majority of the discovered eQTLs are tissue specific (Dimas et al. Science 2009), a finding compatible with the tissue specificity of the phenotypic characteristics of genetic disorders. The challenging question for each eQTL is to identify the functional variant that controls the gene expression variation. We hypothesize that CNV of short sequence repeats in the human genome contributes to the gene expression variation of some genes. Our laboratory has previously identified that rare expansions of a dodecamer repeat (CG4C G4CG) in the promoter region of the CSTB gene on chromosome 21q lead to silencing of the gene, resulting in progressive myoclonus epilepsy (Lalioti et al Nature 1997). The majority of alleles in the human population contain either 2 or 3 copies of this dodecamer repeat. Since the large expansion to more than 200 repeat copies results in silencing of the CSTB gene, we hypothesized that the common 2 or 3 copy variation may be a causative cis-eQTL for CSTB expression variation. We used PCR to genotype the repeat length and quantified CSTB expression by TaqMan qRT-PCR in 170 lymphoblast and fibroblast samples from the GenCord collection. Although there is considerable variation in expression of this gene in the normal population, we observed that in lymphoblasts repeat length is strongly correlated with CSTB expression (p=3x10-11), with individuals homozygous for the 3-repeat allele showing ~2-fold higher expression than individuals homozygous for the 2-repeat allele. In fibroblasts a weaker effect was observed (p=0.03), showing that this effect is cell-type specific. Examination of both genotyped and imputed SNPs within 1Mb of CSTB revealed none that were significantly correlated with CSTB expression. Therefore, the dodecamer repeat represents the strongest cis eQTL for CSTB in lymphoblasts, explaining 23% of expression variation. We conclude that polymorphic tandem repeats likely represent the causative variation of a fraction of eQTLs in the genome.

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The functional effects of *CCL3L1* copy number. *D. Carpenter, R.I. McIntosh, R. Pleass, J.A.L. Armour.* Genetics, University of Nottingham, Nottingham, Notts, United Kingdom.

Copy number variation (CNV) is a frequent form of variation throughout the human genome. It is now apparent that CNVs contribute to the variation observed between individuals and can influence both gene expression[1] and human disease progression.

CCL3L1 encodes the chemokine MIP-1α isoform LD78β which functions as a chemotactic mediator of the immune system, and is the most potent known ligand for the CCR5 receptor. Furthermore copy number variation of *CCL3L1* has been implicated in HIV progression and has the potential to influence other immune disorders.

This study examines the functional consequences of copy number variation of *CCL3L1*. Monocytes were isolated from 53 independent UK samples with no known clinical phenotypes and, after stimulation with LPS, secreted protein and cellular mRNA were isolated. Genomic DNA extracted from remaining leukocytes was used for determination of *CCL3L1* copy number by the paralogue ratio test (PRT)[2]. A time trial assessment was performed to optimise the time point for protein and mRNA collection.

Levels of MIP-1α protein were determined by sandwich ELISAs of the cell supernatants and real time PCR was used to determine transcripts levels of mRNA. The protein and mRNA results suggest that there is some influence of copy number on gene expression but only at higher copy numbers, and that there may be other factors influencing gene expression of *CCL3L1*.

1. Stanger et al. 2007. Science, 315; 848-853
2. Walker et al. 2009. Genomics, 93; 98-103.

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Transcriptional Regulation of ERBB3, a gene essential in neural crest development. *M.K. Prasad[1], J. Cronin[2], S.K. Loftus[2], W.J. Pavan[2], A.S. McCallion[1].* 1) Johns Hopkins University, Baltimore, MD; 2) National Institutes of Health, National Human Genome Research Institute, Bethesda, MD.

The neural crest (NC) is a transient multipotent population of cells in vertebrate development that give rise to a variety of cell types including peripheral neurons and glia, melanocytes, craniofacial skeleton and smooth muscle. The molecular pathways governing neural crest development are of immense interest due to the large number of neurocristopathies seen in patient populations. ERBB3 is one many genes that are essential for normal NC development. Studies in mouse knockouts and zebrafish mutants have established a role for ERBB3 in Schwann cell development. However, recent studies have also suggested a role for ERBB3 in melanocyte development. Although not required for their specification, it is required for later stages of development in culture. ERBB3 is also required for maintaining the proliferative and migratory properties of melanocyte precursors, and is activated in melanomas. In order to better understand the regulation of ERBB3 during melanocyte development and melanoma progression, we have begun a comprehensive analysis of the regulatory landscape at this locus. To date, we have tested the regulatory activity of eight evolutionarily conserved sequences in vitro in 2 human melanoma cell lines (UACC-1022 and BLA) and a mouse melanocyte cell line (melan-A). Five of eight sequences showed greater than 3-fold luciferase activity relative to a promoter only construct. We then tested regulatory activity of these sequences in vivo using a Tol2 based transgenic zebrafish assay. Preliminary results from mosaic embryos indicate that most (7/8) sequences have regulatory activity, of which two direct expression in a manner consistent with ERBB3 expression in peripheral glia and epidermal cells. We will describe the progress in our efforts confirming the regulatory activity of these and other sequences at ERBB3 in germline transmitted transgenic fish. Our long-term goal is to determine key factors involved in regulating the expression of these sequences in the melanocyte lineage.

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The role of the transcription factor TCF7L2 in type 2 diabetes. *J.L. Hall[1], L. Prokunina-Olsson[2], V. Weaver[1], J.A. Wood[3], B. LaCroix[1], N.T. Turner[1], M. Rolfe[1], D. Delaney[1], D. Bloom[1], M.K. Thomas[3].* 1) Department of Medicine, University of Minnesota, Minneapolis, MN; 2) Lab of Translational Genomics, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Bethesda MD; 3) Lab of Molecular Endocrinology, Massachusetts General Hospital, Boston, MA.

Genetic variants within the TCF7L2 gene have been identified as the strongest common risk factors for the development of type 2 diabetes (T2D). Using a systems biology approach, we identified a neuroendocrine specific isoform of TCF7L2 that was associated with the T2D risk alleles (Prokunina-Olsson et al, 2009). This unique neuroendocrine isoform was found in human thalamus, hypothalamus, occipital lobe, gut, pancreas, and pancreatic islets. RTQPCR and in situ hybridization confirmed the neuroendocrine expression pattern for this TCF7L2 isoform in mice. To further test the biological mechanisms through which TCF7L2 regulates metabolic homeostasis, we utilized the TCF7L2 heterozygous mouse model, as the TCF7L2 -/- mouse dies at birth. TCF7L2 heterozygous mice exhibited an impaired glucose tolerance phenotype on a low fat diet in response to an intraperitoneal glucose test ($n= 5$, $p < 0.015$). These findings align with a previously identified association in non-diabetic individuals harboring the TCF7L2 risk allele and impaired glucose tolerance. Circulating ghrelin levels were also decreased in the TCF7L2 heterozygous mice ($n = 3$, $p < 0.03$), similar to individuals harboring the TCF7L2 T2D risk allele. Expression of the neuroendocrine specific isoform of TCF7L2 was decreased in TCF7L2 heterozygous mice, and correlated with decreased ghrelin mRNA levels in the gut (n=4-5 TCF7L2 heterozyous and wild type mice per group, $p < 0.037$). The neuroendocrine specific TCF7L2 isoform also correlated with expression of additional metabolic regulation genes CART ($r = 0.45$, $n = 41$, $p <0.0032$) and glucagon ($r = -0.4$, $n=41$, $p <0.0096$) in the gut of TCF7L2 heterozygous and wild type mice. In addition, this TCF7L2 isoform correlated with expression of cell proliferation and survival regulators in the gut including cyclinD1 ($r = 0.5$, $n = 41$, $p < 0.00087$), bcl-2 ($r= 0.74$, $n = 41$, $p < 0.0001$), and c-myc ($r = 0.41$, $n=41$, $p < 0.0077$). In conclusion, TCF7L2 deficiency results in impaired glucose tolerance and decreased circulating ghrelin levels in mice. These findings are similar to humans with the T2D risk alleles. Moreover, loss of the neuroendocrine specific isoform of TCF7L2 is associated with decreased mRNA expression of ghrelin, cyclin D1, bcl-2, and c-myc in the gut. Taken together, these findings may have important implications for a newly identified isoform of TCF7L2 in regulating metabolism through a neuroendocrine specific axis.

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RFX6, a novel gene necessary for the β-cell phenotype. *N. Taleb, D. Levi, H. Zouk, C. Polychronakos.* Departments of Pediatrics and Human Genetics, McGill University, Montreal, Québec, Canada.

Introduction: Our group has recently identified RFX6 as a novel gene necessary for the development of the endocrine pancreas, using homozygosity mapping and high throughput sequencing on several probands with neonatal diabetes mellitus (Nature, Feb 2010). Interestingly, many family members, obligatory or potential heterozygous carriers of RFX6 mutations, have been diagnosed with diabetes (type 1, 2 or gestational, glucose intolerant). This, added to its sustained specific expression in the adult pancreatic islets, implies a role for RFX6 as a phenotype-maintaining gene in the insulin producing beta-cells. Methods and results: To examine the role of RFX6, a transcription factor, in the post-natal pancreas two different hypotheses have been tested. Hypothesis 1- RFX6 expression is modulated by glucose concentration. In vivo glucose stimulation effect on the expression of RFX6 was studied in C57 mice. We treated mice with intraperitoneal glucose (Dextrose 50%) injection at 3 g/kg body weight and euthanased them at one hour. In four different pairs, the expression of RFX6, tested by real-time PCR, significantly increased in the stimulated versus unstimulated mice (2.3 ± 0.5 fold increase, $p = 0.03$). Hypothesis 2- RFX6 determines insulin synthesis and/or release. We knocked RFX6 down in Min6 cells, an established mouse β-cell cell line model for glucose stimulated insulin secretion (GSIS) using si-RNA. A static glucose stimulation experiment (0.5 vs 25 mM Glucose for half an hour) was carried out at 48 hours post-transfection. Insulin peptide levels were measured by ELISA kit. GSIS percentage from baseline was significantly reduced in the knock-down samples compared to negative controls ($292.0\pm48.7\%$ vs $192.5\pm52.1\%$, $p=0.008$). There was no significant difference in the total insulin content between the two groups. RNA-microarray is generated from the knock-down cells and will be analysed for the effect of RFX6 as a transcription factor on other genes in beta-cells. Conclusion: According to our data, RFX6 plays a role in GSIS, a main function of beta-cells. More experiments are being carried to characterize the interaction of RFX6 with other genes. Our study will hopefully add to our genetic understanding of the function of beta-cells and the pathophysiology of diabetes mellitus.

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Individual variation in insulin response. *I.X. Wang[1,2], V.G. Cheung[1,2].* 1) Howard Hughes Medical Institute; 2) Departments of Pediatrics & Genetics, University of Pennsylvania.

Insulin sensitivity varies greatly among individuals, which underlies susceptibility to diseases including metabolic and polycystic ovary syndromes. In this study, we measured the extent of natural variation in insulin response, and investigated the molecular mechanisms of how human cells respond to insulin at the signal transduction and gene expression levels. Insulin induces intracellular kinase signaling that mediates diverse cellular processes, such as gene expression. Using primary fibroblasts from 35 unrelated and age-matched healthy individuals, we measured insulin-induced 1) phosphorylation of signaling factors; and 2) changes in gene expression. We identified signaling factors and nearly 3,000 genes (ANOVA, $p<10\text{-}6$) that are involved in insulin response. Signal transduction initiates insulin response pathways; however, despite their importance, the levels of induction of key factors including ERK and AKT vary greatly among individuals. For example, following insulin treatment, the phosphorylation of ERK1/2 differs by >20 fold among our subjects. We further showed that the variability of signal transduction is functional; it leads to variation in downstream gene expression. The level of ERK1/2 phosphorylation is correlated with induction of about 500 genes ($r>0.4$, $p<0.01$). We confirmed that these genes are targets of ERK by pharmacologic inhibition studies using U0126. Annotations of these ERK target genes showed that they are involved in primary metabolism and cell cycle regulation. In this presentation, I will describe results from our study and discuss the implications for insulin resistance and associated human diseases.

1580/F

Length of GT repeats in intron 2 of the JME-associated gene BRD2 affects alternative splicing. *Q. Cui[1], E. Shang[2], D. Greenberg[2, 3], D. Wolgemuth[1, 4, 5].* 1) Departments of Genetics and Development and Obstetrics and Gynecology, Columbia University Medical Center, NEW York, NY; 2) Division of Statistical Genetics, Department of Biostatistics, Mailman School of Public Health, Columbia University Medical Center, NEW York, NY; 3) Department of Psychiatry, Columbia University Medical Center and New York State Psychiatric Institute, NEW York, NY; 4) The Herbert Irving Comprehensive Cancer Center, Columbia University Medical Center, NEW York, NY; 5) The Institute of Human Nutrition, Columbia University Medical Center, New York, NY.

Juvenile Myoclonic Epilepsy (JME) is among the most common types of Idiopathic Generalized Epilepsy and has a complex genetic inheritance. Linkage and association analysis led us to the double- bromodomain-containing gene BRD2, which is implicated in a variety of cellular processes, including regulating gene expression. Our gene-targeting studies showed that homozygous Brd2-/- mice are embryonic lethal, importantly, with profound abnormalities in neural development. Heterozygous Brd2+/- mice exhibit enhanced sensitivity to chemically-induced seizures and abnormal neural architecture. We also discovered that intron 2 of BRD2 contains a highly conserved, alternatively spliced exon, which introduces a premature termination codon in the resulting mRNA. Further, we identified polymorphisms in the lengths of GT-repeats in intron 2 of BRD2 that are associated with JME. Variation in the length of GT-repeats has been shown to affect alternative splicing events in other experimental models. We now test the hypothesis that the polymorphisms in intron 2, in particular JME-associated variants in GT repeat-length identified in patient DNA, affect alternative exon splicing and the levels of the corresponding transcripts, and hence, the production of functional BRD2 products. We have generated 10 mini-gene constructs containing exon2-intron2-exon3 of BRD2, incorporating different GT dinucleotide repeat lengths (13, 4, 3, 2 and 1) in the presence or absence of GTAA (another polymorphism located 22 bp upstream of the GT repeats). These mini-genes were transiently transfected into 293T cells (embryonic kidney) and SH-SY5Y cells (neuroblastoma). Splicing activity was detected by RT-PCR analysis using primers that discriminate between the regularly spliced mRNA and the exon 2a-containing alternatively spliced mRNA. In 293T cells, shortening of the GT repeats significantly increased the proportion of the alternatively spliced BRD2 mRNA product. In SH-SY5Y cells, this phenomenon only exists in mini-genes lacking the GTAA. We therefore conclude that the length of the GT repeats in intron 2 of BRD2, which is polymorphic in the human population and associated with JME, affects the alternative splicing event, and hence, the production of functional BRD2 protein. The data suggest that aberrant expression of BRD2 in neuronal populations contributes to seizure susceptibility, likely through mechanisms involving haplo-insufficiency.

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Identification of a novel transcript of ARMS2 gene in human retina. *G. Wang[1], W.K. Scott[1], P. Whitehead[1], B.L. Court[1], S.G. Schwartz[3], J.L. Kovach[3], K.L. Spencer[2], A. Agarwal[4], J.L. Haines[2], M.A. Pericak-Vance[1].* 1) John P. Hussman Institute for Human Genomics, Dr. John T. Macdonald Foundation Department of Human Genetics, University of Miami Miller School of Medicine, Miami, FL; 2) Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 3) Bascom Palmer Eye Institute, university of Miami Miller School of Medicine, Miami, FL; 4) Department of Ophthalmology, Vanderbilt, Nashville, TN.

Variations in gene ARMS2 (Age-related macular degeneration susceptibility 2, also known as LOC387715, MIM 611313) at the chromosome 10q26 locus is strongly associated with risk of age-related macular degeneration (AMD). However, it remains unclear of what the basic function of ARMS2 is in human eye. We recently systematically analyzed ARMS2 transcription in human retina. We first performed 5'RACE (Rapid amplification of cDNA ends) and 3'RACE assays to characterize ARMS2 transcripts. The 5'RACE found that the transcription start site (TSS) of ARMS2 in human retina is 220bp upstream compared to the annotation in public database (NCBI GRCh37). And 3'RACE confirmed that the polyadenylation site in retina matches the annotation in the database (NCBI GRCh37). Whether the extended 5'UTR contains any local cis-acting regulatory elements need more studies. We validated this 5' end extended transcripts by RT-PCR and sequencing. We then conducted quantitative PCR to measure ARMS2 mRNA level in dissected retina and choroid/retinal pigment epithelium (RPE) samples. The preliminary data show that ARMS2 transcript is preferentially and significantly expressed in choroid/RPE compared to retina (fold change»11, P<0.05). In conclusion, the transcription start site of the ARMS2 gene in human eyes is 220bp upstream from the annotated TSS. ARMS2 is preferentially expressed in the choroid/RPE than in the retina, suggesting a potential function of ARMS2 in the RPE and choroid. The results of this study are critical to further analyze the function of ARMS2 in AMD pathogenesis in the future.

1582/F

Genetic variants and monoallelic expression of surfactant protein D in inflammatory bowel disease. *Z. Lin[1], G. John[1], J.P. Hegarty[1], A. Berg[2], W. Yu[1], Y. Wang[1,3], A. Kelly[1], B.Z. Peterson[3], L.S. Poritz[1,3], J. Floros[4,5], W.A. Koltun[1].* 1) Surgery, Pennsylvania State University College of Medicine, Hershey, PA; 2) Public Health Sciences, Pennsylvania State University College of Medicine, Hershey, PA; 3) Cellular and Molecular Physiology, Pennsylvania State University College of Medicine, Hershey, PA; 4) Pediatrics, Pennsylvania State University College of Medicine, Hershey, PA; 5) Obstetrics and Gynecology, Pennsylvania State University College of Medicine, Hershey, PA.

Surfactant protein-D (SP-D) belongs to the collagen-containing C-type (calcium dependent) lectins. It plays an important role in linking adaptive and innate immune cell functions in the first line of the host defense. SP-D primarily studied in the lung is widely distributed on mucosal surfaces of various tissues. It is most likely involved in extra-pulmonary host defenses. SP-D is expressed in the gastrointestinal tract, and SP-D polymorphisms have been associated to inflammatory bowel disease (IBD) recently. IBD comprised of two main forms of Crohn's disease (CD) and ulcerative colitis (UC) is a chronic and often debilitating disorder of the gastrointestinal tract. Although more than 32 genes have been associated with IBD, but only account for 20% of genetic risky factors. Here we studied the genetic association and allele expression of the two non-synonymous SP-D SNPs rs721917 (C/T Met11Thr) and rs2243639 (G/A Ala160Thr) in 256 IBD cases (123 CD and 133 UC) and 376 unrelated healthy individuals from an IBD population from Central PA. Case-control analysis revealed a significant association of rs2243639 with susceptibility to CD (OR=1.5531, 95% CI= 1.14992-2.0983, p=0.003570), but not UC (OR=1.0300, 95% CI=0.7645-1.3845, p= 0.8836), and no association of rs721917 with CD (p=0.328) or UC (p=0.218). Using intestinal tissues from 19 individuals heterozygous for each SNP, we compared allele expression of these two SNPs between diseased and matched normal tissues. The SNP rs2243639 exhibited balanced biallele expression while rs721917 exhibited a differential allele expression (balanced biallelic 37%, imbalanced biallelic 45%, and dominant monoallelic 18%). Monoallelic expression is widely observed in human, it was assessed that more than 5% of genes were subjected to random monoallelic expression. Monoallelic expression can lead to differences in expression level of gene and alleles. Different expression of mutated allele may affect gene function and phenotype outcome. Comparison of allelic expression pattern between diseased and matched normal tissues, 13 out 19 individuals (14 UC, 5 CD) showed a similar pattern. The six patients exhibiting a different pattern were all UC patients. From the present study we suggest that factors determining the incomplete penetrance of a genetic mutation should be addressed in genetic association studies, especially for complex human diseases such as IBD.

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Fine mapping and expression studies implicate *KIF5A* as the gene responsible for association of the 12q13 locus with Rheumatoid Arthritis. *A. McClure, S. Eyre, W. Thomson, J. Worthington, A. Barton, UKRAG Consortium.* Arthritis Research UK , The University of Manchester, Manchester, United Kingdom.

Background: Association of the chromosome 12q13 region with rheumatoid arthritis (RA) has now been confirmed in two independent studies and a meta analysis ($p=3.6x10^{-9}$). The associated variant is located in intron 15 of the *KIF5A* gene, a surprising candidate for RA as the expression of KIF5A has been reported to be confined to neurons.

Objective: The aim of this study was to investigate the 12q13 region further to identify the most likely RA susceptibility gene within the region.

Methods: Fine mapping of the 12q13 region surrounding the associated variant (~400kb) was carried out by genotyping tag SNPs, capturing all of the known variation across the region (r2>0.8) in 1076 RA cases and 890 controls. SNPs showing greater effect sizes than rs1678542 were then genotyped in a larger independent validation cohort consisting of 3633 RA cases and 2908 controls. The expression of KIF5A and two other candidate genes in the region (PIP4K2C and CYP27B1) was investigated by QRT-PCR. RNA was extracted from whole blood collected from 52 RA patients (genotype at rs1678542: major allele homozygous n=20, heterozygous n= 19, minor allele homozygous n=13) and cDNA was synthesised.

Results: Fine-mapping identified 5 SNPs exhibiting greater effect sizes than rs1678542. However only rs1678542 was significantly associated (p= 0.004, OR 0.90, 95% CI 0.84-0.97) in the validation cohort. Expression studies confirm the expression of KIF5A in whole blood. Gene expression levels were then analysed according to genotype at the most associated SNP (rs1678542). Expression of KIF5A was significantly associated with genotype (p=0.001) whereas expression of two other candidate genes in the region was not.

Conclusion: We have confirmed that the strongest association with RA at 12q13 is tagged by rs1678542 in intron 15 of KIF5A. We have confirmed expression of KIF5A in whole blood of RA cases and have shown for the first time that expression of KIF5A, but not other genes in the region, is influenced by genotype at rs1678542. Although validation is required the findings suggest that KIF5A may be the RA susceptibility gene in the region as all other SNPs tagged by rs1678542 map within the gene.

1584/F

A novel role for hMSH5 (MutS Homolog 5) which localizes into mitochondria after oxidative stress and stimulates mitochondrial DNA repair. *S. Bannwarth[1,2], A. Figueroa[1,2], K. Fragaki[1,2], L. Destroismaisons[3], F. Lespinasse[1], M. Abbassi[1], F. Vandenbos[4], L.A. Pradelli[5], J-E. Ricci[5], A. Rötig[6], J-F. Michiels[4], S. de Vries[7], C. Vande Velde[3], V. Paquis-Flucklinger[1,2].* 1) LBPG, CNRS UMR 6267/Inserm U998/UNS, Medicine School, Nice; 2) Department of Medical School, CHU Nice, Nice; 3) Center of Excellence in Neuromics (CENUM), University of Montreal, Montreal; 4) Department of Neuropathology, CHU Nice, Nice; 5) Inserm, U895, C3M, Nice; 6) Inserm U781, Paris; 7) Division of Molecular Biology, The Netherlands Cancer Institute, Amsterdam.

MutS Homolog 5 (MSH5) does not participate in mismatch repair but is essential for meiotic recombination in germ cells. MSH5 and MSH4, another MutS homolog, form a heterodimeric complex that is likely involved in the processing and/or the stabilization of meiotic recombination intermediates. We report a novel function for human MSH5 that may have a fondamental role in protecting the mitochondrial genome (mtDNA) from oxidative damage. We show that hMSH5 is mainly associated with the outer mitochondrial membrane in basal conditions and that Msh5 deficiency causes respiratory chain defect in knock-out mice. However, the role of the MSH5 protein is particularly important under conditions of oxidative stress. Reactive oxygen species (ROS) production is decreased in rotenone-treated HeLa cells over-expressing *hMSH5* gene. And in response to DNA damage induced by H2O2, a fraction of hMSH5 localizes inside mitochondria and stimulates mtDNA repair. The hMSH5 protein physically interacts with mitochondrial helicase Twinkle and DNA polymerase gamma, two proteins involved in mtDNA replication and repair. Therefore, *hMSH5* is a new candidate gene for mitochondrial diseases linked to mtDNA instability. Furthermore, we observed subsarcolemmal accumulation of hMSH5 in cytochrome c oxidase-negative muscle fibers of patients carrying mtDNA deletions, suggesting that this protein may accumulate into mitochondria in response to mitochondrial dysfunction in vivo. Our data indicate a novel role for MSH5 in protecting mitochondrial genome by supporting mtDNA repair with polymerase γ and Twinkle and providing a mechanism to suppress ROS amplification vicious circle.

1585/F

Variants controlling expression of multiple genes in the insulin/IGF1 signaling pathway. *D. Hu, S. Huntsman, E. Ziv.* Dept Medicine, Institute for Human Genetics, Univ of California, San Francisco, San Francisco, CA.

The insulin/IGF1 signaling pathway is involved in the aging process in several model organisms. Common variants of genes in this pathway have been associated with human longevity in some studies. Studies of expression as a Quantitative Trait Locus (eQTL) may help identify genetic variations that control expression and may be candidates for association with more complex traits. We performed an association analysis for 35 genes in the insulin/IGF1 signaling pathway in order to identify loci affecting expressions of these genes. Gene expression and genotype data for 58 European samples were used from previously published studies (Cheung et al, Nature, vol 437, 2005). The association of a genetic variant with gene expression was tested by linear regression. The p value cutoffs for cis effect (+/- 5kb of a gene) and trans effect are 0.01 and 1E-7, respectively. Once we identified one transcript controlled by a SNP at a genome wide significance level, we evaluated significance for other transcripts using a permutation procedure. We found evidence for trans-regulation for IGF1, AKT1, IGFBP2, and IGFBP6. Among these trans effects, a genetic variant (rs10243691) for MAGI2 on chromosome 7 is associated with the expression of AKT1. A SNP (rs873078) for DOK5 (IRS6) on chromosome 20 is strongly associated with IGFBP2 (p<1E-10) and more weakly with IGFBP5 (p=0.009). Two SNPs (rs9470112 and rs11967333) that are associated with IGFBP6 (p<1E-9) also show association with IGF1 and PIK3CA (p=0.003 and p=0.001). SNPs rs651825, rs678957, rs11216795, and rs12282334 that are associated with KL (p<1E-7) might be associated with GH1 (p=0.002) and INSR (p=0.008). In addition to trans effects, we have observed weak evidence for cis-regulation for INSR, IRS1, PI3KR3, PI3KCB, and SHC1. Our results suggest that several loci, acting in trans, control multiple transcripts in the insulin/IGF-1 pathway and are good candidates for biological processes controlled by this pathway.

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An evaluation of Limp-2 in patients with Gaucher disease and myoclonic epilepsy. *N. Gupta, A. Velayati, JH. Choi, W. Westbrook, J. Depaolo, BK. Stubblefield, E. Sidransky, N. Tayebi.* Section on Molecular Neurogenetics, Medical Genetics Branch/NHGRI, NIH, Bethesda, MD.

Lysosomal integral membrane protein type 2 (LIMP-2) is responsible for the proper sorting of glucocerebrosidase, the enzyme deficient in Gaucher disease (GD), to the lysosome. Mutations in LIMP-2 were identified in patients with some forms of myoclonic epilepsy (ME). A subgroup of patients with neuronopathic Gaucher disease develop myoclonic epilepsy. We investigated whether development of ME in type 3 GD is related to alterations in Limp-2, studying two groups of patients with GD,13 patients with myoclonic epilepsy and 18 without myoclonic epilepsy, as well as 40 normal controls. The promoter region, all 12 exons and the flanking intronic regions of the Limp-2 gene (Scarb2) were sequenced in each of the patients and controls. One patient with GD/ME was found to have a heterozygous mutation in exon 12. Two other GD/ME patients had intronic mutations, although no effect on splicing was demonstrated using RT-PCR and cDNA sequencing. Furthermore, one polymorphism in exon 1 and 21 polymorphisms in introns were identified among patients and controls. Two polymorphisms are also present in the promoter region of Scarb2. The frequency of these polymorphic changes did not differ significantly between patients with GD with or without ME or controls. We also studied glucocerebrosidase and Limp-2 RNA expression in fibroblasts from patients with GD/ME, 20 with GD and 20 controls using real-time. The results demonstrated down-regulation of Limp-2 in the patient with the exonic mutation and up-regulation in individuals carrying an intronic mutation 1100bp upstream of exon 4 as compared to controls. Protein expression studies, cell-based assays and the confocal microscopy are being employed to evaluate the functional significance of the exonic and intronic mutations identified. These studies should clarify to what extent Limp-2 is associated with myoclonic epilepsy in patients with neuronopathic Gaucher disease.

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***Drosophila* deficient for porin provide a model to identify and dissect functions of VDAC.** *R. Masand, Z. Li, Y. Huang, B.H. Graham.* Department of Moleular and Human Genetics, Baylor College of Medicine, Houston, TX.

The voltage-dependent anion channel (VDAC or porin) is an integral membrane protein present in the mitochondrial outer membrane (MOM). VDACs are the predominant determinant of MOM permeability but also integrate mitochondrial function and other cellular pathways by interacting with various mitochondrial and cytoplasmic proteins; however, many details of these functions as well as the interacting pathways remain poorly understood. VDACs have also been implicated to play a pathogenic role in several human diseases including cancer, diabetes, Alzheimer's and cardiac ischemia-reperfusion injury. Flies mutant for *porin* (the predominant VDAC in *Drosophila*) demonstrate energy metabolism defects, neurologic dysfunction with abnormal mitochondrial distribution in motor neurons and male infertility. The ultimate goal of this project is to exploit the genetic versatility of *Drosophila* to dissect and identify VDAC functions and interacting pathways by identifying modifiers of *porin* mutant phenotypes using both candidate genes and unbiased genetic screens.

Aconitase activity analysis from isolated mutant mitochondria suggests that porin deficiency deficiency results in increased mitochondrial reactive oxygen species (ROS). A microarray analysis of gene expression has identified significant altered expression of a subset of cytochrome P450 genes in *porin* mutants. As cytochrome P450 monooxygenases can produce ROS through NADPH dependent oxidation reactions and as *porin* mutants have increased ROS,these P450 genes are potential candidate modifiers of *porin* mutant phenotypes. To evaluate their potential as genetic modifiers of *porin*,-cytochrome P450 genes with confirmed altered expression in *porin* mutants are being screened through systematic overexpression and knockdown in the *porin* mutant background to observe potential modifying effects on *porin* mutant phenotypes. In addition,a pilot deficiency screen has identified multiple deletions that suppress male infertility in *porin* mutants,including one deletion that also suppresses neuronal dysfunction. A chemical mutagenesis screen designed to identify suppressors of *porin*-deficient male infertility has been initiated with two confirmed suppressors recovered to date. Identification of suppressors of *porin* mutant phenotypes will provide insight into VDAC functions as well as identify potential novel therapeutic targets and strategies for multiple human diseases.

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Ribosomal stress and the p53 network: Impaired ribosome biogenesis due to ribosomal protein deficiency leads to p53 induction in zebrafish. *N. Kenmochi, A. Chakraborty, H. Torihara, T. Uechi.* Frontier Sci Res Ctr, Univ Miyazaki, Miyazaki, Japan.

Ribosomes, the molecular factories that carry out protein synthesis, are essential for every living cell and ribosomal proteins (RPs) play important roles in the formation of a functional ribosome. Recent findings indicate that disruption of ribosome biogenesis, induced either by decrease of an RP or mutations in rRNA processing factors, activates the p53 pathway presumably through a nucleolar stress. Studies in cell lines have identified several RPs as key mediators of this new ribosomal stress-p53 signaling pathway because of their ability to directly bind to MDM2, thus inhibiting the MDM2-p53 regulatory loop. RPL11 has been identified to be the most important among these MDM2-interacting RPs. To investigate the consequences of L11 depletion in vivo, and its physiological relevance to p53 activity, we knocked down the *rpl11* gene in zebrafish and analyzed the p53 response. Contrary to the cell line-based results, our data indicate that L11 deficiency activates the p53 pathway in zebrafish. The L11-deficient embryos (morphants) displayed morphological defects mainly in the developing brain and died within 6-7 days post-fertilization. TUNEL assay indicated that these developmental defects are due to increased cellular apoptosis, which was p53 dependent, as revealed by increased transcript level of *p53* and its down stream target genes in the morphants. We also observed upregulation of ccng1 (encoding cyclin G1), a p53-responsive gene involved in G1-S cell-cycle transition, in the morphants indicating that a cell-cycle arrest precedes the apoptotic pathway. In addition, *in situ* analysis revealed enhanced expression of *p53* and its target genes specifically in the head region of the morphants. The morphants displayed altered rRNA levels and upregulation of several ribosome-associated nucleolar proteins, indicating an impaired ribosome biogenesis. Interestingly, these nucleolar proteins were also enriched in the head region of the morphants that displayed morphological abnormalities and high levels of *p53* expression. These results suggest that ribosomal dysfunction due to the loss of L11 activates p53 to prevent improper embryonic development. Deficiency of any RP could lead to p53 induction, suggesting a general response of a cell against ribosomal stress.

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Interactive expressions of HtrA1 and VEGF in human vitreous and retinal pigment epithelial cells through a NFκB-related mechanism. *C.P. Pang[1], T.K. Ng[1], G.H.F. Yam[1], D.T.L. Liu[2], S.W.Y. Chiang[1], L.J. Chen[1], P.O.S. Tam[1], T.Y.Y. Lai[1].* 1) Department of Ophthalmology & Visual Sciences, The Chinese University of Hong Kong, Hong Kong, HKSAR, China; 2) Hong Kong Eye Hospital, Hong Kong, HKSAR, China.

Purpose: High temperature requirement factor A1 (HtrA1) is associated with exudative age-related macular degeneration (AMD), which is an angiogenic retinal disease related to vascular endothelial growth factor (VEGF) and a leading cause of serious visual impairment affecting the elder populations in developed countries. In this study, we investigated the interactions of HtrA1 and VEGF. **Methods:** The vitreous levels of HtrA1, VEGF and PEDF were determined in vitreous samples of 55 unrelated Chinese patients who underwent ocular surgeries. Expressions of HtrA1 and VEGF were studied interactively and under stress conditions in cultured human fetal retinal pigment epithelial (RPE) cells. **Results:** Significant association of vitreous humor levels of HtrA1 with VEGF was found (Pearson's correlation coefficient test; r = 0.650, $p = 7.91 \times 10^{-8}$). Vitreous levels of HtrA1 were higher in patients with retinal vascular and inflammatory diseases than those with traumatic injury ($p < 0.04$). However, neither HtrA1 nor VEGF regulated each other's expressions in the incremental analysis. Like HtrA2, HtrA1 was involved in cellular stress response, which also induced VEGFA expression. Meanwhile, HTRA1 expression was substantially reduced in the presence of MG132, a proteasome and NFκB inhibitor. **Conclusions:** Our results revealed an association between the vitreous humor levels of HtrA1 and VEGF. They are both involved in cellular stress response and modulated by a NFκB-related mechanism. There is therefore a direct role of HtrA1 in the pathogenesis of angiogenic diseases.

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Functional analysis of *BBS3* and *BBS3L*, a novel *BBS3* transcript, in syndromic and non-syndromic retinal degeneration in animal models and humans. *P.R. Pretorius[1, 2, 3], M.A. Aldahmesh[4], Q. Zhang[1, 3], C.C. Searby[1, 3], D.Y. Nishimura[1, 3], F.S. Alkuraya[4, 5, 6], E.M. Stone[3, 7], K. Rahmouni[8], D.C. Slusarski[2], V.C. Sheffield[1, 3].* 1) Dept Pediatrics, Univ Iowa, Iowa City, IA; 2) Dept Biology, University of Iowa, Iowa City, IA; 3) Howard Hughes Medical Institute; 4) Dept Genetics, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia; 5) Dept Pediatrics, King Khalid University Hospital and College of Medicine, King Saud University, Riyadh, Saudi Arabia; 6) Dept Anatomy and Cell Biology, College of Medicine, Alfaisal University, Riyadh, Saudi Arabia; 7) Dept Ophthalmology and Visual Sciences, University of Iowa, Iowa City, IA; 8) Dept Internal Medicine, University of Iowa, Iowa City, IA.

Bardet-Biedl syndrome (BBS) is a heterogeneous, autosomal recessive disorder characterized by retinal degeneration, obesity, learning disabilities, congenital anomalies, and an increased incidence of hypertension and diabetes. Seven BBS proteins form a stable complex known as the BBSome that promotes ciliary membrane elongation. Other BBS proteins have chaperonin homology and play a role in BBSome formation. BBS3, an ADP-ribosylation factor (ARF)-like small GTPase (ARL6), is not part of the BBSome complex. We identified a second longer transcript of *BBS3*, designated *BBS3L*, which is expressed primarily in the eye. To gain insight into BBS3 disease mechanisms, we created mouse and zebrafish models of both *BBS3* and *BBS3L*. *Bbs3*$^{-/-}$ mice have retinal degeneration, increased body fat, elevated blood pressure and severe hydrocephalus, whereas *Bbs3L*$^{-/-}$ mice have only a retina phenotype. These models were used to demonstrate that *BBS3L* is specifically required for retinal organization and function in both mouse and zebrafish. To extend this work to humans, genetic mapping and DNA sequencing identified a homozygous missense mutation (A89V) in *BBS3* that results in non-syndromic retinal degeneration. To evaluate the *in vivo* function of the A89V missense mutation in non-syndromic retinal degeneration and BBS, rescue experiments were performed in the zebrafish. Unlike wild-type *BBS3L* RNA, *BBS3L A89V* RNA does not rescue the vision defect seen with loss of *bbs3* in zebrafish; however, *BBS3 A89V* RNA is able to suppress the cardinal zebrafish BBS phenotype of melanosome transport, similar to wild-type *BBS3* RNA. These data demonstrate that the *BBS3L A89V* mutation identified in patients with non-syndromic retinal degeneration is critical and specific for the vision defect. To investigate the function of BBS3, we used *Bbs3*$^{-/-}$ mice to determine whether BBS3 is required for BBSome formation. Loss of Bbs3 does not affect BBSome protein levels or BBSome formation; however, loss of Bbs3 disrupts localization of BBSome proteins to cilia. Our results demonstrate that one of the functions of BBS3 is to target the BBSome to cilia, explaining the phenotypic similarity between Bbs3 knockout mice and BBSome subunit knockout mice. Phenotypic differences between patients can, in some instances, be explained by mutations affecting tissue specific BBS protein isoforms and tissue specific BBSome functions.

1591/F

The Role of SRY Mutations in the Etiology of Gonadal Dysgenesis in Patients with 45,X/46,XY Disorder of Sex Development and Variants. *M.Y. Nishi, E.M.F. Costa, S.B. Oliveira, B.B. Mendonca, S. Domenice.* Div Endocr. Lab Horm LIM42, Faculdade de Medicina da Univ Sao Paulo, Sao Paulo, Brazil.

Background- The potential involvement of SRY in abnormal gonadal development in 45,X/46,X,der(Y) patients was proposed following the identification of SRY mutations in a few patients with Turner syndrome (TS). However, its exact etiological role in gonadal dysgenesis in patients with Y chromosome mosaicisms has not yet been clarified. Aims- To screen for allelic variation in SRY in a large cohort of patients with disorders of sex development (DSD) due to chromosomal abnormalities with 45,X/46,X,der(Y) karyotype. Patients- 27 patients [14 with TS and 13 with mixed gonadal dysgenesis (MGD)] harboring 45,X/46,X,der(Y) karyotypes were selected. Methods- Genomic DNA was extracted from peripheral blood leukocytes of all patients and from gonadal tissue in four cases. SRY coding region was PCR-amplified and sequenced. Results- We identified only one polymorphism (c.561C>T) in a 45,X/46,XY MGD patient, which was detected in blood and in gonadal tissue. Conclusion- Our results indicate that mutations in SRY are rare findings in patients with Y chromosome mosaicisms. Therefore, a significant role of mutated SRY in the etiology of gonadal dysgenesis in patients harboring 45,X/46,XY karyotype and variants seems very unlikely.

1592/F

ATP7B variants in Wilson disease: impact on protein function. *J.R. Pon, G. Macintyre, L. Davies, D.W. Cox.* Department of Medical Genetics, University of Alberta, Edmonton, Alberta, Canada.

Wilson disease (WND) is an autosomal recessive disorder caused by mutations in *ATP7B*, a gene encoding an integral membrane copper transporting ATPase. Copper accumulation ultimately results in life-threatening liver and/or neurological impairment. Treatment is essential, and consists of copper chelation and inhibition of copper uptake. Mutation detection facilitates early diagnosis and treatment, reducing symptoms and the need for liver transplant. Since our discovery of the *ATP7B* gene in 1993, more than 590 potential WND-causing *ATP7B* variants have been identified worldwide (www.wilsondisease.med.ualberta.ca). However, due to incomplete gene sequencing and lack of population control data, some variants may be ethnic group specific SNPs or rare normal variants. Functional testing is necessary to confirm designation as disease-causing (DV). ATP7B contains several functional domains and DVs have been identified in each domain. We have tested eighteen variants, using a stable transfectant mammalian cell system. The tested variants are located within either the ATP binding domain for autophosphorylation (N-D) or the transmembrane domains (TM), required for membrane integration and copper transport. Full length *ATP7B* variant cDNAs were stably transfected into Chinese hamster ovary (CHO) cells. Wild type (WT) human ATP7B protects CHO cells from copper toxicity and each variant cell line was assayed for copper resistance. The five N-D variants exhibited reduced copper resistance: P1052L, G1149A, I1184T, V1262F, and P1273Q. Nine of thirteen TM variants tested were also impaired: G710S, A727D, A874V, R969Q, A971V, L1305P, Y1331S, I1336T and L1371P. The deficits of G1149A and L1371P were severe. The remaining TM variants, P840L, D918N, R919G and T974M, exhibited normal copper resistance. In response to excess copper, WT ATP7B is trafficked in copper-loaded vesicles for copper extrusion at the plasma membrane. Using immunofluorescent microscopy, trafficking defects were observed for P840L, P1052L, G1149A, I1184T, V1262F, L1305P, I1336T, and L1371P. On Western blots, variants with trafficking defects showed reduced ATP7B levels. T974M, D918N and R919G confer WT ATP7B protection and traffic normally, and are designated suspected non-disease causing variants. All other tested variants likely contribute to the WND phenotype. Our studies aid in the designation of variant status and enhance our understanding of ATP7B function.

1593/F

Functional impact of the RAI1 missense and polyQ length mutations identified in Smith-Magenis syndrome patients. *W. Gu[1], M. Heney[1], W. Bi[1], J.R. Lupski[1, 2, 3].* 1) Molecular and Human Genetics, Baylor College Med, Houston, TX; 2) Department of Pediatrics, Baylor College Med, Houston, TX; 3) Texas Children's Hospital.

Genetic variations involving RAI1 (Retinoic acid induced gene 1), including copy number variation (CNV), single nucleotide variation (SNV) and trinucleotide expansion, have been associated with common disease traits. The decrease (1n) in gene dosage of RAI1 (per deletion or point mutation) causes Smith-Magenis syndrome (SMS, MIM 182290), characterized by mental retardation, circadian rhythm distortion, self-aggression and obesity. The increase (3n) of RAI1 dosage results in Potocki-Lupski syndrome (PTLS, MIM610883), which manifests autism. Furthermore, the length of a polyQ repeat in RAI1 protein is associated with phenotypic manifestation of ataxia and drug response in schizophrenia; abnormally increased poly Q length was also reported in one patient with SMS phenotypes. Little is known about the function of RAI1 protein and the pathomechanisms of RAI1 mutations except that RAI1 is a nuclear protein and can act as a transcriptional co-activator. So far, twelve heterozygous point mutations and one poly Q length variation in RAI1 have been reported in SMS patients who did not have a heterozygous deletion of 17p11.2. Nine of the twelve point mutations are frameshift or nonsense alleles, and thus likely loss of function mutations; the functional consequences of the three missense alleles and poly Q variation have not been examined prior to this study. We cloned the full length human RAI into the expression vector pEGFP-C (Clontech) and pCMV-BD (Stratagene). Similar to the mouse Rai1, human RAI1 is localized in the nucleus and it can activate transcription. The three disease-related missense mutations of RAI1 (c.4685A>G [Q1562R], c.5423G>A [S1808N] and c.3634A>G [G1212S]) and the 18Q (instead of 14Q in wild type allele) variant were engineered into the constructs using the Quick-change site-directed mutagenesis kit (Stratagene). These mutations do not change the nuclear localization of RAI1 but appear to affect its transcription activity.

1594/F

Molecular characterization of phenotypes associated with PEX7 deficiency: Rhizomelic Chondrodysplasia Punctata type 1 (RCDP1) and adult Refsum disease (ARD). *S. Jiralerspong[1], A. Moser[2], S. Steinberg[2], N. Braverman[1].* 1) Dept. of Human Genetics, McGill University-Montreal Childrens Hospital Research Institute, Montreal, Quebec, Canada; 2) Dept. of Neurogenetics and Neurology, Kennedy Krieger Institute, Johns Hopkins Med. Ctr., Baltimore, Maryland, USA.

RCDP1 is a peroxisome biogenesis disorder caused by defects in PEX7, the peroxisome receptor for PTS2 targeted matrix proteins: thiolase, phytanoyl-CoA hydroxylase (PhyH) and alkylglycerone-phosphate synthase (AGPS). PEX7 defects mislocalize these enzymes to the cytosol where they are inactive. The severity of the clinical phenotype directly correlates to tissue plasmalogen levels and therefore, amounts of AGPS transported into the peroxisome. We previously reported 3 phenotype groups, severe, intermediate and mild, based on residual plasmalogen levels. In the mild group, plasmalogens are near normal and the phenotype no longer resembles RCDP, but instead resembles ARD, due to a deficiency of PhyH. We used protein modeling to show that mutations predicted to disrupt the β-propeller organization of PEX7 were associated with severe phenotypes, whereas mutations with less structural impact were associated with milder phenotypes. In the present study, we evaluated amounts of AGPS, PhyH and PEX7 proteins in 24 RCDP1 fibroblast lines, covering all 3 phenotypic groups, by immunoblotting methods. PTS2 proteins undergo N-terminal peptide removal inside the peroxisome to generate a smaller protein distinguishable by size. We show that 30-60% of AGPS is correctly targeted inside the peroxisome in the mild patient group, whereas AGPS import appeared absent in the severe and intermediate groups. We suggest that AGPS import in the intermediate group is below the sensitivity of western analysis, but accounts for residual tissue plasmalogen levels in this group. For PhyH, 10-20% is imported into the peroxisome in both mild and intermediate groups, but negligible in the severe group. We found reduced amounts of PEX7 protein generated by the majority of alleles in each group. The highest recovery of AGPS and PhyH import occurred in an ARD patient, shown to express residual amounts of wild type PEX7 transcript from a 'leaky' splice site mutation. To determine if conformational correction of the PEX7 missense proteins could be encouraged by chemical chaperones, we treated the cell lines with trimethylamines but did not find any improvement in PTS2 protein import. We conclude that certain PEX7 missense alleles as well as residual amounts of wild type PEX7 can rescue the RCDP phenotype by restoring sufficient (≥30%) AGPS import. These same PEX7 alleles, however, although associated with some PhyH import, cannot prevent the development of ARD over time.

1595/F

***Rai1* haploinsufficiency causes reduced *Bdnf* expression resulting in hyperphagia, obesity, and altered fat distribution in mice and humans with no evidence of metabolic syndrome.** *S.H. Elsea[1,2], B. Burns[2], K. Schmidt[2], S.R. Williams[2], S. Kim[2], S. Girirajan[2,3].* 1) Department of Pediatrics, Virginia Commonwealth University, Richmond, VA; 2) Department of Human & Molecular Genetics, Virginia Commonwealth University, Richmond, VA; 3) Department of Genome Sciences, University of Washington, Seattle, WA.

Smith-Magenis syndrome (SMS) is a genetic disorder caused by haploinsufficiency of the *retinoic acid induced 1 gene* (*RAI1*). In addition to intellectual disabilities, behavioral abnormalities, and sleep disturbances, a majority of children with SMS also have significant early-onset obesity. To study the role of RAI1 in obesity, we investigated the growth and obesity phenotype in a mouse model haploinsufficient for *Rai1*. Data show that *Rai1*$^{+/-}$ mice are hyperphagic, have an impaired satiety response, and have altered abdominal and subcutaneous fat distribution, with *Rai1*$^{+/-}$ female mice having a higher proportion of abdominal fat when compared to wild type female mice. Expression analyses revealed that *Bdnf*, a gene previously associated with hyperphagia and obesity, is downregulated in the *Rai1*$^{+/-}$ mouse hypothalamus, and reporter studies that show RAI1 directly regulates the expression of *BDNF*. Even though the *Rai1*$^{+/-}$ mice are significantly obese, serum analyses do not reveal any evidence of metabolic syndrome. Supporting these findings, a caregiver survey revealed that even though a high incidence of abdominal obesity is observed in females with SMS, they did not exhibit a higher incidence of metabolic syndrome compared to that in the general population. We conclude that *Rai1* haploinsufficiency represents a single gene model of obesity with hyperphagia, abnormal fat distribution, and altered hypothalamic gene expression associated with satiety, food intake, behavior, and obesity. Linking RAI1 and BDNF provides a thorough understanding of the role of RAI1 in growth and obesity and insight into the complex pathogenicity of obesity, behavior, and sex-specific differences in adiposity.

1596/F

Looping Interactions and Anatomical Specificity of Distant *Bmp2* Enhancers. *E.M. Broeckelmann, S. Pregizer, D.P. Mortlock*. Vanderbilt University, Nashville, TN.

Bone Morphogenic Proteins (BMPs) are members of the TGF-β superfamily of secreted signaling molecules that are not only vital factors in pattern formation and morphogenesis during early embryonic development but also play critical roles in the maintenance of health throughout all stages of adult life. For example, both *Bmp2* and *Bmp4* are among top GWAS hits on colorectal cancer, and *Bmp2* polymorphisms have been implicated in the pathogenesis of bone disorders such as osteoporosis and osteoarthritis. The osteogenic properties of BMP2 have been studied extensively and are highlighted by the fact that *Bmp2* conditional knockout mice exhibit reduced bone density and suffer spontaneous fractures that are unable to heal, indicating that BMP2 is indispensable for the endogenous repair process in bone. We have previously shown that *Bmp2* gene expression is controlled by numerous distant cis-regulatory elements in a tissue- and time- specific manner. Specifically, transgenic analyses in mice have identified a 656bp evolutionarily conserved element (ECR1) 156kb downstream of the promoter, which functions as *Bmp2* enhancer in osteoblasts. However, while ECR1 drives robust transgene expression in most endochondral bones, it exhibits remarkable anatomical specificity in that it is inactive in cranial intramembranous bones, suggesting the existence of (an) additional osteoblast enhancer(s). In order to further elucidate the role of ECR1 in various osteoblast cell populations *in vivo*, we deleted ECR1 from a *Bmp2*-containing BAC transgene, whose expression is analyzed in transient transgenic mouse embryos. Preliminary data suggest that ECR1 is essential for transgene expression in all bones, although in isolation it is only sufficient to drive expression in a specific subset of osteoblasts. Further evidence for the involvement of multiple enhancers in osteoblast-specific *Bmp2* expression was obtained in a Chromosome Conformation Capture (3C) assay, which demonstrated direct looping interactions of the *Bmp2* promoter with not only ECR1 but also with two additional candidate loci in the large gene desert surrounding *Bmp2*. Ultimately, a detailed understanding of both the regulatory elements governing *Bmp2* expression in bone and the molecular mechanisms underlying their function will be essential for the development of new therapies for the treatment of various human bone disorders.

1597/F

Promoter-enhancer interaction is responsible for tissue specific expression of OTC, the disruption may cause the manifestation of ornithine carbamoyltransferase deficiency. *O. Luksan[1], M. Jirsa[1], M. Hrebicek[2], L. Dvorakova[2].* 1) Laboratory of Experimental Hepatology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic; 2) Institute of Inherited Metabolic Disorders, First Faculty of Medicine, Charles University in Prague, and General University Hospital in Prague, Czech Republic.

Ornithine carbamoyltransferase (OTC, EC 2.1.3.3) is a homotrimeric enzyme catalyzing the synthesis of citrulline in the second step of the urea cycle. The OTC is expressed exclusively in the liver and the gene is located on Xp21.1. OTC deficiency (OTCD; OMIM 311250) is the most common inherited urea cycle disorder manifesting in a spectrum of severity ranging from neonatal hyperammonemic coma followed by death to later onset of milder symptoms or asymptomatic course. The published data bring evidence that mutation analysis fails in 20-25% of cases. The reason for the disease manifestation in these cases may be a genetic defect in regulatory regions of the gene.

We predicted the human promoter and enhancer elements from homology with rat and mouse and performed functional studies of both regulatory regions using dual luciferase reporter assay. The transcriptional activity of the promoter and the promoter - enhancer interaction was tested in three human hepatocellular lines (Hep-G2, HuH-7, PLC/PRF/5) and in the human embryonic kidney cells HEK293. Functional studies showed that the presence of the promoter increases the expression of the reporter gene significantly, but with no difference in both liver and kidney cells. The interaction promoter - enhancer contributed to high tissue specific expression of *OTC* in the liver while no increase of luciferase expression was observed in the HEK293 cells. We studied the effect of a single nucleotide substitution c.-366A>G located in the promoter of a female patient with mild symtoms of OTCD. The promoter variation did not affect the function of the promoter alone but it disrupted the interaction of the promoter with the enhancer, which was observed as 50% decrease of the transcriptional activity.

Our data indicate that full transcriptional activity of human *OTC* promoter depends on an upstream enhancer. The promoter - enhancer interaction contribute to tissue specific expression of *OTC* in the liver and the disruption can lead to the manifestation of OTCD. Therefore the regulatory regions should be included in genetic testing of OTC deficiency.

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1598/F

Specific interactions of the Williams-Beuren syndrome-associated protein GTF2IRD1 suggest a role in chromatin reorganization. *S.J. Palmer[1], J. Widagdo[1], K.M. Taylor[1], S. Bontempo[2], P.W. Gunning[3], E.C. Hardeman[1].* 1) School of Medical Science, Neuromuscular and Regenerative Medicine Unit, University of New South Wales, Sydney, NSW, Australia; 2) Muscle Development Unit, Children's Medical Research Institute, Westmead, NSW, Australia; 3) School of Medical Sciences, Oncology Research Unit, University of New South Wales, Sydney, NSW, Australia.

Williams-Beuren syndrome (WBS) results from a hemizygous microdeletion within chromosome 7q11.23 involving 28 genes. Its features typically involve characteristic physical abnormalities and a set of cognitive and behavioural symptoms that are collectively called the Williams syndrome cognitive profile (WSCP). Haploinsufficiency of elastin (encoded by *ELN*) causes the supravalvular aortic stenosis. The remaining physical and neurological symptoms have not yet been attributed to specific genes, but it is assumed that they are due to haploinsufficiency for a subset of the remaining 27. Genotype/phenotype correlations in patients with smaller deletions have mapped the typical craniofacial dysmorphology and WSCP to a pair of evolutionarily-related DNA-binding proteins, GTF2IRD1 and GTF2I. We have generated *Gtf2ird1* knockout mouse lines by deletion of exon 2 and replacement with a LacZ reporter allowing us to map expression and examine the consequences of gene inactivation. These mice show developmental abnormalities and cognitive impairments that reflect aspects of the disease. In an effort to understand the underlying biochemistry of these features, we have developed studies that focus on GTF2IRD1 function. We have demonstrated that GTF2IRD1 undergoes post-translational modification through attachment of SUMO to identified lysine residues. SUMOylation is catalysed via interactions with the E3 SUMO ligase PIASX and the E2 ligase UBC9, which occurs at separate well-conserved domains of the protein. In addition, we have identified a series of nuclear proteins that are capable of interacting with GTF2IRD1 using yeast 2-hybrid screening. Several of these novel target proteins have been implicated in chromatin reorganization via histone methylation, acetylation or ubiquitination. We propose that GTF2IRD1 nucleates a complex of proteins that are targeted to specific regions of the genome in order to repress gene expression via specific modifications of the underlying histones. Homology between GTF2I and GTF2IRD1 and adjacent chromosomal locations of the encoding genes clearly indicate a common ancestral origin. Therefore, it is highly probable that they share functional similarities. We are currently investigating whether this includes sharing similar protein-protein interactions.

1599/F

In utero knockdown of FlnA leads to periventricular nodular heterotopia in rats. *A. Carabalona[1,2], S. Beguin[1,2], E. Pallesi-Pocachard[1,2], C. Quere[4], A. Allain[4], E. Buhler[2,3], N. Dagoneau[4], I. Coupry[4], A. Gelot[5], C. Goizet[4], A. Represa[1,2], C. Cardoso[1,2].* 1) INMED, INSERM, Faculté des Sciences de Luminy, Marseille, France; 2) University de la Mediterranee, Marseille, France; 3) INSERM, Plateforme Postgenomics-INMED, Marseille, France; 4) Laboratoire de Génétique Humaine, Université Victor Segalen Bordeaux 2, Bordeaux, France; 5) Département de Neuropathologie, Service d'Anatomie et Cytologie Pathologiques, Hopital A. Trousseau, Paris, France.

Cortical malformations are important causes of mental retardation and account for 20-40% of drug-resistant epilepsy in childhood. The advancement of high-resolution imaging has facilitated the in vivo identification of a large number of brain malformation phenotypes. Periventricular Nodular Heterotopia (PNH) is one of these cortical malformations caused by defective neuronal migration resulting in abnormal positioning of post-mitotic neurons. These ectopic neurons remain in the periventricular zone, close to their birth place, and form nodules of heterotopic grey matter. Clinical manifestations range from asymptomatic to intractable epilepsy and mental retardation. To date, two genes have been identified to cause PNH. Mutations in FLNA (Xq28) and ARFGEF2 (20q13) are responsible for X-linked bilateral PNH (Fox et al. 1998) and a rare autosomal recessive form of PNH is associated with microcephaly (Sheen et al. 2004). Although the link between PNH and epilepsy has been well established, the physio-pathological mechanism responsible for epileptogenesis remains obscure. In addition, the limited availability of human samples and the lack of genetic animal models that mimic PNH have both delayed our understanding of the underlying pathological mechanism. Thus, It is unclear whether the epileptogenic foci involve ectopic neurons or normotopic cortex or both. To address this issue, we have developed an animal model that reproduces a FlnA gene-driven PNH phenotype. Ectopic masses of grey matter lining the lateral ventricle are obtained by knocking-down FlnA in the rat developing neocortex using in utero RNA interference. The reliability of this genetic model in reproducing a PNH phenotype allows us, to study nodular formation and intrinsic cellular organization during development.

1600/F

Disruptions of spatacsin and/or spastizin functions lead to motor neuron axonal outgrowth defects in the Danio rerio. *E. Martin, C. Yanicostas, A. Maouedj, A. Brice, N. Soussi-Yanicostas, G. Stevanin.* CR-icm, INSERM / UPMC UMR_S 975, NEB, Paris, France.

The hereditary spastic paraplegias (HSP) are characterized by the degeneration of the cortico-spinal tracts leading to a progressive spasticity of lower limbs. A common autosomal recessive HSP associates various additional signs to this spasticity: mental retardation and/or cognitive deficits, neuropathy, cerebellar ataxia and an abnormal brain MRI with thin corpus callosum (TCC) and white matter hyperintensities. Two genes, SPG11 and SPG15, coding for the spatacsin and the spastizin proteins, respectively, have been identified as responsible for the majority of these complex HSPs. All, except one, mutations found in both genes result in an abnormally truncated protein, suggesting that both diseases are due to loss of protein functions. Furthermore, SPG11 and SPG15 have similar expression profiles, shown by in situ hybridization in adult rat brain and an identical associated phenotype, suggesting that both proteins are involved in a common pathway. However, the spatacsin and spastizin functions are still unknown. Here we used the Danio rerio (zebrafish) to explore these functions during development. We first demonstrated that the expression profiles of both genes established by RT-PCR and in situ hybridization are similar and that their mRNA are ubiquitously expressed from the first developmental stages to adulthood but at a higher levels in the brain. We then used a morpholino-based gene knockdown approach. The down regulation of spatacsin or spastizin resulted in a frequent curly tail phenotype in injected embryos leading to reduced or abolished motility of morphant animals. Morpholino injections in zebrafish strains expressing GFP in motorneurons or oligodendrocytes demonstrated that the phenotype was due to aberrant motor neuron axonal outgrowth. To test whether the spatacsin and spastizin proteins are involved in a common pathway, we co-injected SPG11 and SPG15 morpholino oligonucleotides at doses that did not markedly perturb locomotor activity and tail phenotype when either morpholino was injected individually. The co-injected embryos presented with curly tails and reduced locomotor activity. Together, these data suggest that SPG11 and SPG15 are acting in a common pathway involved in development of the spinal motor neurons. These models should help us to decipher both spatacsin and spastizin functions and may represent powerful tools for therapeutic trials.

1601/F

Functional characterization of a conserved intronic element in hypertension- associated gene, STK39. *J. Chen, S. Dorff, Y. Chang.* Division of Endocrinology, U. of Maryland, SOM, Baltimore, MD.

The WNK-SPAK-NCC signaling pathway has recently been appreciated for its essential role in maintaining ion and osmotic homeostasis, as well as blood pressure (BP). SPAK is a key kinase in this signaling pathway, responding to activation by upstream WNK kinases and activating downstream cation-chloride cotransporters. Using a genome-wide association scan approach, we previously reported that common variants in STK39, the gene encoding SPAK, are associated with baseline BP. The most promising functional candidate among the BP-associated SNPs in STK39 is located in an intronic conserved element, CE5. In this study, we examined the potential role of CE5 in regulating SPAK expression. We demonstrate that CE5 binds nuclear protein(s) from multiple cell-lines in an allele-specific manner. Mass Spectrometry sequencing of these proteins revealed that multiple components of the splicing machinery interact either directly or indirectly with CE5. How CE5 functions in splicing was tested by insertion of CE5 into the second intron of human beta-globin minigene. We found a novel alternate splice form of mRNA in which CE5 is partially integrated into the mature mRNA. This novel mRNA represents approximately 15% of total mature mRNA, with an increase in abundance when additional splicing regulatory elements are present in the same intron. Tissue-specific alternate splicing of STK39 has been previously reported, although the underlying regulatory mechanism has not been identified. Studies to determine if CE5 is involved in tissue-specific splicing of SPAK in vivo, and if this function is altered by the sequence variant associated with higher BP, are underway to provide insight on how SPAK expression can influence BP.

1602/F

Frequency and function of a common IgE heavy chain (IGHE) variant. *J.M. Reynolds[1], D.B. Lloyd[2], K.G. Spink[3], J.F. Zobel[4], G.I. Johnston[5].* 1) DNA and BioFluids RCoE , Pfizer, Inc., Groton, CT, USA; 2) CVMED Research Unit, Pfizer, Inc., Groton, CT, USA; 3) Research Enabling Group, Pfizer, Inc., Sandwich, Kent, UK; 4) Bioprocess Analytic, Pfizer, Inc., Chesterfiled, MO, USA; 5) Molecular Medicine, Pfizer, Inc., Sandwich, Kent, UK.

Amino acid changing polymorphisms have the potential to alter the structure and function of a protein as well as influence sensitivity to targeted therapies. In the quest for the treatment of severe asthma, blocking the activity of IgE using a monoclonal antibody has been identified as a targeted therapy. A search of the public data revealed several SNPs located in the heavy chain of IgE (IGHE). However, when the IGHE sequence was aligned to one of it's pseudogenes, IGHEP1, it was observed that most of the putative SNPs were located at a position of sequence disagreement between IGHE and IGHEP1. To determine the true variation in the CH3 and CH4 domains of IGHE , we resequenced these regions in DNA from an ethnically diverse panel of 102 individuals using primers specific for IGHE. Our findings suggest that many of the amino acid changing SNPs for these regions reported in public databases were misidentified due to co-amplification of non-conserved alleles of IGHE and IGHEP1. In addition, we have identified novel amino acid changing SNPs not previously reported. The most common of these SNPs was analyzed for its ability to be bound by a marketed IgE therapy and it was found to have binding properties similar to wild-type IgE. Despite this observation, development of other novel antibodies should be tested against this variant to avoid finding a potential pharmacogenetic effect in the clinic.

1603/F

SplicePort: Analysis of Genetic Variation Affecting the Splicing of Precursors to Messenger RNA. *S. Mount[1, 2].* 1) Dept. Cell Biology and Molecular Genetics, University of Maryland, College Park, MD; 2) Center for Bioinformatics and Compuational Biology, University of Maryland, College Park, MD.

SplicePort (see spliceport.org) is a splice-site analysis tool that makes splice-site predictions for submitted sequences, and which allows browsing of predictive signals and motif exploration. The SplicePort algorithm is based on a feature generation algorithm that produces accurate splice site prediction on human pre-mRNA sequence data using predictive features selected from a large space of sequence based features (1). We are using SplicePort to systematically examine human sequence variants, primarily single nucleotide polymorphisms, for their effect, or potential effect, on the splicing of precursors to messenger RNA. The goal of this analysis is to assess the contribution of these variants to genetic disease susceptibility. SplicePort score changes due to sequence variants of potential significance are compared with the distribution of score changes associated with high frequency variants. Preliminary data indicate that a surprising fraction of minor alleles at polymorphic sites have the potential to affect splicing. Candidate variants will be assessed experimentally by reverse transcription, polymerase chain reaction and sequencing, or by mining publicly available human RNA sequence data. 1. Dogan RI, L Getoor, WJ Wilbur and SM Mount. 2007. SplicePort -- An interactive splice site analysis tool. Nucleic Acids Research. PMID 17576680.

1604/F

Expression of chitin binding domain of mouse chitinase in Escherichia coli and characterization of its properties. *A. Kashimura, M. Kojima, M. Sakaguchi, Y. Sugahara, F. Oyama.* Applied Chemistr, Faculty of Engineering, Kogakuin University, Hachioji, Tokyo, Japan.

Chitinases hydrolyze chitin, a polymer of N-acetyl-D-glucosamine, which is present in a wide range of organisms, including fungi, insects, and parasites. Although chitin is not present in mammals, chitinase genes were found in both human and mouse genomes. It has been known that chitinase activity is significantly increased in plasma from patients with Gaucher disease, an autosomal recessive lysosomal storage disorder. Recent researches have shown that elevated expression of chitinases is closely associated with progression of allergy and asthma. The pathophysiological role for the mammalian chitinases remains unproven. To understand how chitinase interacts with chitin and their derivatives, we expressed the chitin binding domain (CBD, the C-terminal ~80 amino acids) of chitinase in Escherichia coli as a fusion protein. The recombinant fusion protein with the CBD domain bound to chitin beads, whereas fusion protein without it did not, indicating that the recombinant CBD bound to chitin. Thus, this recombinant protein can be used to elucidate detailed biological functions of the chitinases.

1605/F

Molecular Genetics Study on HCMV Clinical Isolates from Guangzhou: the Gene Structure and Function of UL136 and UL145. *B. Wang, JJ. Hu, HH. Su, JC. Ding, YY. Guo, CA. Zhao, XZ. Zhang.* Paediatrics Dept., Maternal and Children's Hospital of Guangdong, Guangzhou, Guangdong, China.

BACKGROUND Human cytomegalovirus (HCMV) is the most important cause of congenital infection. It will develop to neonate anomaly, deafness, mental retardation and hydrocephalus. When it occur to immunosuppression children or adults, will lead to severe disease. The clinical low-passage isolate which is capable of pathogenesis, comparing with avirulent high-passage laboratory strain, it contains the UL/b' area. This unique structure, including at least 19 open reading frames (ORF): UL133 to UL151, is supposed to be a crucial area for HCMV pathogenicity. Now we know litter about two of the ORFs, UL145 and UL136. PURPOSE To investigate the genetic characterization and gene function of human cytomegalovirus UL145 and UL136 genes in low passage clinical isolates from Guangzhou China. METHOD The clinical isolates of HCMV were segregated from the urine sample collected from those infants with intra-uterus HCMV infection from Guangzhou. Using Multiplex PCR, we identify HCMV. After cloning UL145 and UL136 genes, amplifying the genes and sequencing, we analyzed its genetic characterization. Finally, we predict their post-translation modified site entering ProtParam web site. RESULT Two strains of low-passage isolate have been obtained. The completed sequence of UL145 and UL136 gene have submitted to GenBank, and gained GenBank accessions (No.: DQ180367, DQ180381, DQ180377, DQ180389). The aberration rate of DNA and amino acid sequences of UL145 is 0.7-1.5% and 0.7-2.2% respectively, and the UL136 is 2.9% and 1.7-3.6% respectively. And UL145 is predicted to have 1 PKC phosphorylation site, 2 CK II phosphorylation site and a zinc finger analogic structure, and is identified has mRNA expression. UL136 is predicted to exist PKC phosphorylation site, N-Myristoylation site, cAMP- and cGMP-dependent protein kinase phosphorylation site and Tyrosine kinaseII phosphorylation site. CONCLUSION The DNA and deduced amino acid sequences of the two genes show great conservation, regardless its polymorphism. UL145 protein is predicted to be a intranuclear cell factor. While UL136 protein is predicted to act as a factor related to membrane receptor mediated signal transduction.

1606/F

The Fanconi anemia pathway regulates telomeric recombination and telomere DNA synthesis in ALT-immortalized human cells. *M. Komosa[1, 2], H.A. Root[1, 2], A. Larsen[1, 3], D. Bazett-Jones[1, 3], M.S. Meyn[1,2,4].* 1) Program in Genetics & Genome Biology, Hospital for Sick Children, Toronto, ON, Canada; 2) Dept of Molecular Genetics, University of Toronto, Toronto, ON, Canada; 3) Dept of Biochemistry, University of Toronto, Toronto, ON, Canada; 4) Dept of Paediatrics, University of Toronto, Toronto, ON, Canada.

Fanconi anemia (FA) is an inherited disorder characterized by bone marrow failure, congenital malformations and cancer. A core complex of FA proteins is required to promote FANCD2 monoubiquitination and foci formation during S-phase and in response to DNA damage. Other FA proteins, including the FANCJ ATP-dependent DNA helicase, function independently of FANCD2. To understand the role(s) of FA proteins in DNA replication and recombination, we study the involvement of FA proteins in ALT, the recombination-dependent telomere maintenance pathway used by telomerase-negative immortalized human cells. We find that FANCD2, FA core complex proteins, and FANCJ form nuclear foci that colocalize with telomeric foci in ALT-dependent, but not telomerase-positive or primary cells. FA protein colocalization with telomeric DNA occurs primarily within ALT-associated PML bodies (APBs). Electron spectroscopic imaging of APBs suggests that they contain extrachromosomal telomeric repeat DNA (ECTR) and that FANCD2 depletion leads to chromatin intrusions within APBs that may represent dysfunctional telomeres. FANCD2 depletion also results in ALT-specific increases in ECTR DNA synthesis, telomere dysfunction induced foci, telomere entanglements, and recombination events between sister telomeres, as well as decreased cell viability. We previously found that telomere abnormalities in FANCD2-depleted ALT cells occur via a BLM-dependent, RAD51-independent mechanism. We now report co-depletion of BLM with FANCD2 partially rescues cell viability, suggesting that the induced telomere abnormalities trigger increased cell death. In addition, telomeres in ALT, but not telomerase-positive cells, can display a FISH staining pattern characteristic of expressed fragile sites. FANCD2 depletion results in an ALT-specific increase in “fragile” telomeres, suggesting that FANCD2-associated telomere abnormalities arise during replication of ALT telomeric DNA. In contrast to FANCD2, depletion of FANCJ results in a significant decrease in the number and intensity of detectable foci of telomeric DNA, telomeric binding proteins, and BRCA1. Additionally, co-depletion of FANCJ with FANCD2 partially suppresses amplification of ECTR DNA in FANCD2-depleted cells. Together our results suggest regulation of ALT telomeric DNA replication/recombination by the FA pathway is complex, with FANCJ and BLM required to promote replication/recombination, while FANCD2 and the core complex restrain these events.

1607/F

***Gtf2ird1*$^{-/-}$ mice do not show genotype-specific changes in gene expression during brain development.** *J. O'Leary, L.R. Osborne.* Departments of Molecular Genetics and Medicine, University of Toronto, Toronto, ON, Canada.

Williams-Beuren Syndrome (WBS) is an autosomal dominant neurodevelopmental disorder caused by hemizygous deletion of a 1.5 Mb region on chromosome 7q11.23. The clinical symptoms are numerous and include behavioural and cognitive components. One of the deleted genes, *GTF2IRD1*, shows widespread expression during development and genotype-phenotype studies in patients with atypical deletions of 7q11.23 implicate this gene in the neurological features of WBS. *Gtf2ird1*-targeted mice have features consistent with the WBS phenotype, namely reduced innate fear and increased sociability so to identify neural targets of GTF2IRD1, microarray analyses were performed comparing gene expression in the whole brains of *Gtf2ird1*$^{-/-}$ and wildtype (WT) mice at embryonic day 15.5 and at birth. Overall, the changes in gene expression in the mutant mice were not striking, with most changes falling in the range of 0.3 to 2 fold. Real-Time PCR was used to verify the expression levels of candidate genes identified as altered in the *Gtf2ird1*$^{-/-}$ mice and examination of verified genes revealed that they were all located on chromosome 5, within 50 Mb of *Gtf2ird1*. Since gene targeting may have affected local gene expression, we used siRNA to knock-down *Gtf2ird1* in two mouse neuronal cell lines (Neuro2A and N1E-115) and analyzed the genes identified from the microarray, but none were altered. The initial targeting of the Gtf2ird1 locus was done in R1 ES cells, which are derived from 129 strains, and the mice were backcrossed onto CD1. As the region around the targeted locus will retain a 129 genotype, we hypothesized that the gene expression differences in the *Gtf2ird1*$^{-/-}$ mice were actually the result of differential expression between mouse strains. Expression of the altered genes in *Gtf2ird1*$^{-/-}$ mice was found to be the same as in WT 129S1/SvImJ mice, and significantly different from CD1 WT mice. In addition, analysis of strain-specific SNPs within genes that showed altered expression, demonstrated that the *Gtf2ird1*$^{-/-}$ mice were homozygous for 129 alleles within this region of chromosome 5. In summary, we have been unable to find any in vivo neuronal targets of this putative transcription factor, despite its widespread and robust expression in the developing rodent brain. It seems likely, therefore, that GTF2IRD1, like its protein family member GTF2I which regulates calcium entry, may have an alternate role in the cells of the developing brain.

1608/F

The C-Terminus of P/Q type Voltage Gated Calcium Channel Alpha1A subunit (Cav2.1) modulates the transcription of other neuronal genes. *L. Veneziano[1], E. Mantuano[1], G. Maresca[1], I. Arisi[2], R. Brandi[2], M. D'Onofrio[2], P. Imbrici[3], M. Frontali[1], A. Felsani[1].* 1) Institute of Neurobiology and Molecular Medicine, National Council of Research. Rome, Italy; 2) European Brain Research Institute (EBRI). Rome, Italy; 3) Department of Internal Medicine. Section of Human Physiology. University of Perugia, Italy.

The CACNA1A gene on chromosome 19p13 encodes the α1A subunit of P/Q type voltage-gated Ca2+ channels (Cav2.1) expressed mainly in the cerebellum (particularly in cerebellar Purkinje and granule cells) and (presynaptically) at the neuromuscular junction. Mutations in this gene are responsible for three rare autosomal dominant neurological disorders: Episodic Ataxia type 2 (EA2), Familial Hemiplegic Migraine type 1 (FHM1) and Spinocerebellar Ataxia type 6 (SCA6) due to the expansion of a CAG repeat at the 3'end of the gene. The Cav2.1 α1A subunit is a four domain transmembrane protein of about 280KDa with cytoplasmic N- and C-terminal tails. The cytoplasmic C-terminus plays regulatory roles in the gating and trafficking of many ion channels. The C-terminus of the Cav2.1 α1A subunit, which represent the 25% of the entire protein (about 700 aminoacids) and contains the CAG repeat expanded in SCA6, plays a regulatory role in gating and trafficking and contains residues involved in channel inactivation and modulation induced by intracellular signalling proteins. Recently, the C-terminal tail was shown to be cleaved in vivo and conveyed into the nucleus, suggesting its involvement in regulating some transcriptional processes. In order to elucidate the role of the Cav2.1 C-terminal tail as possible transcription factor, gene expression arrays were carried out in HEK293 cell line wild type and over-expressing Cav2.1 C-terminal tail. Total RNA, extracted from these cells, was analyzed using Agilent 44K whole human genome oligo microarray system. Gene expression in HEK293 cells wild type and over-expressing Cav2.1 C-terminal tail was compared and for genes resulted up or down-regulated more than 5 folds, the expression profile was checked by using standard qRT-PCR analysis. These results, for the first time, provide evidence that Cav2.1 C-terminus, beside modulating channel activity, regulates the expression of genes important for the neuronal excitability such as gap junctions, ion channels, signalling proteins.

1609/F

TP63, TFAP2A, and ADH1C are potential transcriptional targets of interferon response factor 6 (IRF6). *D. Zemke, B. Schutte.* Michigan State University, East Lansing, MI.

Variation in the interferon response factor 6 (IRF6) gene has been shown to cause and contribute risk for cleft lip and palate. IRF6 is a member of the IRF family of transcription factors, however the genes regulated by IRF6 are currently unknown. In this study we used a candidate gene approach to identify genes that are direct transcriptional targets of IRF6. Potential target genes were selected based upon multiple criteria, including expression in relevant tissues, mutant phenotype resembling that of mice lacking Irf6, altered expression in tissues lacking Irf6, and the presence of an IRF6 DNA binding site. Additional candidates were selected based upon the results of genome wide association studies for nonsyndromic cleft lip and/or palate. Among the candidate genes, TP63, TFAP2A, and ADH1C were found to contain sequences bound by the IRF6 DNA binding domain in vitro, as determined by electrophoretic mobility shift assays. Since previous studies demonstrated that p63 and AP2α regulate expression of Irf6, our data suggests a potential transcriptional feedback loop between these transcription factors. The investigation of IRF6 and its targets may lead to a greater understanding of the mechanisms by which the palate is formed. In addition, the targets of IRF6 may be potential candidates for the cause of other clefting syndromes or isolated cleft of the lip and/or palate.

1610/F

A multi-species conserved sequence located 9.7 kb upstream of *IRF6* is sufficient to recapitulate endogenous expression in epidermis, limb and craniofacial tissues. *W.D. Fakhouri[1], L. Rhea[2], M.M. Dunnwald[2], B.C. Schutte[3].* 1) Microbiology and Molecular Gen, Michigan State University, East Lansing, MI; 2) The University of Iowa, Department of Pediatrics, Iowa City, Iowa; 3) Microbiology and Molecular Gen, Department of Pediatrics, Michigan State University, East Lansing, MI.

DNA variation in interferon regulatory factor 6 (*IRF6*) causes, and contributes risk for, orofacial clefting, including a common DNA variant (rs642961) that is located in MCS9.7, a multi-species conserved sequence (MCS) that is 9.7 kb upstream from the *IRF6* transcription start site. MCS9.7 possesses enhancer activity. We hypothesized that the MCS9.7 is sufficient to recapitulate the endogenous expression of *IRF6* during embryo development. We generated three independent stable transgenic lines of mice that contain a *lacZ* reporter gene under the control of MCS9.7. Expression of *lacZ* was determined at E9.5 to E17.5 of embryonic development using X-gal staining of whole embryos and of cryosectioned tissues. In E9.5 embryos, we detected *lacZ* expression in the periderm cells. At E10.5 and E11.5, *lacZ* was expressed in the epithelium of lateral and medial nasal prominences, in the epithelium of the maxillary and mandibular prominences, and in the apical ectodermal ridge of fore- and hind-limbs. At E13.5 and E14.5, *lacZ* expression was detected in the nasal pit, hair follicles, eyelid, corneal ectoderm, epithelial tongue, incisor tooth germ, submandibular gland and secondary palatal shelves. OCT transverse sections showed that *lacZ* is also expressed in the medulla and shaft of hair follicles and the sebaceous glands. In E17.5 skin, MCS9.7 was expressed in the spinous, granular, and cornified layers of the epidermis. In adult ear and footpad, we observed *lacZ* expression in the granular and cornified layers of the epidermis, and occasionally in spinous cells. A similar pattern was also observed in the footpad. In both the tongue and the palate, *lacZ* expression was detected in the suprabasal layers, however, only in patches. In conclusion, our results indicate that MSC9.7 element is sufficient to recapitulate endogenous expression of IRF6 in epidermis, limb and craniofacial tissues during embryonic development between E9.5-E17.5. However, we also observed *lacZ* expression in the neuroepithelium of hindbrain, the 3rd branchial arch, the somites and corneal ectoderm. Further studies are needed to determine whether endogenous *IRF6* is expressed in these latter tissues.

1611/F

Characterization of a LINE-1 retrotransposition intermediate. *P. Mandal, J. Goodier, H. Kazazian.* Gen, Univ Pennsylvania, Philadelphia, PA.

Long Interspersed Nucleotide Element-1s (LINEs1 or L1s) are the most active human retrotransposons, occupying, 17% of the human genome. Although most L1s are not able to retrotranspose, about 100 remain potentially active. A potentially active L1 is 6.0 kb in length and contains a 5' UTR, two open reading frames (ORF1 and ORF2) and a 3' UTR. ORF1 encodes a 40 kDa nucleic acid binding protein, whereas ORF2 encodes a 150 kDa protein with endonuclease (EN) and reverse transcriptase (RT) activities. Although the function of the ORFs is not completely understood, both are critical for the retrotransposition process. A model of L1 retrotransposition proposes that L1 ORF1p and ORF2p bind in cis with L1 RNA and form an L1 ribonucleoprotein particle (L1-RNP) in the cytoplasm. The L1-RNP then enters the nucleus and finds its target site where the L1 RNA is converted to a new genomic DNA copy. Previous studies to characterize human L1-RNPs relied on differential centrifugation of extracts from HeLa cells transfected with an active epitope-tagged L1. Although informative, these studies probably examined L1-RNPs in association with co-concentrating molecules not involved in the retrotransposition process. Here we have developed a quick and efficient way to purify L1RNPs by affinity purification. We have fused a Flag epitope at the C-terminus of ORF1p of a wild type active L1. Cell culture based retrotransposition assays show that this epitope-tagged L1 retrotransposes at the same frequency as wild type L1. We transfected this epitope-tagged L1 into 293T cells and prepared cytoplasmic lysate after 72 hours. The cytoplasmic lysate was incubated with anti Flag-agarose beads for 2 hrs and washed extensively with 500 mM NaCl before eluting L1-RNPs by Flag peptide competition. We have detected the 40 kDa ORF1p by Flag Ab and the 150 kDa ORF2p either by using an ORF2p N-terminal Ab or C-terminal Ab. We have also detected the 6kb L1 RNA in the L1-RNPs. We also demonstrated ORF2p RT activity in these same RNPs. We made another construct in which the C-terminus of ORF2p was fused with Flag epitope and repeated the same experiments. We demonstrated the presence of ORF1p, ORF2p, L1RNA and ORF2p RT activity in the RNPs pulled down via the ORF2p Flag-tag. Our ability to efficiently purify L1-RNPs using either an epitope-tagged ORF1p or ORF2p now makes it possible to identify cis-acting cellular factors involved in L1 RNP assembly and the complex process of retrotransposition.

1612/F

Detection and characterization of microsatellite variation in the human genome from 1,000 genomes Pilots 1 & 2. *D.R. Murdock, F. Yu, R.A. Gibbs, P.E. Bonnen, 1000 Genomes Project.* Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX.

Variation in microsatellites contributes to the spectrum of human phenotypic diversity ranging from non-pathogenic traits to causing devastating diseases. There is also evidence in non-human species for a role for microsatellites as a mechanism of rapid evolution. We have developed a methodology for extracting genotypes for microsatellites from next-generation sequence data and applied this method to data from the 1000 Genomes Project. Using low coverage whole genome sequence data pooled across 22 individuals (Pilot 1) we developed high confidence genotypes for 110,769 microsatellites in the human genome. Additionally, we computed microsatellite genotypes for two high coverage individuals (Pilot 2). Using these data we assess the extent of heterozygosity among microsatellites genome-wide and the factors that contribute to this variability. Our method and analyses demonstrate the ability to genotype highly polymorphic, multiallelic variants such as microsatellites and provide insight into the basis for this variability. In addition, we expand the repertoire of variants available for human studies beyond bi-allelic markers and provide a rich substrate for studies into phenotypic variability and disease investigations.

1613/F

Structural-Functional Analysis of *cep290* in Zebrafish Vision. *L.M. Baye[1], X. Patrinostro[1], J.S. Beck[2,4], S. Swaminathan[1], Y. Zhang[2], E. Stone[3,4], V.C. Sheffield[2,4], D.C. Slusarski[1].* 1) Dept of Biology, University of Iowa, Iowa City, IA; 2) Dept of Pediatrics, University of Iowa, Iowa City, IA; 3) Dept of Ophthalmology,University of Iowa, Iowa City, IA; 4) Howard Hughes Medical Institute.

CEP290 is a large, multi-domain gene implicated in several cilia related syndromic disorders including Meckel-Gruber, Joubert, Senor-Loken and Bardet-Biedl Syndrome (BBS). Moreover, *CEP290* is the most frequently mutated gene underlying the non-syndromic blinding disorder Leber's congenital amaurosis (LCA). Using the zebrafish model system, we characterized the developmental role of *cep290* and used a functional assay to identify a region of the CEP290 protein that is required for vision. In the zebrafish embryo the *cep290* transcript is expressed throughout development and localizes to the ganglion, inner nuclear layer and the photoreceptor cells of the retina. To determine the *in vivo* function of *cep290* in the zebrafish, we examined the effects of gene knockdown using an antisense oligonucleotide (Morpholino, MO) designed to generate an altered *cep290* splice product that models a common LCA mutation. We have previously demonstrated that knockdown of *bbs1-12* results in reduction of a ciliated vesicle, the Kupffer's Vesicle (KV) and delays in intracellular transport of zebrafish melanosomes. Consistent with a role in BBS, *cep290* morpholino-injected embryos exhibited defects in the size of the KV and statistically significant delays melanosome transport. Morphologically, the embryos exhibited a curved body axis at increased doses of MO consistent with additional roles of *cep290* in cilia function. Histological analysis of the retina revealed no gross morphological defects; however, knockdown of *cep290* resulted in a statistically significant reduction in visual function. We next examined the function of CEP290 protein domains by co-injection of RNA with the *cep290* MO and then evaluated suppression of morpholino-induced phenotypes. We demonstrate that vision impairment caused by disruption of *cep290* can be rescued by expressing only a portion of the protein; however, the KV and melanosome transport delays were not rescued. Together, these data illuminate a specific region of the CEP290 protein that is functioning in vision and identify a potential gene therapy target for LCA patients with mutations in *CEP290*.

1614/F

The Role of *Robo1* in Retinal Vascular Disorders. *N. Haider[1,2], A. Yang[1], Y. Yuan[1], M. DeAngelis[3].* 1) Dept Gen, Univ Nebraska Med Ctr, Omaha, NE; 2) Dept. Ophthalmology & Visual Sciences, Univ Nebraska Med. Ctr, Omaha NE; 3) Ocular Molecular Genetics Institute and Department of Ophthalmology, Harvard Medical School, Massachusetts Eye and Ear Infirmary Boston, Massachusetts.

Pathological ocular angiogenesis occurs in many vision-debilitating diseases such as retinopathy of prematurity, diabetic retinopathy, and age-related macular degeneration (AMD). The Slit/Roundabout (Robo) system, originally discovered in Drosophila neurons for its role in axon guidance, has been well studied in the brain and other parts of the central nervous system. Although there is some evidence that *Robo1* plays a role in retinal angiogenesis, its exact role in the normal retina is still unknown. We previously reported that SNPs and haplotypes in RAR-related orphan receptor alpha, *RORA*, are significantly associated with neovascular AMD in three diverse populations and furthermore that ROBO1 likely functions in the same pathway with RORA. Using ChIP assays in the normal mouse retina, we report that Robo1 gene expression is modulated by *Rora*. Further, in order to better understand the role of *Robo1* in retinal angiogenesis, we have examined the temporal and spatial expression profile of *Robo1* in the mouse retina and have determined the retinal pathology of the *Robo1* knockout mouse model. Our results demonstrate that *Robo1* is expressed in several layers of the retina including the inner segments of the photoreceptor cells, inner and outer plexiform layers, ganglion cell layer, nerve fiber layer, as well as retinal blood vessels. Our studies suggest a novel role for *Robo1* in retinal angiogenesis and studies using the *Robo1* knockout mouse will allow us to better understand the role of *Robo1* in common blinding retinal diseases such as neovascular AMD and diabetic retinopathy.

1615/F

Human Kir7.1 Channel Mutation (R162W), Associated With Snowflake Vitreoretinal Degeneration (SVD), Disrupts the Lipid Binding Pocket and Results in a Non-Functional Channel. *D.M. Pillers[1, 2], S. Tokarz[1], M. Asuma[1], T. Schoeder[1], A. Sharma[3], A.O. Edwards[4], B. Pattnaik[1, 2, 5].* 1) Dept Pediatrics-Neonatology, University Wisconsin, Madison, WI; 2) Eye Research Institute, University Wisconsin, Madison, WI; 3) Experimental Pathology, Mayo Clinic, Rochester, MN; 4) Institute for Molecular Biology, Univ. of Oregon, Eugene; 5) Ophthalmology, University Wisconsin, Madison, WI.

Background: Hereditary snowflake vitreoretinal degeneration (SVD) is an autosomal dominant disorder that causes degeneration of multiple ocular tissues. SVD is linked to a mutation R162W in the Kir7.1 inwardly rectifying potassium channel. The Kir7.1 channel is located in the apical membrane of retinal pigment epithelium (RPE) and facilitates potassium homeostasis around the photoreceptor outer segment and also facilitates directional fluid flow across the RPE. A similar mutation in rat Kir7.1 forms functional but non-selective channels in CHO cells. Purpose: To understand the cellular physiology phenotype associated with the R162W mutation in human Kir7.1 expressed in mammalian cells. Methods: Wild-type (hKir7.1WT) and mutant (hKir7.1M) were co-transfected into CHO cells along with GFP. Kir channel currents were studied by whole-cell electrophysiology. Bath solution (in mM): 135 NaCl, 5 KCl, 10 HEPES, 10 glucose, 1.8 CaCl2 and 1 MgCl2 pH 7.4. For selectivity bath Na+ was replaced with either K+ or Rb+. Recording electrodes (in mM): 30 KCl, 83 K-gluconate, 10 HEPES, 5.5 EGTA, 0.5 CaCl2, 4 Mg-ATP, and 0.5 GTP adjusted to pH 7.2. Total protein from transfected CHO cells was isolated, subjected to electrophoresis and Western blot analysis using Kir7.1 and βactin antibodies.The blot was visualized using the Odyssey IR system.Results: Cells expressing hKir7.1WT had a resting membrane potential of -45 ± 4.6 mV (n=10) compared to -3.2 ± 1.8 mV (n=15) for the hKir7.1M transfected cells ($p<0.005$). Current-voltage curves for the hKir7.1WT showed typical inward rectification. The inward current of hKir7.1WT had preference for Rb+ over K+ as expected. The hKir7.1M exhibited no current over the whole voltage range in which hKir7.1WT is active. Protein analysis showed that mutant Kir7.1 expression was decreased compared to wild-type. Conclusions: Wild-type Kir7.1 expression in CHO cells results in a highly selective current while the human mutant Kir7.1 did not exhibit any current proving that the mutant fails to form functional channels. This mutation also results in reduced Kir7.1 expression in transfected cells. The R162W mutation lies within the Kir7.1 cytoplasmic lipid-binding pocket. A similar mutation in Kir2.1 results in cardiac electrical instability causing cardiodysrhythmic periodic paralysis. This non-functional Kir7.1 channel may contribute to the classic phenotype of SVD, by distorting RPE transport function and altering RPE physiology.

1616/W

Rapid and Sensitive by Capillary Electrophoresis for Expanded Allele in Friedreich Ataxia Type 1. *P. YESCAS[1], N. MONROY[1], M. LOPEZ[2], E. ALONSO[1].* 1) Department of Neurogenetics, National Institute of Neurology and Neurosurgery Mexico City, Mexico; 2) Biological Systems UAM-Xochimilco, Mexico City, Mexico.

Friedreich's ataxia (FA) is an autosomal recessive disease. FA type 1 is caused by mutations in the gene encoding frataxin (FXN). The most common molecular alteration is the expansion of a GAA trinucleotide repeat in intron 1 of FXN gene. Normal subjects have 5 to 30 GAA repeats, while 98% of affected individuals may have from 70 to more than 1000 repeats. In general the clinical diagnosis of ataxias is difficult, often the FA phenotype is atypical and the techniques used for detection are laborious and require enormous amounts of DNA sample. To implement a screening technique that allows fast and simple detection of expanded FXN alleles. The sample consisted of 18 patients positive for AF type 1, previously confirmed by other techniques, and negative controls. The region flanking the GAA repeat of intron 1 of FXN was amplified triple-primer PCR 100 ng of DNA, where one of them is fluorescent. The amplicons were separated in a capillary electrophoresis genetic analyzer. Each sample was analyzed by triplicate. The technique used allows to distinguish healthy individuals, as well as heterozygous and homozygous FA patients. The results of the 18 cases of AF were consistent with those previously obtained by Southern blot The implemented technique allows rapid screening of expanded alleles in individuals with suspected type 1 FA. We propose that the expansion by triplet repeat in the FXN gene should be investigated in all patients with sporadic ataxia and age of onset before 40 years even though the phenotype is atypical FA. This technique could be used for other types of ataxias. CONACYT 69210.

1617/W

Analysis of the performance of prediction algorithms for the evaluation of missense and splicing variants in a clinical diagnostics context. *C.D. Elzinga, C.D. Braastad, I.D. Karbassi, C. DiVincenzo, C. Moffitt, J.G. Jones, S.D. Batish, E.J. Couchon.* Athena Diagnostics, Worcester, MA.

Missense variants and variants that may have an effect on splicing are commonly encountered by clinical diagnostic labs. Often little or no information is available in the scientific literature to assist in classifying these variants as normal or as pathogenic. Predictive algorithms (i.e. SIFT, PolyPhen, and various splicing algorithms) are commonly used as part of the analysis of these variants of unknown clinical significance. However, the sensitivities and specificities of different algorithms vary, and the performance of individual prediction algorithms vary across genes as well. In order to evaluate the performance of prediction algorithms intended for missense variants (including SIFT and PolyPhen), missense disease variants and known missense polymorphisms (normal variants) from a variety of genes were used as a test pool. Each algorithm was used to produce predictions for these selected variants. Concordance of the predicted effect on normal protein function with the actual effect was used to assess sensitivity, specificity, and other test performance metrics for individual genes and for the algorithm as a whole. Similarly, to evaluate selected splicing algorithms, variants known to have an effect on splicing and known polymorphisms occurring very near known splice sites were used as a splicing test pool. Splicing algorithms were used to produce predictions for variants in the splicing test pool. Where possible, ROC curves were generated to assist in selecting optimal significance thresholds for algorithms tested, and test performance metrics were calculated for each splicing algorithm.

1618/W

Deciphering the Molecular Basis of Neurological Diseases: Screening Tests in the Bedouin Population. *S. Khateeb, O. Barel, G. Narkis, O.S Birk.* Soroka Medical Center and NIBN, Ben Gurion University, Beer-Sheva, Israel.

Our Bedouin community of ~170,000 is characterized by ~60% consanguineous marriages and 8-9 children per couple, leading to a high incidence of autosomal recessive diseases. A comprehensive organization of community education, molecular research and clinical implementation has been set up. We have generated effective novel software that facilitates finding disease-associated loci using Affymetrix SNP arrays, and a unique bioinformatics tool (S2G = Syndrome to Gene) to identify the disease genes within the defined loci. Using these tools, we recently identified 14 novel disease genes, 5 of them for severe neurological diseases: infantile neuroaxonal dystrophy (INAD) is a storage disease caused by mutations in phopholipase PLA2G6; a severe recessive phenotype of mental retardation and cerebral palsy is due to a mutation in UQCRQ of mitochondrial complex 3; Birk Barel genomic imprinting mental retardation syndrome is due to mutation in potassium channel KCNK9; Lethal congenital arthrogryposis can be caused by mutations in either ERBB3 or PIP5K1C, which directs ERBB3 internalization. These findings, with dozens of novel mutations we found in known disease genes, are being implemented as free carrier testing, prenatal diagnoses and pre-implantation genetic diagnoses, leading to a dramatic two-fold reduction in infant mortality in our Bedouin community over the past 4 years.

1619/W

MutaDATABASE, a standardised, centralised, open access database of mutations leading to human genetic disease. *P.J. Willems[1], F. Decouttere[2], S. Bale[3], M. Devisscher[2], W. Van Criekinge[4], R.L. Nussbaum[5].* 1) GENDIA, Antwerp, Belgium; 2) GENOHM, Zwijnaarde, Belgium; 3) GENEDX, Gaithersburg , USA; 4) BIOBIX, University Ghent, Belgium; 5) Department of Medicine, UCSF, San Francisco, USA.

A small fraction of the mutations causing genetic disease have been published in the literature or listed in public mutation databases, as most novel disease mutations identified over the last decade have been identified in clinical diagnostic laboratories. International journals have published individual or lists of mutations without accompanying detailed clinical and/or functional data, which is generally not available to the diagnostic laboratory. Many mutations are not introduced into mutation databases because at present there is no easy or efficient way for a busy diagnostic laboratory to submit novel mutations into a standardized database platform. Furthermore, there is only limited exchange of information about mutations between different diagnostic laboratories. Consequently, the clinical significance of many novel mutations remains undetermined. Furthermore, no standardized, centralized, and open access database of mutations exists that includes well-vetted information on the common disease genes and mutations implicated in human genetic diseases. To address these problems we have developed MutaDATABASE, a standardized, centralized, and free open access database that addresses this need. Included for each disease gene is : i) general information on the disease gene, including genomic sequence, cDNA sequence, amino acid sequence, genomic structure, intron-exon boundaries, functional domains, and function, ii) general information on the diseases associated with mutations in that gene, iii) tables of exon-intron sequences and disease-causing mutations, and iv) figures of the cytogenetic position, genomic structure of the gene, and disease-causing mutations, v) lists of MutaCIRCLES -- labs working on the same disease gene that are linked in community groups. MutaDATABASE has real-time bidirectional communication with a new software interface, MutaREPORTER, which is designed to define, characterize and archive variations in human disease genes. MutaDATABASE currently is supported by hundreds of gene curators and genetic labs.

1620/W

Mitochondrial Genome Sequence Analysis: A custom bioinformatics pipeline substantially improves Affymetrix MitoChip call rate and accuracy. *H.M. Xie[1], J.C. Perin[1], T.G. Schurr[2], J. Baur[2], M. King[3], E. Place[1], C. Clarke[1], M. Grauer[1], J. Schug[2], A. Santani[1], A. Albano[1], C. Kim[1], H. Hakonarson[1,2], X. Gai[1], M.J. Falk[1,2].* 1) The Children's Hospital of Philadelphia, Philadelphia, PA; 2) University of Pennsylvania, Philadelphia, PA; 3) Thomas Jefferson University, Philadelphia, PA.

Mitochondrial genome sequence analysis has become highly relevant to the clinical diagnostic evaluation of mitochondrial disease. Existing methodologies widely differ in cost, complexity, and heteroplasmy detection sensitivity. Further, reports of numerous sequence variants often present a challenge to ready clinical diagnostic interpretation. Here, we report the performance of a bioinformatics software pipeline that achieves much improved mitochondrial genome sequence call rates and accuracy following Affymetrix MitoChip analysis. **METHODS:** A custom bioinformatics software pipeline was created to detect sequence variation. Results are fed into a presentation layer that incorporates a MySQL database backend with a PHP-based web front-end, "MitoSNP", which facilitate rapid SNP presentation and visualization. MitoSNP annotates variants based on gene content, known pathogenicity, and haplogroup-determining SNPs. Gbrowse integration permits visual interpretation in the context of all other publicly available genome annotations. 24 samples were genotyped by Affymetrix MitoChip (v2) and analyzed with this custom pipeline. For comparison purposes, samples included 7 previously analyzed with Sanger sequencing in a clinical laboratory, 1 analyzed by NGS, 1 with a 2 base pair deletion, 1 with a 5 Kb deletion, 2 harboring known heteroplasmic mutations, and 1 from a rare haplogroup. **RESULTS:** An average call rate of 99.97% was achieved with our pipeline. In comparison, Affymetrix GSEQ software provided an average call rate of 98.63% for the same data set. We successfully identified 2 known heteroplasmic mutations and an additional 21 potential heteroplasmic mutations from all samples. GSEQ software could not detect 1 of the 2 heteroplasmic mutations despite overcalling other potential heteroplasmic mutations that we confirmed were not present by NGS analysis. As expected, the 2 base pair deletion was not detected but the 5 Kb deletion was clearly illustrated. Comparison of calls for 7 samples previously sequenced in a clinical laboratory revealed a total of 19 discordant calls, which translates to an upper bound of 0.016% for the estimated error rate. **CONCLUSIONS:** Affymetrix MitoChip analysis using a revised bioinformatics pipeline offers desirable sensitivity and accuracy for whole genome mitochondrial sequencing at a very low cost. This pipeline provides an attractive alternative to classic Sanger and other whole genome mitochondrial sequencing technologies.

1621/W

Can genetic-guided screening help the prevention of lung cancer? *B. Peng[1], J. Su[1], M. Foy[2], O. Gorlova[1], C. Amos[1].* 1) Dept Epidemiology, Univ Texas MD Anderson CA Ctr, Houston, TX; 2) Brown Foundation Institute of Molecular Medicine, University of Texas Health Science Center at Houston, Houston, TX 77030.

Because of concerns about the unknown degree of over-diagnosis, risks associated with radiation exposure, and follow-up of very small indeterminate nodule, early detection of lung cancer using chest X-ray, computed tomography, and sputum cytology screening of high-risk individuals is currently not recommended. However, with the identification of a number of risk factors associated with this disease, it is possible that the benefit of screening in high-risk populations, defined by those with strong family history or presence of specific biomarkers and a heavy smoking history, could outweigh the cost and make screening a useful tool in the prevention of cancer among these individuals. Using a microsimulation model that simulates populations with realistic smoking histories and individual lung cancer risk models, we introduced explicit genetic risk factors to a two-stage clonal expansion model and simulated populations with realistic individual, familial and population level properties. Hypothetical genetic risk factors following different disease models are used to compensate for the missing heritability of lung cancer. We applied different cancer screening strategies to various high-risk populations defined by smoking patterns and individual genotype profile and compared the benefits, harms and costs of these strategies using a cost model.

1622/W

Copy Number Analysis of the FH Gene is a Valuable Tool in Diagnosing Hereditary Leiomyomatosis and Renal Cell Cancer Syndrome and Fumarate Hydratase Deficiency. *J. Higgs[1], J. Boyle[2], A. Mroch[2], J. Flanagan[2], S. Suchy[1].* 1) GeneDx, Gaithersburg, MD; 2) Sanford Health, Sioux Falls, SD.

Germline mutations in the FH gene, encoding the TCA cycle enzyme fumarate hydratase, are associated with both an autosomal dominant syndrome that includes skin and uterine tumors and/or renal cell cancer: hereditary leiomyomatosis and renal cell cancer (HLRCC), and with an extremely rare autosomal recessive inborn error of metabolism: fumarate hydratase deficiency (FHD). Identification of FH mutations completes the search for a diagnosis, enables accurate risk assessment and genetic counseling, allows for prenatal diagnosis and carrier testing, and for HLRCC permits the identification of additional family members and establishment of cancer surveillance programs prior to the onset of symptoms. While most disease-associated mutations are identified by sequence analysis of the FH gene, large intragenic deletion/duplications of one or more exons that have been reported in HLRCC and FHD will not be detected by sequencing. Copy number analysis of the FH gene was performed on 15 individuals at GeneDx by quantitative PCR, custom oligonucleotide array with exon-level resolution (ExonArrayDx), or by multiplex ligation-dependent probe amplification, and 4 deletions of one or more exons were identified (27%). These included a novel deletion of exon 6 found in a single patient via array CGH. In one family with a previous child with FHD and an apparently homozygous missense mutation in the FH gene, a second pregnancy presented with ventriculomegaly and agenesis of the corpus callosum. Parental testing revealed that one parent did not have the missense mutation present in the proband but instead had a large deletion in the FH gene. Identification of the deletion allowed for prenatal diagnosis and establishment of carrier status in the siblings. Enzymatic testing performed after delivery confirmed the diagnosis of FHD. Deletion/duplication testing has a high detection rate for FH-related disorders particularly after sequence analysis fails to identify the mutation(s), enables the accurate diagnosis of individuals affected with HLRCC and FHD, and establishes carrier status in individuals at risk for HLRCC-related tumors prior to the onset of symptoms.

1623/W

Longitudinal Changes in Patient Distress following Interactive Decision Aid Use among BRCA1/2 Carriers. *G.W. Hooker[1], K.G. Leventhal[1], T. DeMarco[1], B. Peshkin[1], C. Finch[1], E. Wahl[2], J. Rispoli-Joines[3], K. Brown[4], H. Valdimarsdottir[5], M.D. Schwartz[1].* 1) Cancer Control ,Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC; 2) Section of Genetics and Metabolism, Albany Medical Center; 3) University of Maryland, Greenebaum Cancer Center; 4) Department of Genetic and Genomic Sciences, Mount Sinai School of Medicine; 5) Department of Oncological Sciences, Mount Sinai School of Medicine.

Background: Increasingly, women with a strong family history of breast cancer are seeking genetic testing as a starting point to making significant decisions regarding management of their cancer risks. Individuals who are found to be carriers of a BRCA1 or BRCA2 mutation have a substantially elevated risk for breast cancer and are frequently faced with the decision of whether or not to undergo risk reducing mastectomy. **Objective:** In order to provide BRCA1/2 carriers with ongoing decision support for breast cancer risk management, a computer-based interactive decision aid was developed and tested against usual care in a randomized controlled trial. **Design:** Following genetic counseling, 214 female (aged 21-75) BRCA1/2 mutation carriers were randomized to Usual Care (UC; N=114) or Usual Care plus Decision Aid (DA; N=100) arms. UC participants received no further intervention; DA participants were sent the CD-ROM based decision aid to view at home. **Main Outcome Measures:** The authors measured general distress, cancer specific distress and genetic testing specific distress at 1-, 6- and 12-month follow up time points, post-randomization. **Results:** Longitudinal analyses revealed a significant longitudinal impact of the DA on cancer specific distress (B= 5.54, z = 2.74, p = 0.006) which varied over time (DA group by time; B = -2.19, z = -2.45, p = 0.01) and on genetic testing specific distress (B = 5.38, z = 2.38, p = 0.02) which also varied over time (DA group by time; B= -2.38, z = -2.43, p = 0.02). Individuals randomized to UC reported significantly decreased distress in the month following randomization, whereas individuals randomized to the DA maintained their post-disclosure distress over the short-term. By 12-months, the overall decrease in distress between the two groups was similar. **Conclusion:** This report provides new insight into the long-term longitudinal effects of DAs, and raises possibilities for mechanisms of deliberative decision making.

1624/W

VUS Predict, a web based tool for classifying *BRCA* variants of uncertain significance. *A. Toland[1,4,5], L. Senter-Jamieson[2,4,5], K. Sweet[2,4,5], S. Gnanadesikan[3,5], D. Newman[3,5].* 1) Human Cancer Genetics; 2) Cancer Genetics Program; 3) Information Warehouse; 4) Comprehensive Cancer Center; 5) The Ohio State University, Columbus, OH.

Approximately 7% of individuals who undergo genetic testing of *BRCA1* and *BRCA2* are found to carry a variant of uncertain significance (VUS). There are a number of different algorithms used to classify these variants, many of which require intensive statistical analysis and the availability of genetic test results for the *BRCA* VUS for multiple family members. We developed a user-friendly algorithm to classify *BRCA1* and *BRCA2* variants in individuals with breast and/or ovarian cancer which utilizes readily available tumor histopathology and evolutionary conservation data. We tested our algorithm using 50 deleterious *BRCA1* and *BRCA2* mutations and 21 neutral polymorphisms. We had 98% sensitivity and 76% specificity to predict classified BRCA sequence changes. (It is important to note that the majority of deleterious mutations tested using the model were nonsense or truncating.) To make this algorithm widely available, we developed a web-based tool called VUS Predict, which genetic counselors and other genetic professionals can use to obtain evidence that a *BRCA* VUS may be deleterious or neutral. VUS Predict requires VUS sequence information; histopathological data from the patient who has the variant as well as breast and/or ovarian cancer; and age of diagnosis in order to estimate the odds of a VUS being deleterious. Information from only one patient is necessary to estimate odds but the ability of the program to classify an uncertain variant as neutral or deleterious improves with the number of unique individuals entered with the particular VUS. On-going studies will further evaluate the predictive power of this tool. Our goal is to continue to enhance VUS Predict so that all types of *BRCA* variants might be evaluated, including putative splice variants, which are likely to be an important class of variants.

1625/W

Arginine Missense Mutation Concentration in the Ig Loop of SCN1B: Epilepsy Testing Results from a Clinical Reference Laboratory. *I.D. Karbassi, S.D. Batish, J.G. Jones, C.D. Elzinga, C. DiVincenzo, C.D. Braastad.* Athena Diagnostics/Thermo Fisher Scientific 377 Plantation Street Worcester, MA 01605.

SCN1B is a voltage-gated ion channel auxiliary beta subunit that associates non-covalently with the alpha subunit. Beta subunits have been shown to regulate channel kinetics and cell surface expression of alpha subunits and mutations in SCN1B may result in generalized epilepsy with febrile seizures plus, Brugada syndrome 5, and other defects in cardiac conduction. Providing a differential diagnosis through genetic testing of SCN1B (available since 2007 from Athena Diagnostics as a single test or within a panel) in a young patient guides proper treatment, given that anticonvulsant drugs exacerbate seizures in these patients. SCN1B, a 218 amino acid protein, contains a signal peptide, one transmembrane domain, an intracellular C-terminus and an extracellular Ig-like domain. Within the Ig-like domain there is an Ig loop region that is thought to be defined by a disulfide linkage between C40 and C121. This loop has been shown to be involved in critical interactions with cell-adhesion molecules and also with other SCN1B molecules. There are currently five published disease-associated variants and all are found within, or just outside, the Ig- loop: R85C, R85H, IVS2-2A>C (causes a deletion of I70 to E74), C121W (abolishes the disulfide bond), and R125C (associated with Dravet if seen as homozygous). The majority of these variants affect arginine residues (the deletion caused by IVS2-2AC encompasses R72). Since the extracellular portion of SCN1B is structured into beta-sheets, arginines may play a role in maintaining beta-sheet integrity through hydrogen bonding or even in stabilizing protein interactions occurring in this region. There are a total of seven arginines contained within the Ig-loop (with the eighth, R125, located just outside). These arginines are highly conserved among 12 species (from human to zebrafish), with R46 and R89 being completely conserved. We at Athena have observed each of these arginines (with the exception of R60) changed to another amino acid in individuals whose physicians have referred them for febrile seizures evaluation. Interestingly, the majority of these changes have been from an arginine to a cysteine or histidine. This pattern suggests pathogenicity, but there is limited published data available on the variants that affect SCN1B and cause disease. As a leader in genetic testing for epilepsy, Athena is in a unique position to collaborate with researchers to better assess clinical pathogenicity of SCN1B sequence variants.

1626/W

Rapid detection of the 22q11.2 deletion by Pyrosequencing. *D. Koontz, S. Nikolova, M. Gallagher.* Centers for Disease Control, Newborn Screening and Molecular Biology Branch, Atlanta, GA.

Identification of microdeletions of the chromosome 22q11.2 region is important to the diagnosis of DiGeorge (DGS) and Velocardiofacial (VCFS) syndrome, also referred to as 22q11.2 deletion syndrome (22q11DS). Much clinical heterogeneity is associated with deletions in this region, which can be variable in size. Most individuals (~90%) with 22q11DS have a common 3.0Mb deletion that contains more than 45 known genes, and another 8% carry a 1.5Mb nested deletion. A minority of cases contain smaller atypical deletions. The conventional method for 22q11.2 deletion detection is fluorescence in situ hybridization (FISH) with commercial probes. Molecular-based methods include microsatellite typing, microarray-based comparative genomic hybridization, multiplex ligation-dependent probe amplification (MLPA), and TaqMan real-time quantitative PCR. However, most of these methods require a minimum of 20-100ng of high quality DNA. We developed a Pyrosequencing assay suitable for lower quantity DNA from buccal swabs that requires as little as 1.0ng input DNA. Results can be obtained in a single run with one set of PCR and Pyrosequencing primers. PCR primers target the human ubiquitin fusion-degradation gene (UFD1L) within the commonly deleted 3.0 Mb region and nested 1.5 Mb region. Determination of relative copy number of the deletion was obtained by co-amplification of an intergenic region on chromosome 18 with 97% sequence homology to the 22q11 target. Two sequence differences in the co-amplified amplicons were used to distinguish chromosome 18 (Chr18) from chromosome 22 (Chr22) sequence. The Chr18-specific peak heights were used as two copy references, and genotype calls are derived from quantitative peak height ratios between Chr18-specific and Chr22-specific peak heights. Assay optimization was performed on Coriell DNA from clinically diagnosed and FISH-confirmed DGS (n=2) and VCFS (n=1) patients and on multi-ethnic DNA samples from the Coriell Variation Collection (n=186). Further assay validation was performed on paired blood and buccal specimens from patients with FISH-confirmed 22q11.2 deletion (n=20) and buccal samples from normal subjects who presumably lack the deletion (n=118). Pyrosequencing results showed 100% concordance with FISH data, and reproducibility was high. This method represents a reliable and low-cost alternative to other methods for the detection of 22q11.2 microdeletions, especially when DNA quality and quantity is limited.

1627/W

Identification of Loss of Function *SPRED1* copy number changes using RT-PCR, Multiplex Ligation-dependent Probe Amplification and Quantitative PCR. *E. Spencer[1], J. Ramsey[1], F.M. Mikhail[1], C. Fu[1], R. Vijzelaar[2], H. Feret[3], E. Zackai[3], A. Shugar[4], M.S. Meyn[4], G. Bellus[5], S. Kivirikko[6], M. Pöyhönen[7,8], L. Messiaen[1].* 1) Genetics, University of Alabama, Birmingham, Birmingham, AL; 2) MRC-Holland, Amsterdam, The Netherlands; 3) Division of Human Genetics, Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, PA; 4) Division of Clinical & Metabolic Genetics, The Hospital for Sick Children, Toronto, Ontario, Canada; 5) Department of Genetics, Children's Hospital, Aurora, CO; 6) Department of Clinical Genetics, Helsinki University Central Hospital, Helsinki, FInland; 7) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 8) Dept of Clinical Genetics, HUSLAB, Helsinki, Finland.

Legius syndrome, is a recently identified autosomal dominant disorder caused by Loss of Function (LOF) mutations in the *SPRED1* gene, with individuals mainly presenting with multiple café-au-lait macules (CALM), freckling and macrocephaly. So far, only minor lesion LOF *SPRED1* mutations have been identified as the cause of this syndrome. To determine if Copy Number Changes (CNCs) are a cause of Legius syndrome, we have used a Multiplex Ligation-dependent Probe Amplification (MLPA) assay covering all *SPRED1* exons in a cohort of 320 NF1-negative patients presenting with multiple CALM in whom no minor lesion LOF *SPRED1* mutation had been found by cDNA sequence analysis of the entire *SPRED1* coding region. Four different deletions were identified by MLPA and confirmed by quantitative PCR, reverse transcriptase PCR and/or Array CGH: a deletion of exon 1 and the *SPRED1* promoter region in a proband and two first-degree relatives; a deletion of the entire *SPRED1* gene in a sporadic patient; a deletion of exon 2-6 in a proband and her father; and a deletion of ~6MB of chromosome 15 in a sporadic patient. Patients presented with multiple CALM with or without freckling, but fulfilled no other NF1 diagnostic criteria. These results indicate the need for dosage analysis to complement sequencing-based *SPRED1* mutation analyses.

1628/W

Genetic Laboratory Testing Without Clinical Correlation: A Recipe for Misdiagnosis. *H. Welsh[1], L. Zhang[2], S. Yu[2], H. Ardinger[1].* 1) Section of Genetics, Children's Mercy Hospitals and Clinics, Kansas City, MO; 2) Cytogenetics Laboratory, Children's Mercy Hospitals and Clinics, Kansas City, MO.

A chromosome analysis was ordered by an endocrinologist to rule out Turner syndrome in a 12-year-old girl with short stature. This testing revealed a 46,XX,del(11)(q24q25) karyotype, consistent with the diagnosis of the Jacobsen Syndrome. The phenotype of Jacobsen Syndrome includes growth deficiency, microcephaly, mental retardation, craniofacial anomalies, joint contractures and cardiac defects. The family was advised of the cytogenetic findings and referred for a genetics evaluation and counseling for Jacobsen syndrome. On physical examination, the patient was found to be at the <3rd percentile for height and at the 40th percentile for head circumference. The remainder of her physical exam was normal, with the exception of an inability to pronate or supinate her forearms and difficulty with fine motor skills. There were no dysmorphic features. There was no developmental delay and the family reported her to be a straight A student. Because of the inconsistency between her phenotype and her cytogenetic findings we obtained a skeletal survey, a second chromosome analysis with FISH studies and a microarray analysis. The skeletal survey was suggestive of the diagnosis of Leri-Weil Dyschondrosteosis (LWD), which is typically associated with a mutation or deletion of the SHOX gene located on Xp. With this information in mind, we asked the Cytogenetics Lab to carefully study the X chromosome. The studies in our laboratory revealed a nonrciprocal, unbalanced translocation between one chromosome X short arm and one chromosome 11 long arm. The karyotype is 46,X,der(X)t(X;11)(p22.32;q24.2),del(11)(q24.2).ish der(X)t(X;11)(DXYS129-,SHOX-,DXZ1+,D11S2071+), del(11) (D11Z1+,D11S2071-,DXYS129-,SHOX-), which indicates a genetic imbalance for monosomy of Xp22.32-Xpter with loss of one copy of the SHOX gene. Microarray analysis confirmed a 4.33 Mb terminal deletion of one chromosome X short arm and identified an additional 1.11 Mb deletion at the 11q24.2 breakpoint. SHOX haploinsufficiency is associated with LWD, which is the diagnosis most consistent with her clinical presentation. The translocation of the segment of 11qter to Xp masked the deleted segment of Xp and led to an erroneous initial diagnosis that was quite concerning to the family. This case demonstrates the need for clinical correlation with laboratory results prior to devising treatment and management plans.

1629/W

Perceived Risk, Anxiety and Sharing Behavior in Response to Personalized Risk Information in a Cohort Study. *E.S. Gordon, C.B. Stack, N. Gharani, T.J. Schmidlen, M.F. Christman, M.A. Keller.* Coriell Institute for Medical Research, Camden, NJ.

In the new era of direct-to-consumer testing (DTC), risk perception may influence the patient's decision to share their genomic results with their health care provider (HCP) thus influencing not only lifestyle decisions but direct medical care as well. In the present study, we delivered personalized risk results for coronary artery disease (CAD) based on a validated genetic risk variant (rs1333049 on 9p21.3), family history, smoking status, and diabetes status through a secure web based portal to participants in the Coriell Personalized Medicine Collaborative. Participants reported perceived risk and decision to share results with a HCP in a follow up survey distributed to participants 90 days after they had viewed their result. Thirty-three percent (472 of 1437) of eligible participants completed the follow up survey. The majority of respondents were Caucasian (95%), female (69%), with a bachelors degree or higher (72%), high incomes (50% >$100,000) and an average age of 51 years. Overall, survey respondents closely mirrored non-responders with respect to demographics, though responders were older (mean age 51 vs. 48, p<0.001) and a larger proportion of responders were female (69% vs. 62%, p<0.001). Each of the factors included in the CPMC risk report for CAD were associated with overall perceived risk of CAD: genotype (p<0.001), family history (p<0.001), diabetes status (p=0.007) and smoking status (p=0.026). Participants with zero copies of the genetic risk variant rated their overall risk of CAD higher if they also had a family history of CAD (p-interaction = 0.0076). The decision or intention to share results was associated with perceived risk (p<0.001); 51%, 62%, and 74% of participants who rated their risk of CAD as low, average, and high, respectively, reported that they had shared or planned to share results. Older age (p=0.001) and higher BMI (p=0.009) were also associated with increased rates of sharing. No association was found between the decision to share results with a HCP and having 0, 1, or 2 copies of the genetic risk variant (p=0.73). These findings suggest that participants are making behavioral decisions, specifically related to sharing results with a HCP, based on their perception of their risk. Autonomous decisions regarding sharing have the potential to lead to missed medical intervention for those who underestimate their risk and elect not to share and overuse of the health care system among the worried well.

1630/W

Comprehensive variant analysis using the Variant Analysis Tool. *R.K. Hart[1], S.E. Brenner[2].* 1) Center for Computational Biology, 324G Stanley Hall, University of California, Berkeley, CA 94720; 2) 461 Koshland Hall, University of California, Berkeley, CA 94720.

The analysis of genomic variants in clinical settings frequently involves entering variants into multiple tools and manually integrating results. This process is time-consuming and error-prone. We are developing the Genome Commons Variant Analysis Tool (VAT), an open source, open access portal that facilitates the analysis of variants by clinical geneticists and genetic counselors. With the VAT, clinicians will be able to enter a variant once and obtain up-to-date data from public sources and tools.

Given a single nucleotide or protein variant specified in standard Human Genome Variation Society mutation syntax, the current VAT prototype aggregates disparate publicly accessible data and tools. Data sources presently referenced include UniGene, PubMed, dbSNP, RefSeq, Homologene, GeneTests and GeneReviews. Links to expression data, genome browsers, LSDBs, and pathogenicity assessment tools will be available. Additional facilities will be added to accommodate requests from users and to keep up with the evolution of tools for genome interpretation.

The VAT improves the reliability, accuracy, completeness, and currency of information used to assess the pathogenicity and pathogenic mechanisms of clinical variants by using up-to-date data and eliminating the need to manually enter variation specifications. The VAT is an initial tool being developed by the Genome Commons, a project that aims to provide open source and open access interoperable tools for the interpretation of individuals' genomes.

1631/W

Expanding access to cardiovascular genetic counseling through integration of services within cardiology specialty clinics. *P.L. Arscott, C.M. Rigelsky, R.T. Moran.* Genomic Medicine Institute, Cleveland Clinic Foundation, Cleveland, OH.

As the genetic basis for a growing number of cardiovascular (CV) conditions is revealed and genetic testing becomes increasingly available, the demand for genetic counseling (GC) for inherited CV conditions will increase. Traditionally, this was accomplished through referral to genetic centers where a patient may be seen by both a genetic counselor and a geneticist. In order to respond to increasing demand for these services, we developed a model for integrating GC services into various CV practices within a large health center. Patients were referred for GC by a managing physician. Testing, referrals, and clinical follow up were based on collaboration between the referring physician and genetic counselor. Clinical oversight was provided by a clinical geneticist. The appointment with a genetic counselor was billed using the 96040 reimbursement code, or self-paid. Evaluation of the clinical services was performed using information collected for clinical operations for CV GC outpatient appointments between September 2009 and May 2010. A total of 160 patients were seen within the 9-month period. Patients were referred for personal or family history of aneurysms or connective tissue disorder (55%), cardiomyopathy (34%), arrhythmia (4%), or other CV condition (6%). Genetic counselors collaborated with 57 referring physicians from multiple departments. Nearly half of the patients (n=76) were referred by seven physicians. Referring physicians were predominantly cardiologists (81%) and referrals from non-cardiologists tended to be for a familial mutation. Fifty-eight percent of patients with HCM and 41% of patients with aneurysms had genetic testing as a result of the appointment. GC Services provided in direct collaboration with managing physicians facilitate access to genetic risk assessment and genetic testing for a wide variety of CV conditions. A small group of specialists consistently referred for GC services, while others referred few patients even though their patient population includes those with high genetic risk. These findings highlight areas for growth such as increasing awareness and education about our services. Systematic approaches to increase referrals for specific CV indications are being explored. The direct collaboration with physicians in multiple specialty areas is expected to increase recognition of available services and to facilitate the use of genetic information in the management of heritable CV conditions.

1632/W

Level of risk and change in risk are determining factors in patient decisions regarding prenatal diagnosis following positive first or second trimester maternal serum screening for Down syndrome. *K. Borsack, A. Vimb, H. Lichtenstein, V. Fudulu, N. Nakata.* Genzyme Genetics, Los Angeles, CA.

OBJECTIVE: Prenatal maternal serum screening (MSS) provides patients with individualized risks for Down syndrome as well as other fetal conditions. This allows patients to make informed decisions regarding prenatal diagnosis (PNDx) via chorionic villus sampling (CVS) or amniocentesis. The purpose of our study was to determine if numeric risk, magnitude of change in numeric risk (from a priori age related risk), and/or other maternal factors correlate with patients' decisions to undergo PNDx. **STUDY DESIGN:** We conducted a retrospective study of singleton pregnancies referred to Genzyme Genetics for genetic counseling services from 2007 through 2008 with an indication of MSS results (including first trimester combined screening, Sequential 1 and 2 screening, and integrated screening) positive for Down syndrome. We compared numeric risk determined by screening, the magnitude of change in risk compared to a priori age related risk, and maternal factors including maternal age and pregnancy history (gravidity, parity, pregnancy loss and termination) for patients who elected to undergo invasive testing and for patients who declined invasive testing. **RESULTS:** The study population included 1,794 patients. Both post-screening risk and magnitude of change in risk were noted to be significant predictors of a patient's uptake of invasive testing. Also significant was a lower average maternal age for patients who had second trimester screening compared with those who had first trimester screening (34.3 years vs. 36.6 years). Other findings included: greater uptake of CVS over amniocentesis in screen positive patients (72% vs. 59%), greater uptake of PNDx in patients with screen positive first trimester results compared with second trimester results (64% vs. 56%), and little correlation with pregnancy history as a determining factor about invasive testing. **CONCLUSION:** In our study population we observed that among patients with either positive first or second trimester MSS results for Down syndrome, numeric risk and magnitude of change in risk are significant factors that influence patients' decisions to pursue PNDx. Both factors have a positive correlation with the decision to pursue PNDx. Women with greater risk and/or higher magnitude of change in risk who have had first trimester MSS screening are more likely to pursue PNDx, most commonly via CVS if testing is available.

1633/W

Where there are no genetic services: a study of patients suffering from Ushers Syndrome in Bangladesh. *E. Haque[1], S. Rozario[2].* 1) Clinical Genetics, Ferguson-Smith Centre for Clinical Genetics, Glasgow, Scotland, United Kingdom; 2) Cardiff University, Cardiff, United Kingdom.

Genetic counselling is available in the western world to support patients with genetic conditions and their families through difficult decision making processes and understanding of their condition. However, not much is known about the quality of life in countries where there are no genetic services. This study was conducted in Bangladesh as an MSc Dissertation and was designed to reach those patients and their families who suffer from genetic diseases but due to a total lack of Genetic services and appropriate genetic counselling may have difficulties in making an informed decision. Semi-structured interviews were undertaken with various members of six families with children suffering from Ushers Syndrome and thematic analysis was used to analyse the data. Family members that took part in the study had no knowledge of hereditary diseases or how they can be passed on. Most participants resorted to trying alternative therapies (e.g. homeopathy) and religious healing to try and make their children well as there was overwhelming evidence of stigma associated with disability. No psychosocial support has been provided for any of the participants or their families. The quality of life lead by these patients and their families were generally poor. It was observed that most families were facing financial troubles due to the cost of the medical care of their children. The findings are in stark contrast to the basic services and facilities taken for granted in countries like the USA and UK. They show that living with a genetic condition in a country where there are no genetic services can be distressing for patients and their families. Better services and better understanding of Genetics needs to be put in place in order to improve their quality of life and making sure they are not victims of stigmatization.

1634/W

“This is not what I was expecting...” - Revealing a personal genetic disorder throughout prenatal counseling for an affected offspring: A counseling challenge. *S. Horowitz[1], S. Shkedi[1], A. Shaag[1], J. Zlotogora[2], V. Meiner[1].* 1) Department of Human Genetics, Hadassah-Hebrew University Medical Center, Jerusalem, Israel; 2) Department of Community Genetics, Ministry of Health, Jerusalem, Israel.

Autosomal dominant disorders frequently present with variable expression and reduced penetrance. Thus, a healthy parent may learn that he carries a faulty gene only after the discovery of abnormal findings in his offspring during or after pregnancy. Oral-facial-digital (OFD) syndrome is a genetically heterogeneous condition with extensive pleiotropy. The common form is inherited as an X-linked dominant disorder with variable expression. Most of the carriers express minor variable symptoms, hence might be unaware of their risk for passing on an abnormal gene. We report a 25 year old individual that was referred for genetic counseling following termination of a multi-malformed pregnancy. Upon intake of personal history, she reported having a “short pinky” and that when being one year old “some kind of an oral operation” was performed. Following clinical examination OFD syndrome was suspected and further molecular studies were issued. OFD1 screening led to the identification of a disease causing mutation. In light of these findings this individual had not only to deal with her grief and mourning for her recent pregnancy loss, but also with feelings of guilt and uncertainty regarding her own personal health perception and future reproductive plans. This is one of many other similar genetic counseling scenarios in which the counselor is confronted by a prenatal issue of concerned parents about an offspring’s health situation which leads to diagnosing an actual clinical genetic disorder in one of the parents. Such incidence presents a critical unique challenge for the genetic counselor in helping the parents to cope with the psychosocial and personal aspects of their current circumstances. In addition, various social perceptions of the role of genetic counseling in health promotion within distinct ethnic groups will be discussed.

1635/W

Mutation Risk Associated with Paternal and Maternal Age in a Cohort of Retinoblastoma Survivors. *M.B. Mills[1], L. Hudgins[2], R.R. Balise[3], R.A. Kleinerman[4].* 1) Master’s Program in Human Genetics and Genetic Counseling, Stanford University School of Medicine, Stanford, CA; 2) Department of Pediatrics, Division of Medical Genetics, Stanford University School of Medicine, Stanford, CA; 3) Health Research and Policy, Stanford University School of Medicine, Stanford, CA; 4) Radiation Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Rockville, MD.

Autosomal dominant conditions such as achondroplasia are known to be associated with advanced paternal age, and it has been suggested that retinoblastoma (Rb) also exhibits a paternal age effect. Research on the relationship between advanced paternal and maternal age and risk of Rb has been reported for several countries, including the United States (U.S.), with mixed findings. To our knowledge this is the first study to look at risk of a *de novo* mutation in relation to parental age for 3 categories of Rb: Rb resulting from a *de novo* mutation, sporadic Rb, and familial Rb inherited from an affected parent.

A cohort of 262 retinoblastoma patients was retrospectively identified at one institution, and data were collected from parents of 160 patients (65.3%) via telephone interview. Mean parental ages were calculated for the general U.S. population. Two sets of hierarchical stepwise logistic regression models were built to look for increased odds of a *de novo* mutation related to older parental age compared to sporadic and familial Rb. The modeling strategy looked for effects of continuous increasing maternal and paternal age controlling for ethnicity and age of the other parent. To assess nonlinear age effects, 5-year age increases were analyzed. All differences between means were compared using either one sample t-tests or one-way ANOVAs.

Our results showed that both mean maternal and paternal ages for *de novo* mutations and sporadic Rb were similar as well as statistically significantly higher than the general U.S. population means, whereas parental ages for familial Rb were not significantly different. There were no significant differences between mean maternal and paternal ages for the comparison between each of the 3 Rb classification groups. For each 5-year increase in paternal age, we found increased odds of having a *de novo* mutation, but these findings were not statistically significant.

Our analysis suggests a weak paternal age effect for *de novo* Rb mutations, however we have insufficient data for investigating paternal age over age 40 to test this hypothesis further. The similarity of mean maternal and paternal ages for *de novo* Rb mutations and sporadic Rb was unexpected and deserves further attention.

1636/W

The future of recurrence risk counseling for complex diseases: a paradigm for counseling incorporating recent molecular findings and discussion of the challenges. *C. Nichols, A. Chakravarti.* Inst Gen Med, Johns Hopkins Univ, Baltimore, MD.

In the past, recurrence risk counseling for isolated multifactorial congenital anomalies, such as Hirschsprung disease (HSCR), involved quoting an ~3% recurrence risk for siblings, with little refinement available for individual families. Noted heterogeneity in risk based on factors such as severity, familiality, and gender has allowed more refined empiric risks, yet individualized risk assessment is often still not possible. Molecular genetic and epidemiological studies in recent years have identified chromosomal variants in ~12% of HSCR cases and rare sequence variants in 11 genes, at least 6 of which are implicated in isolated HSCR. Three common variants are implicated thus far, including a well-characterized RET enhancer variant that increases risk by 4-8 fold. While more detailed studies are needed to clarify the effects of variants, much promise for the use of these findings in risk assessment has been proposed. We consider how this new genetic information will be used in the future. Using HSCR as a complex disease model, we present a paradigm for recurrence risk counseling for complex diseases that incorporates recent findings of rare and common variants, with discussion of the advances, limitations, and counseling challenges through examples. In this paradigm, cases are categorized through phenotypic assessment and genetic testing into those due to syndromic occurrence, rare variants, or common variants. This will allow refinement of level of recurrence risk and potential for phenotypic heterogeneity by group. Limitations and counseling challenges to discuss include arduousness of categorization due to variable expressivity within and between families, highlighting the importance of careful family history and phenotypic assessment. Reports of allelic heterogeneity and pleiotropy also leave remaining uncertainty about potential manifestations that may need to be discussed with families. Interpretation of rare variants and combinations of common and rare variants will be challenging, as will decisions about which tests to offer as numerous genes and types of variation are implicated and cost effectiveness must be considered. Whether testing, particularly for common variants, provides useful information and improved counseling to families in comparison to available empiric risks based on phenotypic and family history information will need to be assessed through family perception and outcome studies.

1637/W

Reflections on genetic counseling for type 2 diabetes mellitus risk in primary care. *W.M. Scott[1], L.A. Killeya-Jones[2], A. Cho[2], S. Joy[2], G. Ginsburg[2].* 1) School of Nursing, Duke University, Durham, NC, 27710; 2) Duke Institute for Genome Sciences & Policy Durham, NC 27708.

Advances in the identification of genomic makers for type 2 diabetes mellitus (t2dm) and the development of commercially available tests suggest that genomic susceptibility testing for this disease may soon be available in primary care settings. Importantly, the potential of personal genomics to increase primary prevention of t2dm relies upon patients' interest in testing. We report a very high level of interest in testing in a general clinic sample enrolled in an RCT assessing the clinical utility of genetic risk testing for t2dm. We discuss the procedure for delivering the genetic results within the scope of a 40-min counseling visit (RCV) with a trained risk counselor (RC) at one of two study sites. We report reflections on participants' responses to genetic risk information as part of their overall standard risk assessment. Participants were randomized 1:1 to receive genetic results at RCV approx. 2 mths post baseline or 12 mths post RCV. Findings reported herein are based on 157 participants' RCVs (approx 50% randomized to receive their genetic results, 1/157 not interested in genetic testing). The aim of the RCV is to inform patients' perceptions of their t2dm risk. The RC is a master's-prepared, nationally-certified family nurse practitioner (NP) with 20 yrs experience as a primary care clinician. The RC uses a semi-structured format to present an overview of diabetic pathophysiology and related risk factors, followed by a discussion of the individual patients' risk factors. To facilitate this discussion, pts receive a color-coded risk profile including FPG, family history, BMI, age, and race. A genetic risk estimate is included for pts randomized to receive genetic risk results (# high-risk alleles and combined genetic risk estimate). The RC reports genuine interest from study pts about the role of genetic testing in healthcare. Participants expressed disappointment when they discovered they would not receive their genetic results at the RCV but looked forward to receiving them at the end of the study. 100% were engaged, with none reacting with negative responses upon learning their individualized genetic risk scores. Most have expressed a willingness to engage in health modifying behaviors, because or in spite of, receiving information on personal t2dm genetic risk. This underscores the value of offering genetic testing/counseling for t2dm in primary care practices in an effort to reduce incidence and improve quality of life.

1638/W

Changes in physical activity but not diet following disclosure of pleiotropic information during genetic risk assessment for Alzheimer's disease: Findings from the REVEAL Study. *K. Christensen[1], J.S. Roberts[1], W.R. Uhlmann[2], P.J. Whitehouse[3], T. Obisesan[4], D.L. Bhatt[5], L.A. Cupples[6], R.C. Green[7] on behalf of the REVEAL Study Group.* 1) Department of Health Behavior & Health Education, University of Michigan School of Public Health, Ann Arbor, MI; 2) Department of Internal Medicine & Human Genetics, University of Michigan, Ann Arbor, MI; 3) Memory & Aging Center, Case Western Reserve University/University Hospitals of Cleveland Memory & Aging, Cleveland, OH; 4) Department of Medicine, Howard University, Washington, DC; 5) VA Boston Healthcare System, Brigham & Women's Hospital, and Harvard Medical School, Boston, MA; 6) Departments of Biostatistics & Epidemiology, Boston University School of Public Health, Boston, MA; 7) Departments of Epidemiology, Neurology & Medicine (Genetics Program), Boston University Schools of Public Health & Medicine, Boston, MA.

Background: Little is known about the impact of disclosing pleiotropic information during genetic susceptibility testing for a specific disease. The ε4 allele of APOE is a risk factor for both Alzheimer's disease (AD) and coronary artery disease (CAD). As part of a series of multi-site randomized controlled clinical trials, the Risk Evaluation and Education for Alzheimer's Disease (REVEAL) Study explored how learning about this pleiotropic association during a genetic risk assessment for AD affects diet and physical activity.

Methods: Subjects randomized into a control arm received their APOE genotypes and an AD risk estimate (range: 6%-70%) based on the gender, ethnicity, family history, and APOE genotype. Subjects randomized to a 'pleiotropy' arm were also informed that APOE ε4 carriers were at increased risk for CAD. Overall physical activity and diet were assessed before and after testing using 7-item versions of the physical activity subscale of the Rapid Assessment of Physical Activity and the Dietary Risk Assessment. Logistic regression was used to determine whether subjects in the pleiotropy arm were more likely than subjects in the control arm to report improvements in physical activity and diet at the one-year follow-up compared with baseline after controlling for APOE genotype, gender, race, and family history of AD.

Results: A total of 257 subjects were enrolled at four study sites (median age 58; 55% female; 16% African American; 71% with affected first degree relatives). Of the pleiotropy arm, 35% showed improvements in physical activity compared with 24% of the control arm. Of the pleiotropy arm, 53% showed improvements in diet compared with 42% of the control arm. Logistic regression confirmed that differences were significant for physical activity (OR=1.99, p=.02), but not for diet (OR=1.50, p=.13).

Conclusions: Findings suggest that pleiotropic information shared during a genetic risk assessment for AD can motivate improvements in physical activity. Such information may not be sufficient to motivate changes in diet, although analyses did not target diets specific to CAD or AD prevention. Overall, the data suggest that pleiotropic associations shared during a genetic risk assessment for a specific condition may have power to motivate additional health behavior changes.

1639/W

Use and misuse of genetic molecular testing: one institution's experience. *H. Toriello.* Gen Services, Spectrum Hlth, Grand Rapids, MI.

In 2009, the CDC published a document entitled "Good Laboratory Practices for Molecular Genetic Testing for Heritable Diseases and Conditions". In that article, it was noted that "no studies have determined the number of molecular genetic tests performed that could be considered unwarranted or unnecessary". As part of my role as consultant to the laboratory at our institution, I undertook a project whereby I kept a database of all molecular genetic tests that were sent out by our referral laboratory. I was able to access information on 135 molecular genetic tests which were sent to a reference laboratory other than Mayo Medical Laboratories (I had no access to that data). The results of my study are cause for alarm, at least for our own institution. Among this group of 135 tests, a genetic counselor was involved with the patient in only 28 cases (21%). Many of the tests in which a counselor was NOT involved included presymptomatic testing for hereditary cancer. I also sought to determine the utility of testing. Overall, 67 of this group were considered unwarranted or unnecessary, with an additional 11 considered questionably appropriate. That means that only 42% was justifiable. There were many reasons for a particular test being considered unwarranted or unnecessary, and included but were not limited to, ordering a test highly unlikely to be positive and ordering a molecular test when a diagnosis had been established biochemically. In addition, there were 29 cases which could be considered compliance issues. For example, research samples were sent without going through our IRB (with no evidence that anyone explained the consent form). Additionally, a few "shaky" situations were discovered, such as the physician stating that the results of testing were required before surgery was done, but then that surgery was done anyway before results were back; and repeat testing when a molecular mutation had previously been found. Unfortunately, this dismal state of affairs also extended to post-testing situations. There were three cases in which incorrect interpretations were provided to patients by the physician ordering the test. In conclusion, my findings provide evidence that genetics professionals clearly need to take a more central role in the provision of molecular genetic testing to patients. As a result of my study, a protocol for review will be instituted before any testing is allowed to be sent via our referral laboratory.

1640/W

Carrier Screening of Recessive Genetic Disorders by Next Generation Sequencing. *C.J. Bell, D.L. Dinwiddie, N.A. Miller, S.L. Hateley, B.J. Rice, S.F. Kingsmore.* National Center for Genome Resources, Santa Fe, New Mexico.

Orphan Mendelian recessive diseases are individually rare, but collectively constitute a major healthcare burden, causing significant childhood morbidity and mortality. 20-30% of all infant deaths and 11% of pediatric hospital admissions are related to genetic disorders. In collaboration with the Beyond Batten Disease Foundation, the National Center for Genome Resources is developing a carrier screening test for a panel of more than 400 recessive genetic conditions that cause death or catastrophic illness in childhood. The intent of this screen is prevention; it is to be targeted at couples considering pregnancy—with appropriate counseling prospective parents having mutations in the same gene will be able to make informed family planning choices. The test uses enrichment of the exons, splice junctions and selected intronic segments of the genes, followed by multiplexed next-generation sequencing. Automated bioinformatic analysis is used to identify carrier status and zygosity for known and novel mutations. To identify the appropriate target enrichment technology for the carrier screening test, a total of 24 samples from carriers of 17 autosomal recessive diseases and 1 X-linked recessive disease were enriched for 437 genes using Agilent SureSelect, RainDance, febit, and Olink enrichment techniques and subjected to multiplexed deep sequencing using the Illumina GA IIx. The 24 samples contained 42 previously characterized mutations including 18 SNPs, 12 short in/dels, and 7 gross deletions impacting both coding and splicing elements. The four enrichment techniques were evaluated for % sequencing reads on target, fold enrichment and mutation detection, with a desired sequencing threshold of at least 20x coverage of at least 99% of targeted nucleotides based upon 500 Mb of aligned sequences, less than 1% of targeted nucleotides with zero coverage, and very high sensitivity and specificity for heterozygous variant detection. Based on these experiments we selected RainDance as the enrichment platform. The final choice of sequencing platform is yet to be made. Initial estimates indicate that members of the sample population carry mutations in 4-5 of our genes of interest. The next phase of the program is to determine sensitivity and specificity of the test for the major classes of mutations in a larger cohort of individuals. The panel currently contains 422 genes causing 430 such diseases. The results to date will be presented.

1641/W

Mutation Analysis of SLC26A4 for Pendred Syndrome and Non-Syndromic Hearing Loss by High Resolution Melting. *N. Chen, I. Schrijver.* Dept Pathology, Stanford Univ, Palo Alto, CA.

Pendred syndrome and DFNB4 are associated with autosomal recessive congenital hearing loss. Up to 50% of patients with Pendred syndrome/DFNB4 carry SLC26A4 mutations. Extensive allelic heterogeneity necessitates analysis of all exons and splice sites to identify mutations for individual patients and sequencing is the gold standard for clinical mutation detection. Denaturing high performance liquid chromatography (dHPLC) is more cost-effective and less time-consuming, but it cannot distinguish homozygous mutations from wild-type sequences. Such mutations require the mixing of wild-type DNA with a patient sample to form heteroduplexes. High resolution melting (HRM), a straight-forward and fast scanning method, is capable of detecting both heterozygous and homozygous mutations cost-effectively. We developed a closed-tube clinical HRM mutation detection method specific for the SLC26A4 gene. Twenty-eight amplicons were designed to cover all 21 SLC26A4 exons and splice junction sequences. Using these amplicons, HRM analysis detected every one of the 47 variants initially identified by diagnostic sequencing. Subsequently, a 384-well diagnostic plate format was designed for routine clinical testing. On each plate, 6 adjacent wells were reserved for each of the 28 amplicons. On one diagnostic plate, up to three patient samples could be tested per run. To evaluate within-run and run-to-run variations, a diagnostic plate with DNA from one patient was run 4 times to measure melting temperature (Tm) of each well. Therefore, 6 wells of each amplicon in each run were analyzed for within-run variations. Run-to-run variations of each amplicon were compared in the 4 replicated runs. Tm analysis of the results indicated that the standard deviation (SD) of within-run variation was less than 0.09°C in 26 of the 28 amplicons. For the other two amplicons, one had one run with an SD of 0.1°C and one had two runs with an SD of 0.11°C. The SD between runs was less than 0.04°C for all 28 amplicons. Subsequent blinded HRM testing of more patient samples detected all variants identified by diagnostic sequencing. Among the variants detected, there were new variants that were not included in the initial set of 47. In conclusion, HRM is a reliable, simple and cost-effective method for SLC26A4 gene mutation detection.

1642/W

A rapid closed-tube method for screening point mutations in Duchenne muscular dystrophy (DMD). *P.S. Lai[1], O.S. Yim[1], S.K.H. Tay[1], P.S. Low[1], E.P.H. Yap[2].* 1) Dept Pediatrics, National Univ Singapore, Singapore, Singapore; 2) DSO National Laboratories, Defence Medical and Environmental Research Institute, Singapore.

Duchenne Muscular Dystrophy (DMD) is an X-linked recessive disorder. Effective mutation screening for families affected with DMD will allow for genetic counseling, carrier screening and molecular diagnosis. Determination of the precise type of mutations present in patients can also contribute towards the identification of cohorts amenable for different types of genetic treatment approaches such as antisense splicing or nonsense codon suppression. However, screening of the DMD gene responsible for this disorder presents special challenges due to its large size and complexity. The most common and easily detectable mutations in DMD are gross deletions or duplications. However, point mutations account for the remaining 40% of patients. Conventional screening for point mutations utilizes PCR-based DNA sequencing and more than 100 amplification reactions are needed to analyze the entire gene coding regions. This study reports a method for rapid screening of point mutations in the DMD gene using High Resolution Melt (HRM) analysis. Amplification and screening are carried out in the same tube without any additional post-PCR steps of reagent addition or manipulation of products. HRM analysis was found to be sensitive and accurate in identifying mutations in DMD amplicons ranging from 130-200 bp. Carrier screening could be carried out in families with mutations. A patient and three female carriers were identified in a family carrying an exon 60 nonsense mutation (UGA stop codon). The HRM results were confirmed to be accurate by re-sequencing. Subsequent genetic correction assays for the nonsense codon were carried out using aminoglycoside-induced translational readthrough approach. A construct carrying this exon 60 nonsense mutation in a reporter gene vector was assayed for luciferase activity after treatment with different concentrations of gentamycin, paromomycin and tobramycin at 24, 48 and 72 hours. Highest readthroughs of about 18% was observed using 2.0 mg/ml gentamycin at 48 hours treatment indicating gene correction during translation. Our results show that HRM screening can identify point mutations, in particular nonsense mutations which are amenable for genetic correction by a drug-induced translation readthough approach. It can thus be used for rapid molecular diagnosis, carrier screening and identification of cohorts suitable for future therapeutic trials.

1643/W

Rapid identification of common β-thalassemia mutations in the Chinese population using duplex or triplex amplicon genotyping by high-resolution melting analysis. *Z. Ren, X. He, M. Xu, C. Xiong.* Shanghai Inst Med Gen, Shanghai Children's Hosp, Shanghai Jiaotong Univ School of Medicine, 24/1400 West Beijing Rd, Shanghai 200040, China.

β-Thalassemia is one of the most prevalent inherited diseases in China. To date, over 20 β-thalassemia mutations have been identified in the Chinese population, and four mutations (CD41-42 (-4 bp), IVS-2-654 C®T, CD17 A®T, and -28 A®G) account for approximately 90% of the cases. Therefore, the exploration of simple, reliable, and rapid approaches for molecular detection of these common mutations is important for prevention and early diagnosis of the disease. High-resolution melting (HRM) analysis is a new technique for mutation detection that has the advantages of rapidity, accuracy, and convenience. Building on one-amplicon genotyping by HRM analysis, we developed duplex and triplex amplicon genotyping to simultaneously identify these common β-thalassemia mutations in patients or carriers. Two or three sets of primers were combined to conduct duplex or triplex amplicon genotyping that distinguished a variety of genotypes by HRM based on the melting curve shapes. 71 DNA samples from β-thalassemia traits or patients were analyzed using the described approaches and 65 were identified to carry the four common β-thalassemia alleles including 56 heterozygous mutations (23 for CD41-42 (-4 bp), 18 for IVS-2-654 C®T, 11 for CD17A®T, and 4 for -28 A®G), 3 homozygous mutations for IVS-2-654C®T, and 6 compound heterozygous mutations [CD41-42 (-4 bp)/ IVS-2-654C®T (four cases), -28 A®G /CD17A®T (one case), IVS-2-654C®T /CD17A®T (one case)]. The whole procedure for mutation detection was completed within only half an hour. The results derived from HRM analysis were fully in accordance with sequencing. We suggest this rapid and accurate method for molecular screening to detect the common β-thalassemia mutations in the Chinese population.

1644/W

Preferential amplification due to DNA secondary structure - a potential cause of diagnostic error. *Y. Wang, S.J. Steinberg, B.A. Karczeski, G.R. Cutting.* DNA Diagnostic Lab, Johns Hopkins Univ, Baltimore, MD.

Molecular diagnosis based on the polymerase chain reaction (PCR) is widely used in clinical laboratories. While PCR has revolutionized diagnostics, it has some shortcomings such as preferential amplification that can lead to errors. We investigated mechanisms underlying preferential amplification of exon 3 (e3) in the α-ENAC gene (*SCNN1A*) in a Pseudohypoaldosteronism type 1A patient and her parents. Initial test results for the proband revealed apparent homozygosity for adjacent nucleotide changes (c.540_541delGCinsTT). Parental testing revealed paternal heterozygosity for the sequence variation and apparent maternal homozygosity for wildtype. Heterozygous SNPs flanking e3 in the mother excluded a maternal deletion. Re-testing revealed heterozygosity in the proband for 4 of 9 assays. Use of multiple primer pairs excluded polymorphism within the primer sequence as the cause of the inconsistency. Since this region of the *SCNN1A* gene is GC-rich, we considered whether a structural feature affected the ability of *Taq* polymerase to copy e3 bearing the wild-type sequence. G-Quadruplex and i-Motif structures predicted by QGRS Mapper and Quadfinder were excluded. However, the MFold algorithm revealed that the two mutated nucleotides reside within a predicted stem-loop structure, and sequence bearing c.540_541GC was predicted to form a more stable stem-loop structure (ΔG= -10.53) than the sequence bearing c.540_541TT (ΔG= -8.2). To test the secondary structure predictions, we altered the annealing temperature of the PCR reaction, theorizing that the higher temperature would destabilize the stem-loop structure, thereby facilitating amplification of the wild-type sequence. Indeed, increasing the annealing temperature from 62° C to 68° C resulted in consistent amplification of both alleles from the heterozygous patient and her father (n=6). Furthermore, decreasing the annealing temperature by 2° C to 60° C resulted in only mutant sequence being amplified (n=6). These observations highlight a previously unrecognized source of mis-genotyping of a rare allele due to differential amplification of mutated and wild-type sequence. For this reason, segregation analysis should be employed for any rare sequence variant that appears homozygous to exclude diagnostic error due to preferential PCR amplification.

1645/W

Receiving genetic results: exploration of this experience in familial epilepsy. *D.F. Vears[1], K. Dunn[2], S.A. Wake[2,3], I.E. Scheffer[1,4].* 1) Epilepsy Research Centre, University of Melbourne, Austin Health, Melbourne, Australia; 2) Department of Paediatics, University of Melbourne, Melbourne, Australia; 3) Genetic Health Services Victoria, Royal Children's Hospital, Melbourne, Australia; 4) Department of Paediatrics, University of Melbourne, Royal Children's Hospital, Melbourne, Australia.

Statement of purpose: Rapid advances in genetic technology have lead to gene identification for many inherited disorders. There is little information, however, on the impact of gene identification for individuals and families with genetic conditions. This study explored the experience of receiving a genetic result in people with familial epilepsy in whom a genetic cause was identified. **Methods:** Using a targeted sampling strategy, individuals who had participated in a previous study identifying familial epilepsy genes and who had received a positive genetic test, were invited to participate. A wide selection of family members were invited to participate. In-depth, semi-structured interviews were conducted. Interviews were audio-taped and transcribed. The data underwent content and thematic analysis. **Results summary:** 20 individuals from 3 families with different epilepsy syndromes and different epilepsy genes were interviewed across 14 interviews. Multiple generations within families were represented. The mean time of receiving the genetic result prior to the study was 10.9 years (range 5-14 years). Three major themes were identified: (i) living with epilepsy; (ii) clinical utility of the test; and (iii) 'talking about the family genes'. How epilepsy affected the individual influences the impact of receiving genetic results and the use of genetic information for reproductive decision-making. While altruism can influence decisions to participate in studies of gene identification, people also want to know what this means for them and how this may help them and their families. It cannot be assumed that family members communicate about their epilepsy. Information that may benefit others in the family may not be shared and understanding of genetic inheritance may be limited. **Conclusions:** While this study focused on familial epilepsy, the findings are relevant to other genetic disorders. The results can inform the development of guidelines for genetic result disclosure, in the context of the research study setting, and more broadly. They also reveal important insights into the family experience of genetic conditions and communication within families, and can be used to improve genetic counselling for individuals and families.

1646/W

Our Experience of Paternity Tests. *M. Korabecna[1], A. Horinek[2].* 1) Faculty of Medicine in Pilsen, Charles University, Czech Republic; 2) 1st Faculty of Medicine, Charles University, Prague, Czech Republic.

AIM: We selected randomly 100 cases from our database to demonstrate the extent of tasks they are solved by Czech laboratories involved in paternity testing. We evaluate the efficiency of our approach based on DNA profiling with PowerPlex 16 not only in classical trios but also in motherless files and deficient files in which the putative father of a person is not available and the paternity testing is dependent on the DNA analysis of his relatives. METHODS: DNA samples were isolated from buccal swabs using QIAamp DNA Blood Mini Kit (Qiagen). For STR analysis, PowerPlex16 (Promega) and AmpFlSTR Yfiler PCR (AppliedBiosystems) were used. The results of STR analysis on autosomal loci were evaluated using the program Familias (Egeland et al., For Sci Int 2000). RESULTS: We found 34 % of trios (accused man, mother and child) in which paternity was excluded, 59% trios in which paternity was practically proven, 2% of motherless files (only putative father and child were tested) and 5% of deficient files in which we tested different relatives of putative fathers - most often their parents but also their siblings and children. In cases of excluded paternity, the inconsistencies were found in 94.11% in more than seven polymorphisms using PowerPlex 16. In such cases, inconsistencies in 8 polymorphisms were detected in 26.5%, in 9 polymorphisms in 14.7%, in 10 polymorphisms in 20.6%, in 11 polymorphisms in 11.5 %, and in 12 polymorphisms in 11.8%. The exclusion was based most often on inconsistencies on loci D21S11, D18S51 and Penta E (76.5% each), vWA (70.6%), and FGA (67.6%). Inconsistencies on some loci were founded more rarely - in TPOX (38.2%),in CSF1P0 (41.2%)and D16S539 (44.1%). In cases in which paternity has been practically proven, probability of paternity reached the values W=99.999999% in 30.5%, W= 99.99999% in 27.1%. The values of paternity index (PI) over 10^{10} were obtained in 8.5%.For motherless files, we reported W= 99.999% using PowerPlex 16 only. In deficient cases, the values of posterior probability calculated using Familias and population data for Czech population (STR loci involved in PowerPlex 16) were in interval from 99.9 to 99.999%. CONCLUSION: Our combination of STR analysis together with application of the program Familias seems to be sufficient for the successful solution of all common cases. The posterior probabilities calculated for deficient cases provided evidence for or against paternity in each tested family.

1647/W

Can informed decision making be measured in population carrier screening for fragile X syndrome? *S. Metcalfe[1, 2], A. Ames[1,2], A. Archibald[1,2], J. Cohen[3], J. Emery[4], M. Hill[1,5], M. Martyn[1], O. Ukoumunne[1,2].* 1) Murdoch Childrens Research Institute, Royal Children's Hospital, Melbourne, Australia; 2) Dept Paediatrics, The University of Melbourne, Melbourne, Australia; 3) Fragile X Alliance Inc, and Centre for Developmental Disability Health Victoria, Department of General Practice, Monash University, Melbourne, Australia; 4) School of Primary, Aboriginal and Rural Health Care, University of Western Australia, Perth, Australia; 5) Clinical and Molecular Genetics, Institute of Child Health and Great Ormond St Hospital for Children, NHS Trust, London, United Kingdom.

Informed decision making (IDM) is important so that individuals can make autonomous decisions without coercion or deception. Tools have been developed to measure IDM in prenatal Down syndrome screening, eg the multidimensional measure of informed choice (MMIC), plus a deliberation (D) scale to measure weighing up of the pros and cons (ie IDM = MMIC+D). However these tools have not been applied to carrier screening. We are currently offering carrier screening for fragile X syndrome (FXS) to non-pregnant women from the general population and applying existing measures to examine IDM in this context. 156 women completed a questionnaire at the time of deciding about testing and, if tested, provided a saliva sample. The questionnaire contained measures of FXS knowledge, attitudes and deliberation, as well as other questions derived from the Health Belief Model. The MMIC and IDM models were applied to these data, including that of test uptake (the behaviour). Results of the measures used in the questionnaire were: test uptake (85%); good knowledge (79%); positive attitudes (80%); deliberated (87%). 71% of women made an 'informed choice' (defined as being tested, having good knowledge and positive attitudes or not tested, good knowledge and negative attitudes). 65% of women made an 'informed decision' (defined as being tested, having good knowledge, positive attitudes and deliberated or not tested, good knowledge, negative attitudes and deliberated). A sub-group of 28 women were also interviewed. Interview responses were coded by three independent researchers using content analysis. Responses in the semi-structured interviews were compared with corresponding responses in the questionnaires to triangulate the findings. Interview and questionnaire findings were discrepant in 12/28 cases, ie 12 women were misclassified according to the models. Interviews highlighted factors other than poor knowledge, which contributed to the apparent lack of IDM, primarily with a mismatch between attitudes and behaviour. These factors, such as not planning a family at the time or apparent lack of deliberation because of prior experiences, are not captured by applying these models. To assess IDM in contexts other than prenatal screening, alternate approaches to these models may be needed to account for these other factors.

1648/W

Design of a triplet repeat-primed PCR protocol for identification of fragile X syndrome. *R.C. Niessen, S. Bronowski, P.M. Grootscholten, R.J. Sinke.* Department of Genetics, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands.

The fragile X syndrome is a frequent cause of X-linked mental retardation and is caused by expansion of a CGG repeat in the 5'untranslated region of the fragile X mental retardation gene (*FMR1*). Repeats larger than 200 repeat units induce hypermethylation of the CpG island in the promoter region of *FMR1* which subsequently results in transcriptional silencing of the gene. To confirm or exclude the clinical diagnosis of fragile X syndrome one needs to determine the length of the CGG repeat in the *FMR1* 5'untranslated region. This usually involves PCR and analysis on automated sequencers. In cases where the outcome of the PCR or family history is suggestive for an expansion, Southern blotting is performed to detect larger expansions (>70 repeats). This technique is very labour-intensive, costly and often requires radioactive labeled probes. Currently, no technique is available which can replace Southern blotting completely. Warner et al. developed an alternative technique for Southern analysis, the triplet repeat-primed PCR (TP-PCR) to screen for presence of expanded CAG repeats in myotonic dystrophy patients (J Med genet 1996;33;1022-1026). We adapted this TP-PCR protocol for the detection of expanded CGG repeats in patients suspected for fragile X syndrome. In our protocol, a fluorescently labeled, locus-specific primer flanking the CGG repeat (P1) is used together with a non-specific primer (P4) which primes at any position within the CGG repeat in the first cycles of the PCR. This non-specific primer has a tail-sequence which is a target for a third primer (P3), which is used in the following cycles of the PCR when the non-specific primer is depleted. In this way, PCR fragments of different lengths are generated and produce a characteristic ladder on an automated sequencer. TP-PCR on DNA from carriers of an expanded CGG repeat results in significantly larger 'ladders' - in other words in broader peak patterns - than DNA from non-carriers. By use of this method presence of an expanded *FMR1* allele can be excluded in women who appear to be homozygous for a normal CGG repeat after regular *FMR1* PCR. Here we show that implementation of triplet repeat-primed PCR techniques is a robust, sensitive and cost-effective means of screening for *FMR1* expansions.

1649/W

Toward elimination of Southern blot analysis for fragile X syndrome: A rapid and sensitive PCR-based assay for accurate sizing of FMR1 alleles, and the detection of full mutation CGG expansions. *C. Stolle, T. Tischler, A. Santani.* Molecular Genetics Laboratory, Dept of Pathology & Lab Med, Children's Hosp Philadelphia, Philadelphia, PA.

In a pediatric setting, molecular diagnosis of fragile X syndrome is used to determine if a child has mental retardation or developmental delay due to an expanded, hypermethylated FMR1 allele. For this purpose, Southern blot analysis (SB) has been the only method capable of detecting both expanded alleles and methylation status. However, SB is labor intensive, time consuming, and unreliable for allele sizing. The exact size of alleles in the normal to premutation range is important for risk assessment and diagnosis of fragile X associated tremor and ataxia syndrome and premature ovarian failure. PCR methods may be used to size alleles, but some methods cannot detect expanded alleles and require SB for males with no amplified allele, females with a single allele, potential mosaics, and to determine the methylation status of large premutations. We describe validation testing of a commercially available PCR assay capable of detecting large alleles and accurately sizing alleles in the normal to premutation range. Validation samples consisting of normal, gray zone, premutation, and full mutation alleles from males, mosaics males, and females previously analyzed by SB, as well as DNA samples of known repeat size, were analyzed using the AmplideX™ Gene Specific PCR and/or CGG Repeat Primed PCR reagents (Asuragen) and resolved by capillary electrophoresis (ABI 3730). Allele sizing was performed using Gene Mapper. The observed allele size of DNAs with a known number of CGG repeats exactly matched expected values for alleles in the 20-80 repeat range. Heterozygous females with alleles differing by a single repeat were clearly resolved and accurately sized. One premutation allele exhibited a one repeat difference in size (91 vs 92), suggesting that this assay is capable of sizing alleles with an accuracy of +/- 1 repeat. Detection of full expansions was in 100% agreement with SB results in both male and female samples. As expected, the PCR method was superior to SB in sizing normal, gray zone, and premutation alleles. Differences in allele sizing resulted in reclassification of the allele designation in 6 out of 62 samples. Mosaic males, females with a full expansions, and homozygous females were also correctly identified. This assay enables detection of alleles in all size ranges and accurate allele sizing in the normal to premutation range. Reflex testing by SB may only be required to determine the methylation status of alleles in the high premutation range.

1650/W

Narrowing the approach to identifying genes and characterizing novel genotypes associated with neuromuscular diseases. *A. Ankala[1], J. Kohn[1], S. Bhide[1], C. Bonneman[2], S. Sparks[3], S. Khadilkar[4], M. Hegde[1].* 1) Department of Human Genetics, Emory University School of Medicine, Atlanta, GA; 2) Division of Neurology, The Children's Hospital of Philadelphia, Philadelphia, PA; 3) Department of Pediatrics, Levine Children's Hospital at Carolinas Medical Center, Charlotte, NC; 4) Department of Neurology, Bombay Hospital and Medical Research Centre, Mumbai, India.

Inherited neuromuscular disorders (NMDs) form a large and heterogeneous group of genetic diseases that cause progressive degeneration of the muscles and/or motor nerves that control movements. Most NMDs result in chronic long-term disability placing a significant burden on patients, their families, and the health care system. Affected patients develop multiple and cumulative symptoms during disease progression which may result in premature death from cardiac and respiratory muscle impairment. NMDs are present in all populations, affecting children as well as adults and is estimated that about 1 in 1000 people may have a disabling inherited neuromuscular disorder. The precise diagnosis and treatment of NMDs require a combination of extensive clinical examination and targeted complementary tests: biological analyses, electromyography, imaging, and histological analysis of biopsies. Currently, molecular genetics analyses are performed on known implicated genes both to confirm the clinical diagnosis and to determine the precise genotype for each patient. However, owing to the several factors including frequent overlap of clinical phenotypes, the large number of affected genes, and lack of common mutations, picking the right gene for molecular diagnosis is challenging. This eventually ends in having to perform highly complex, expensive, and time-consuming analyses on several genes. Also, since many of the genes potentially responsible for these diseases are not yet known, many patients remain undiagnosed; to date, these amount to 30-40%. Therefore, in contrast to the current approach, a differential molecular genotyping is required. The new era of medical genetics focused on investigating rare disorders demands the identification of the causative genes and their mutations. We have developed a sequential, cost effective and comprehensive approach to identify new genes associated with NMDs which includes; grouping them according to phenotype, performing multiplex western blot analysis to compare protein expression, followed by gene expression analysis and high throughput next generation sequencing to identify the causative gene. We present here the effectiveness of our strategy with data from two groups; 1. Isolated cases of CMD- like phenotype; 2. A large kindred with multiple affected descendants from a common ancestor. Our ultimate goal is to create a comprehensive map of the muscle genome and proteome, which may enable development of new therapeutics.

1651/W

Investigation of massively parallel targeted exome sequencing for clinical diagnostic testing and gene discovery in primary ciliary dyskinesia. *J. Berg[1,2], K. Weck[1,3], M. Leigh[4], M. Knowles[2], J. Evans[1,2], M. Zariwala[3].* 1) Department of Genetics, UNC Chapel Hill, Chapel Hill, NC; 2) Department of Medicine, UNC Chapel Hill, Chapel Hill, NC; 3) Department of Pathology and Lab Medicine, UNC Chapel Hill, Chapel Hill, NC; 4) Department of Pediatrics, UNC Chapel Hill, Chapel Hill, NC.

BACKGROUND: Primary ciliary dyskinesia (PCD) is an autosomal recessive disorder associated with impaired mucociliary clearance. Ultrastructural studies suggest a common role of molecular defects in components of the cilia, and mutations in nine different genes have been identified in PCD patients. Advances in genetic sequencing technology are revolutionizing genetic research and have the potential to enhance genetic testing for panels of genes associated with genetically heterogenous clinical syndromes. The objective of this study was to investigate the performance characteristics of exon-capture technology coupled with massively parallel sequencing in the diagnostic evaluation of a highly heterogeneous condition such as PCD. METHODS: We performed a pilot study of four individuals with known PCD mutations (three compound heterozygous and one with only a single known mutation) to determine whether we could identify different classes of mutations including missense, splice site, nonsense, small insertions/deletions, and whole exon deletions. We designed a custom exon capture array (Nimblegen) consisting of 2089 exons from 83 genes, including all genes known to be associated with PCD and a large number of candidate ciliary genes. Sequencing of the enriched material was performed using GS FLX Titanium Sequencing (Roche 454). Bioinformatics analysis was performed in a blinded fashion in an attempt to detect the known mutations and validate the process. RESULTS: Four of seven known mutations were readily identified using this methodology. A fifth known mutation was identified after adjusting the bioinformatics handling of known SNPs. A potentially deleterious mutation in a second gene was identified in a patient for whom only one mutation had been previously identified, raising the possibility of digenic inheritance. Numerous other variants were also detected, which may indicate potential genetic modifiers of the PCD phenotype. However, this process failed to detect two known mutations: a single nucleotide insertion and a whole exon deletion. Additional retrospective bioinformatics analysis revealed strong sequence-based evidence for the single nucleotide insertion but failed to detect the whole exon deletion. CONCLUSIONS: Massively parallel sequencing has great potential for both research and clinical diagnostics, but further accuracy and validation are required before widespread adoption in a clinical setting.

1652/W

Molecular Genetics of Carbamoyl Phosphate Synthetase I Deficiency. *L. Chen[1], J. Wang[1], O. Shchelochkov[2], J. Häberle[3], F. Li[1], H. Zhan[1], E. Brundage[1,4], A. Pursley[1], E. Schmitt[1], L. Wong[1].* 1) Dept. of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Dept. of Pediatrics, Division of Genetics, University of Iowa Hospitals and Clinics, , Iowa City, IA; 3) University Children's Hospital Zurich, Division of Metabolism, 8032 Zürich, Switzerland; 4) GeneDx, Gaithersburg, MD.

The urea cycle disorders (UCD) are inherited defects in the disposal of nitrogenous waste produced from the breakdown of protein and other nitrogen-containing compounds. Carbamoyl phosphate synthetase I (CPSI) is the first enzyme and one of the rate limiting steps in the urea cycle. CPSI deficiency is an autosomal recessive disorder and usually results in severe hyperammonemia presenting in the first days of life warranting prompt diagnosis. It is often difficult to distinguish CPSI deficiency from other proximal urea cycle disorders, such as OTC and NAGS deficiencies based upon the clinical presentation and biochemical tests alone. Therefore mutation analysis of the CPS1 gene is important for diagnostic confirmation, prenatal diagnosis, and counseling. A total of 81 samples from patients suspected of having CPSI deficiency were analyzed. All 38 coding exons of the CPS1 gene and their flanking intron regions were PCR-amplified and sequenced. Only four of the 35 identified mutations were previously reported. The novel changes were either protein truncation mutations or predicted to be deleterious missense mutations by the Polyphen and SIFT computer algorithms. Among the identified mutations, 60% (21/35) were missense, 20% (7/35) were small insertion/deletion mutations, and 20% (7/35) were protein truncation mutations (nonsense and splice site changes). Four cases of CPS1 gross deletions were detected by targeted oligonucleotide microarray MitoMet®. The deletion sizes varied from 1.4 kb to 130 kb. We estimate that the incidence of gross deletions in the CPS1 gene to be approximately 2-3%. All four patients in this study presented with hyperammonemia in early infancy, suggesting that CPS1 deletion confers a risk of early-onset CPSI deficiency. We recommend using aCGH to detect gross intragenic deletion when only one mutation can be found in patients with clinical and biochemical features of CPSI deficiency or there are other indications for the presence of intragenic CPS1 deletion. Five patients negative for CPS1 mutations were subsequently found to have mutations in the OTC gene, including two female patients with large deletions involving the OTC gene were detected by MitoMet® oligonucleotide array and three male patients who had point mutations in OTC gene detected by sequencing. Our results underscore the importance of comprehensive molecular testing in order to diagnose and appropriately counsel families with urea cycle disorders.

1653/W

EuroGentest Clinical Utility Gene Cards - expert documents for the evaluation of genetic tests. *A. Dierking, P. Javaher, E. Nyoungui, J. Schmidtke.* Insitute of Human Genetics, Hannover Medical School, Hannover, Germany.

Clinical utility refers to the ability of genetic test results, either positive or negative, to provide information that is of value in the clinical setting. From many different perspectives this is the most important but on the other hand also the most difficult aspect to define in the context of genetic testing. A major challenge is to balance clinical validity, clinical utility and cost-benefit issues. In some cases a test is performing superbly in the laboratory, but is not viable from the clinical or economical point of view. On the other hand some tests are limited in their validity, but nevertheless have great impact on patient and family management. It is therefore important that (1) the specific requirements for a test with regard to clinical validity are defined in the context of their impact on the clinical setting and (2) that the laboratory genetic test is only one of the components of an overall evaluation and/or intervention. EuroGentest, an EU-funded "Network of Excellence" designed a work package establishing and administering guidelines assessing the clinical utility of genetic testing: Clinical Utility Gene Cards (CUGCs). Sharing the main components analytical validity, clinical validity, clinical utility as well as ethical, legal and social issues with the CDC (Centers for Disease Control and Prevention) ACCE model project, the CUGC initiative intends to additionally cover highly context-dependent aspects such as health care system, locally available resources and set priorities. Also unique is the approach regarding guideline publication. International experts are invited, according to their field of expertise and interest, to convene multinational author teams in order to develop a CUGC. After completion all guidelines are freely available on the EuroGentest and ESHG (European Society of Human Genetics) websites and in the European Journal of Human Genetics, where they are published online and in print. As of May 2010, 17 CUGCs have been finalised. Since the beginning of the year 2010, invitations for 250 CUGCs were sent out. Within the next EuroGentest funding period (2011-2013) another 300 documents are planned to be solicited and published and previously existing ones will be revised on an annual basis. With regards to content, the focus of this work lies on Mendelian, mostly rare, disorders. Further inclusion of diseases with varying causes, e.g. chromosomal aberration, is being discussed at present.

1654/W

Molecular genetic testing of podocin (*NPHS2*) mutations in steroid-resistant nephrotic syndrome by high resolution melting curve analysis. *M. Lu[1], A. Mottl[2], K. Weck[1].* 1) Dept. of Pathology and Lab Medicine, UNC at Chapel Hill, Chapel Hill, NC; 2) Dept. of Medicine and Nephrology, UNC at Chapel Hill, Chapel Hill, NC.

Background: Steroid-resistant nephrotic syndrome(SRNS)occurs mainly in children, typically manifests histologically as focal segmental glomerulosclerosis (FSGS) and often progresses to end-stage renal failure. Mutations in NPHS2 have been associated with autosomal recessive SRNS, with important and variable correlations between genotype and clinical features such as age of onset, severity and treatment responses. The detection of NPHS2 mutations is of potential clinical utility as it could prevent unnecessary steroid treatment and allow risk stratification for post-transplant FSGS recurrence. Our goals are to: 1) develop mutation scanning by high resolution melting curve analysis (HRMA) to screen for mutations in FSGS associated genes, and 2) better characterize the spectrum, incidence and genotype-phenotype correlation of mutations in a cohort of well-characterized patients with biopsy proven primary FSGS or SRNS. **Methods:** We developed HRMA and DNA sequencing conditions for all 8 exons of NPHS2. PCR products were tested by HRMA on a LightScanner (Idaho Technologies) and by DNA sequencing on a 3130xl genetic analyzer (Applied Biosystems). The sensitivity and specificity of HRMA for detecting DNA variants were compared with DNA sequencing. **Results:** Analysis of 10 normal control DNA samples and 64 patient samples showed mutation scanning by HRMA had 100% sensitivity and 93% specificity compared to direct DNA sequencing for detection of all DNA variants. Standardized parameters were established from these analyses. Of 64 patients with biopsy proven primary FSGS, we detected NPHS2 variants in three steroid resistant patients: two patients were compound heterozygous for R229Q and another podocin mutation, which typically leads to mild SRNS in young adults. The third patient was homozygous for R138Q, which leads to early onset SRNS and rapid progression to end stage renal disease. One novel missense mutation (Arg10Thr) in an evolutionally conserved region was found in two steroid resistant patients; the clinical significance of this variant has not been established. **Conclusions:** In summary, mutation scanning by HRMA is a sensitive method for detecting variants in NPHS2. Future work includes testing of a larger sample pool of patients with SRNS, in order to further characterize the clinical features and genotype-phenotype correlation of NPHS2 mutations, and establishing standardized procedures for NPHS2 clinical testing.

1655/W

Genetic Counseling and Testing for Hypertrophic and Dilated Cardiomyopathy: Uptake of genetic testing among family members. *E.M. Miller, S.M. Ware.* Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

Up to 70% of adults with hypertrophic cardiomyopathy (HCM) and 30% with dilated cardiomyopathy (DCM) have positive clinical molecular genetic testing. One of the primary benefits of genetic testing is identification of at risk family members, and recent guidelines recommend increased cardiac surveillance in mutation positive, asymptomatic individuals. This study aims to identify uptake of genetic testing among first and second degree relatives of patients with mutation confirmed HCM and DCM. Thirty-seven unrelated patients (31 HCM, 6 DCM) underwent genetic testing for a personal history of HCM or DCM from October 2006-April 2010. Of 37 probands tested, 28 (76%) had positive test results; 57% of probands had a positive family history, with a disease causing mutation identified in 86% (18/21). Of 16 probands without a positive family history at presentation, 63% (10/16) had positive genetic testing results and nine (56%) subsequently had family members clinically diagnosed with cardiomyopathy as a result of their evaluation and recommendation for family screening with echocardiogram. Patients who received a positive test result were encouraged to share their results with family members and >80% received a post-testing counseling letter. Genetic testing was indicated for 132 first and second degree relatives. Forty-two first degree relatives (49%) and 9 second degree relatives (19%) underwent genetic testing. Nineteen relatives received negative results and therefore could be excluded from ongoing cardiac surveillance. Positive genetic testing results were obtained in a high percentage of probands regardless of family history of cardiomyopathy. Genetic testing and cardiac screening both identified previously unrecognized affected or at risk individuals, facilitating appropriate medical intervention. These data are the first analysis of uptake of genetic testing among first and second degree relatives of patients with mutation positive DCM and HCM and demonstrate the importance of genetic counseling and family based care in this population.

1656/W

Small pathogenic copy number changes detected by whole-genome array CGH define novel dosage-sensitive regions. *L. Schmidt, S. Warren, N. Flores, G. Richard, S. Aradhya.* GeneDx, Gaithersburg, MD.

Whole-genome array CGH is a powerful new standard for chromosomal analysis. Its application has become widespread, leading to the discovery of novel genomic disorders. Several of these disorders are due to large copy number changes but some, such as the 17q21.31 or the 1q21 microdeletions, are caused by <1 Mb deletions. We have used whole-genome array CGH to analyze 7,500 cases and identified positive findings in 25% of cases. Of these, 151 cases involved copy number changes < 2 Mb and that had occurred de novo, thus offering the opportunity to define novel dosage-sensitive genomic regions at a high resolution. Of the 151 de novo cases, nearly 30% were associated with newly defined microdeletion syndromes, thereby confirming the validity of this analysis. For example, nine cases involved the Williams syndrome deletion, another nine represented the autism-linked 16p11.2 deletion, seven matched the 17q21 microdeletion, and one corresponded to the 1q21 TAR syndrome microdeletion. The remaining 70% of cases involved either single genes associated with known Mendelian disorders or novel copy number changes affecting genes that, when haploinsufficient, may be associated with a clinical outcome. Many of these genes are not associated with clinical disorders at present and may be sensitive to dosage. As might be expected of pathogenic genomic changes, particularly when affecting a few genes, most findings (~65%) in this data set involved deletions rather than duplications. These data represent an initial precursor to a high-resolution whole-genome dosage map as more cytogenetic array CGH data is accumulated in the coming years.

1657/W

An automated analysis protocol for research of KRAS/BRAF mutation detection for data generated on capillary electrophoresis instruments. *S. Sharp[1], E. Currie-Fraser[1], J. Walker[1], J. Wang[2].* 1) Life Technologies, Foster City, CA; 2) Trimgen, Sparks, MD.

Biomarker research continues to be an important focus in oncology studies, including the role of KRAS and BRAF mutations in CRC and other EGFR-associated cancers. This has lead to increased research of these genes as possible predictive markers and targets for continued study. With this increased interest comes a need for automation of data analysis and report generation to decrease bottlenecks in the research laboratory by reducing manual review time. This poster will present an automated workflow for detection of KRAS and BRAF mutations and concise report generation in sample data generated on capillary electrophoresis instruments using fragment analysis software tools. We will demonstrate how key features in the software, such as sample quality values, allele binning and report analysis, enable this workflow to be a significant improvement over visual scoring methods.

1658/W

Monogenic diabetes due to mutations in 5 MODY genes: Distribution of disease-associated mutations and implications for approaches to molecular diagnostic testing. *C.M. Stanley[1], U. Geigenmuller[2], N. Leach[2], T. Love[2], S.D. Batish[1], M. Chen[1], E. Couchon[1], M. Morra[2], M. Ito[1].* 1) Athena Diagnostics, Worcester, MA; 2) Correlagen Diagnostics, Waltham, MA.

An estimated 2-5% of the 23.6 million people in the United States with diabetes have monogenic diabetes, also known as maturity onset diabetes of the young (MODY), an autosomal dominant disorder caused by a mutation in one of several MODY genes affecting the development and function of the pancreatic β cells. Patients with MODY differ clinically from those with type 1 or type 2 diabetes and genetic testing for MODY should be considered for atypical patients. MODY diagnostic criteria include a family history of diabetes with one family member having been diagnosed before age of 25, negative for autoantibodies, normal weight, noninsulin dependent 3-5 years after diagnosis, noninsulin-resistant and without acanthosis nigricans. Treatment for individuals with MODY differs from treatment for other types of diabetes mainly because many do not require insulin, responding favorably to either oral sulfonylureas or dietary restriction alone. Therefore, a diagnosis of MODY can offer the patient better prediction of disease progression and prognosis, more appropriate treatment, and identification of risks to children or other family members. We report sequencing results for five genes known to cause MODY: *HNF4A* (MODY1), *GCK* (MODY2), *TCF1/HNF1A* (MODY3), *IPF1/PDX1* (MODY4), and *TCF2/HNF1B* (MODY5), which encode transcription factors, with the exception of MODY2 (*GCK*), encoding a glucokinase. Results from 2,427 MODY panel diagnostic reports were used to estimate the positive rate and the relative distribution of the genes that cause MODY. Approximately twenty percent of reports were positive for a previously published or predicted disease-associated mutation. Of these, 59% were positive for MODY2, 32% for MODY3, 3% for MODY1, 3% for MODY4 and 2% for MODY5. Among individuals with MODY, our data support MODY2 as the most common form of monogenic diabetes and MODY3 as the most common form of transcription factor diabetes and though MODYs 1, 4 and 5 are rare, they collectively account for 8% of MODY-positive reports.

1659/W

Patient Contacts, Counseling and RYR1 Genetic Testing Results in an Academic Medical Center. *D. Steele[1], B.W. Brandom[2], J.A. Kant[3], E.E. Smith[1].* 1) Ctr Med Genetics, Magee Woman's Hosp, Pittsburgh, PA; 2) Department of Anesthesiology, University of Pittsburgh Medical Center, Pittsburgh, PA; 3) Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA.

The laboratory received 162 specimens for sequencing of the 19 exons of the ryanodine receptor type one gene (RYR1)in which mutations that cause malignant hyperthermia (MH) have been documented (see www.emhg.org). In those cases referred for testing because of an episode of suspected MH the yield of mutations and variants of unknown significance was 0.38. This included 3 MH deaths. In those cases referred because of positive muscle contracture tests yield was 0.26. This is not statistically significantly different from the yield in suspected episodes. In those cases referred because of a family history of MH yield was 0.29. In cases that were referred to the lab without a known reason for referral the yield was 0.11. Two thirds of the positive results were known MH mutations; one third were variants of unknown significance. However, at least 2 of these variants were found in people with significant pathology consistent with MH. The two most frequent MH mutations were Gly2434Arg and Gly341Arg. A total of 235 contacts were made regarding testing for malignant hyperthermia. 71% of these cases had contact with a board certified genetic counselor. Nineteen had face-to-face genetic counseling sessions. Fifteen families were identified. One family had 3 positive for a familial mutation and 3 negative. Another family had 3 positive for a familial mutation and 1 negative. Of the 8 cases associated with a death 3 were positive for a mutation, 1 for a variant and 1 the sample did not yield a result. One mother tested positive after her son died of a suspected MH episode. By identifying the familial mutation, families have had the opportunity to have targeted RYR1 analysis. Genetic counseling also allowed for recommendations regarding the most appropriate family member to begin testing. This helps to increase the usefulness of the information from the testing and reduce cost for the family. The access to the lab, an anesthesiologist and a genetic counselor has allowed patients and physicians to receive advice on utility of testing for MH, management of patient care and education regarding the North American MH Registry. The RYR1 gene screen with genetic counseling performed at UPMC has been of benefit to MH individuals and families.

1660/W

Experience with Carrier Screening and Prenatal Diagnosis for Sixteen Ashkenazi Jewish Genetic Diseases. *Z. Tan, S.A. Scott, L. Edelmann, L. Liu, M. Luo, R. Kornreich, R.J. Desnick.* Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, NY.

Prenatal carrier screening in the Ashkenazi Jewish (AJ) population has proven an effective disease prevention strategy since enzyme-based carrier detection for Tay-Sachs disease (TSD) began in the early 1970s. Since then, screening panels have expanded as prevalent disease-causing mutations have been identified in the AJ population. Here, we report the AJ carrier frequencies of over 100 mutations in the greater New York metropolitan area AJ population and our experience with the following 16 debilitating recessive disorders: Bloom syndrome (BS), Canavan disease, cystic fibrosis (CF), familial dysautonomia, familial hyperinsulinism, Fanconi anemia group C, Gaucher disease (GD), glycogen storage disease Ia, lipoamide dehydrogenase deficiency, maple syrup urine disease, mucolipidosis type IV, nemaline myopathy (NM), Niemann-Pick disease type A, TSD, and Usher types 1F and 3. Carrier frequencies were determined for individuals of 100% AJ descent and ranged from 0.066 (1 in 15.2; GD) to 0.006 (1 in 168; NM), which were ~15% higher, on average, than those for all screenees. Residual risks among the 100% AJ population ranged from 1 in 281 (GD) to 1 in 13,301 (BS), and the cumulative frequency for all 16 disorders predicted that ~1 in 3.3 AJ will be a carrier for one of these diseases and ~1 in 24 may be a carrier of more than one disease. Among the observed double-heterozygotes, GD and CF were the most commonly carried disorders (1 in 400). Importantly, panel selection among the screenees indicated that the vast majority of AJ individuals (>95%) chose the expanded disease panel, including those disorders with lower carrier frequencies and/or detectability. Carrier screening also identified rare individuals who were homozygous for disease-causing mutations with previously unrecognized clinical manifestations. These studies include prenatal testing results and experience for all 16 disorders (n = 574). Together, these data illustrate the current status, acceptance, and future directions of prenatal carrier screening in the AJ population.

1661/W

Clinical molecular testing for Neurofibromatosis type 1 (NF1)/ Legius syndrome (SPRED1): 88 novel mutations in NF1/SPRED1. *Z. Wang[1, 2], RK. Basran[1, 2], TA. Maher[1], M. Flynn[1, 2], SM. Jamal[1, 2], JM. Milunsky[1, 2, 3].* 1) Ctr Human Genetics, Boston Univ Sch Medicine, Boston, MA; 2) Department of Pediatrics, Boston Univ Sch Medicine, Boston, MA; 3) Department of Genetics and Genomics, Boston Univ Sch Medicine, Boston, MA.

Neurofibromatosis Type 1 (NF1) [MIM# 162200] is one of the most common autosomal dominant disorders affecting ~ 1/ 3,000 individuals. The main clinical features are café-au-lait macules (CALM), cutaneous neurofibromas, lisch nodules, and optic glioma. Legius syndrome is caused by mutations in SPRED1. Legius syndrome is characterized by the presence of CALMs, axillary freckling and macrocephaly without neurofibromas, and is likely underdiagnosed. Accurate diagnosis of NF1 and Legius syndrome is important since NF1 requires different medical management. We describe our experience with samples submitted to our Molecular Diagnostic Laboratory for NF1 testing. Nearly 400 patients (300 probands) from around the world were tested via bidirectional sequencing of the 58 exons of the NF1 gene. Deletion and duplication analysis was performed by MLPA. Targeted prenatal diagnosis of NF1 mutations was completed in 7 families. In our cohort of 300 probands, a total of 130 (43%) were found to have a mutation or an alteration of unknown significance. A total of 120 mutations were identified, of which 87 (72%) are novel changes and the remaining 33 (28%) were previously reported in NF1 patients. The mutation spectrum included small insertions or deletions (31), splice (23), nonsense (33), and missense (34). In addition, MLPA analysis revealed whole exon or entire gene deletions in 8 patients. Mosaicism is suspected in one NF1 patient with a known termination mutation. The pathogenicity of the unreported changes was assessed using Polyphen, Sift and BDGP Splice site software tools, and correlated to the clinical findings. Testing parental samples, when possible, is undertaken to further elucidate the clinical significance of the changes. Of those patients referred for NF1 gene analysis in whom no NF1 mutations were identified, 36 were tested for mutations in the SPRED1 gene by sequencing. Three patients (8%) were found to have a mutation/alteration. The novel alterations identified here in both NF1 and SPRED1 provide a valuable addition to the currently available mutation database and will aid in better interpretation of molecular test results. This study highlights the importance of employing comprehensive sequencing coupled with MLPA in detection of mutations of various types for NF1. Further, our study demonstrates the necessity of SPRED1 testing for NF1 negative patients as a specific molecular diagnosis will allow better anticipatory guidance.

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The utility of exon targeted array CGH in the diagnosis of mitochondrial related disorders. *H. Zhan, F.Y. Li, E. Brundage, A.N. Pursley, J. Wang, L. Wong.* Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Background: Mitochondrial disorders constitute a group of clinically and genetically heterogeneous diseases for which molecular diagnosis has been a challenge. Currently, direct DNA sequencing of candidate genes is the primary technique for the identification of mutations. However, sequencing does not detect large deletions. Oligonucleotide array-based comparative genomic hybridization (oligo aCGH) is presently in clinical use to detect major chromosomal copy number changes. Methods: A custom oligonucleotide array-based comparative genomic hybridization (oligo aCGH) platform was developed to provide both tiled coverage of the entire 16.6-kilobase mitochondrial genome and high-density coverage of a set of nuclear genes involved in mitochondrial and metabolic related disorders, for quick evaluation of copy number changes in nuclear and mitochondrial genomes. A total of 1000 DNA samples from patients suspected of mitochondrial and metabolic disorders were evaluated using this custom exon targeted aCGH. Deletions detected were confirmed by PCR amplification of the deletion breakpoints followed by sequencing. Results: Oligo aCGH identified a total of 61 (6.1%) cases of unrelated individuals with 21 large deletions in urea cycle genes (16 OTC, 4CPS1, and 1 ASS1), 5 in genes (2 DGUOK, 1 each in POLG1, TK2, and MPV17) responsible for mitochondrial DNA (mtDNA) depletion syndromes, 9 cases with deletions in metabolic related genes (SLC25A13, ABCB11, CACT, OCTN2, ALDOB, STS, MCCC1, MECP2, and GK), and 9 cases with large deletions in mtDNA. In almost all cases, the breakpoints were confirmed and determined by PCR/sequencing and the percentage of mtDNA deletion heteroplasmy was also estimated. In addition, 9 and 8 cases, respectively, of large copy number loss and gain involving multiple genes (ranging from 10 kb to 16 Mb) believed to be pathogenic were identified. Conclusions: These examples illustrate the successful utilization of exon targeted custom oligonucleotide arrays to detect either whole gene deletions or intragenic exonic deletions, and deletions in mitochondrial genome. This technology is particularly useful as a complementary diagnostic test in the context of a recessive disease when only one mutant allele is found by sequencing. Furthermore, deletion of as small as a single exon of about 200 bp or as large as entire chromosome can be easily detected using the custom aCGH.

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Cystic Fibrosis mutation screening in a North American population. *V.V. Zvereff, K.J. Friedman.* Center for Molecular Biology and Pathology, Laboratory Corporation of America, Research Triangle Park, NC.

Cystic Fibrosis (OMIM 219700) is one of the most common autosomal recessive genetic disorders. The incidence of CF in the US is estimated to be 1: 3,300 live births (Grody et al. 2001). The disease is caused by mutations in the CFTR gene located at chromosome 7q31.2. Over 1300 CFTR mutations have been identified, but most of these mutations are rare. In April 2001, American College of Medical Genetics (ACMG) and American College of Obstetricians and Gynecologists (ACOG) recommended a panel of 25 mutations for carrier screening of the US population. Since that time, extended panels have targeted specific ethnic populations while maintaining the value of a pan-ethnic screen. To investigate the performance of extended panels in CF population-based screening, we examined data from 1,140,769 cases tested in our laboratory using two mutation screening panels. One panel included 32 of the most common CF mutations (CF32) with 1,061,764 samples tested, and a second panel consisted of 70 mutations (CF70) with 79,005 cases tested. DNA analysis of the mutations on the CF32 panel was performed using multiplex PCR and oligonucleotide ligation assay (Applied Biosystems). Mutation detection technique of the CF70 was based on the Tm Bioscience/Luminex Universal Array Platform using primer extension chemistry. Genotyping data from the CF32 panel identified deltaF508 as the most frequent mutation (69.3%), followed by R117H (12.5%), and G542X (2.34%). A majority of the mutations on the CF32 panel have exceeded the ACMG 0.1% incidence threshold. Only S549R, which is not present on the ACMG CF panel, was observed at a lower frequency (0.07%). Genotyping data from the CF70 panel demonstrated a significantly lower mutation frequency for deltaF508 (58.5%) compared to the CF32 deltaF508 mutation frequency. For the rest of the variants present on both CF32 and CF70 panels, a comparable pattern of mutation frequencies were identified. Other mutations on the CF70 panel, such as D1152H, G622D and L206W, were identified with the frequency 4%, 3.23%, and 2.47% respectively, and 19 other mutations exceeded a frequency of 0.1%. In conclusion, although the clinical utility of extended CF panels is still controversial, their use improves the mutation detection rate for specific mutations within specific target populations.

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Whole Genome Sequencing: Medical and Genetic Counseling Approaches and Challenges. *FM. Facio[1], JK. Teer[1], JJ. Johnston[1], PF. Cherukuri[2], P. Cruz[2], NF. Hansen[2], DA. King[1], SC. Parker[2], HO. Abaan[2], ED. Green[2,3], EH. Margulies[2], JC. Mullikin[2,3], LG. Biesekcer[1].* 1) Genetic Disease Research Branch, Natl Human Genome Res Inst, NIH, Bethesda, MD; 2) Genome Technology Branch, Natl Human Genome Res Inst, NIH, Bethesda, MD; 3) NIH Intramural Sequencing Center, Natl Human Genome Res Inst, NIH, Bethesda, MD.

Introduction: Medical and genetic counseling at the scale of a full genome will be a major challenge of clinical research in the genomic era. The ClinSeq™ study was implemented with the goal of piloting large-scale medical sequencing (LSMS) in a clinical research setting to contribute to the research base for the development of these practices. By sequencing targeted or all regions of 1000 human genomes and returning individual relevant genotype results to research participants, the study aims to investigate the technical, medical, and genetic counseling issues that accompany the implementation of LSMS in the clinical setting. We present the findings associated with the first whole genome sequence of a ClinSeq™ participant. **Methods**: After enrollment in ClinSeq™, participants provide personal and family history of disease, and undergo clinical evaluations focused on cardiovascular health. A ClinSeq™ participant reported a remarkable personal and family history of early onset autosomal dominant atherosclerotic coronary artery disease without hyperlipidemia, and was selected to be the first participant to have his whole genome sequenced. Paired-end Illumina sequencing system was used to generate 50-fold coverage upon alignment. Upon identification of genetic variants of clinical significance the participant was contacted to decide if he wished to learn about these individual genotype results. **Results**: Initial analysis detected ~4.1 million variants including ~3.7 million single nucleotide polymorphisms and ~439 thousand copy number variations. One of the copy number variations found is a deletion of the PMP22 gene associated with Hereditary Neuropathy with Liability to Pressure Palsies. Preliminary analysis of the sequence alterations using the Human Gene Mutation Database identified >40 potential pathogenic variants. Manual curation trimmed this to four. These included variations in genes associated with carrier status for Congenital dyserythropoietic anemia type II (*SEC23B*), Ectodermal dysplasia (*WNT10A*), Pendred syndrome (*SLC26A4*), and Galactosemia (*GALT*). The participant opted to learn these genotype results and met with the team to discuss the medical and genetic meaning of these variants. **Conclusions**: Despite the challenges surrounding interpretation and disclosure of results from LSMS, our results show that this technology can generate genetic test results that are clinically significant and of interest to clinical research participants.

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Whole Genome Sequencing in the Clinical Laboratory. *T.M. Hambuch[1], M. Laurent[1], B. Sickler[1], A. Liao[1], P. Cotter[1], S. Jain[1], Y. Lyan[1], J. Bernd[1], J.M. O'Daniel[1], P. Poggio[1], M.T. Ross[2], D.R. Bentley[2].* 1) Illumina Clinical Services Laboratory, San Diego, CA; 2) Illumina United Kingdom Chesterford Research Park Little Chesterford Nr Saffron Walden Essex CB10 1XL, UK.

The advent of routine whole genome sequencing creates an opportunity to provide an accurate, comprehensive and cost-effective catalogue of germline variation for an individual. The process of sequencing and delivering genomes for individual use must be driven by clinical and educational opportunities balanced by addressing ethical concerns. Using guidelines issued from professional and accrediting agencies as well as an independent ethics board, we developed and launched individual genome sequencing (IGS) as a physician-led service. A physician orders the sequence and obtains informed consent from the individual; the sample is sequenced within a CLIA certified laboratory and after a series of quality checks the sequence is returned to the physician for communication back to the individual. The sequencing platform and process were validated for accuracy and precision, and accredited following review by a College of American Pathologist (CAP) inspection team. Each individual genome is sequenced at >30 fold coverage using paired-end reads of 100 base pairs. Resulting sequence information is provided for >93% of the NCBI36 genome, the remainder being mostly recently duplicated repeats where ambiguous read alignment is not permitted in our ELAND analysis. On average, we detect over 3 million SNPs most of which are previously documented in dbSNP129. The overall accuracy of our base calling is measured as >99.99% and the accuracy for SNP calling is >99.7% based on assessments using multiple methodologies. The aim of the Illumina Clinical Services Laboratory is to make Individual Genome Sequencing accurate, accessible and clinically relevant for physicians and patients through a fully accredited process. Here we have established baseline processes, tools, and policies to maximize the benefit to patients and minimize potential misuse. Additionally, our ongoing efforts to develop clinically relevant interpretation tools for physicians are described in a separate abstract (see M. Ross). Individual Genome Sequencing has the capacity to replace current genetic testing with a near-complete description of the sequence of an individual. Considering this potential, it is essential to engage policy makers and ethicists so that appropriate policies are developed around information access and use of whole genome information.

1666/W

Next generation sequencing - applications for genetic testing. *A. Horvath, C. Wassif, C. Stratakis, F. Porter.* NICHD, NIH, Bethesda, MD.

Next generation sequencing (NGS) technologies are anticipated to have a crucial impact on genetic testing, by providing accuracy at a reasonable cost, tremendous robustness, and a relatively straightforward data analysis. So far, NGS diagnostics-related applications have been limited by the high cost of the individual tests, as well as by a high rate of false-negative and, in particular, false-positive results. Consistent advancements in technology have led to increased speed and read length, allowing for higher accuracy due to extended coverage in a cost-effective setting. We have developed an approach that allows accurate sequence analysis of one or more disease-related genes in multiple patients, and in parallel, generates data for research applications in a single run. The approach utilizes a capture enrichment protocol (Agilent) of a custom, disease-based array, and subsequent re-sequencing on a Solid 4 platform (Applied Biosystems). Thus, a high level of sequence coverage is achieved on 392,574 bp, consisting of the coding and the flanking regions of 1982 exons of 201 genes. The panel of the included genes consists of two major groups: genes related to tumors of endocrine origin, and genes linked to aberrant lipid metabolism. Extending the experience into diagnostic applications of the NGS technologies, along with their constantly improving accuracy, speed, and cost-effectiveness, are expected to bring a significant progress towards clinical applications, including sequencing of numerous genes in an individual with a complex or muti-factoral genetic disorder or complex disease predisposition.

1667/W

An approach to clinical interpretive tools for whole genome sequencing. *M.T. Ross[1], T.M. Hambuch[2], J.M. O'Daniel[2], L.J. Murray[1], D.R. Bentley[1].* 1) Seqeuncing Applications, Illumina, Chesterford, Essex, United Kingdom; 2) Illumina Clinical Services Laboratory, 9440 Carroll Park Dr., Ste. 100, San Diego, CA 92121.

The barriers to accurate human genome re-sequencing have largely been surmounted, enabling considerations of routine clinical applications. Significant challenges remain, however, for interpretation of whole genome data for diagnostic or prognostic purposes. Public data on mendelian variants are held in a disparate set of locus-specific or genomic databases, while the largest repository of human diversity data (dbSNP) currently contains limited information of clinical relevance. The significance of novel coding variants remains unknown in the absence of other medical information. Most importantly, the prediction of phenotype from genotype is complicated by variability in penetrance, expressivity, epigenetic and environmental factors. We have established a clinical service for whole genome sequencing (see abstract by T.Hambuch). To assist physicians in patient management, we explored the challenges of providing genome interpretation tools. We analyzed an individual genome, utilizing available databases. For our approach, we considered five tiers of variant information with varying degrees of immediacy in their clinical impact: (1) known monogenic variants with evidence for disease causation, (2) pharmacogenetic markers, (3) polymorphisms associated with common, complex conditions, (4) novel variants in genes in tier 1, and (5) known tissue-type variants. We focused our efforts on Tier 1, where our starting point was the Human Gene Mutation Database (HGMD). We observed 16 homozygous and 48 heterozygous variants considered by HGMD as "disease causing" in the individual genome most of which were not compatible with a reportedly healthy adult. These variants were manually assessed via literature review, locus-specific databases and function prediction algorithms. The evaluation resulted in a list of 10 variants with sufficient evidence of clinical impact to be considered pathogenic, probably pathogenic or possibly pathogenic. Whole genome sequencing holds tremendous potential for clinically important information throughout the lifespan. The development of interpretation tools is essential to assist the physician in understanding and management of the implications of genome information for the patient. Ongoing discussions of the ethical, legal and social implications around appropriate access and use of this information will guide the further development of the interpretation strategy we present here.

1668/W

Novel Approaches toward a One-Step Comprehensive Molecular Diagnosis of Mitochondrial Disorders. *L. Wong, H. Zhan.* Molecular and Human Genetics, Baylor College of Medicine, Houston, TX. 77030.

Mitochondrial disorders are a group of clinically and genetically heterogeneous diseases that may affect any individual at any age with any form of clinical manifestation. Due to the large number of genes (~1500) involved and the complexity, the disease is undoubtedly under-diagnosed. Currently, clinical molecular analysis is based on the screening of known recurrent mutations and sequencing of a limit number of nuclear genes known to cause mitochondrial disorders. However, more than 90% of patients with mitochondrial disorders are left without a definite diagnosis. In addition, sequence analysis of relevant genes one by one using Sanger's method is too costly and not practical. At the present, the molecular diagnosis of mitochondrial disorders requires the implementation of several different methods including sequencing, quantitative PCR for mtDNA heteroplasmy and depletion, as well as the detection of large deletions by various techniques. It is tedious, expensive, and often not definitive because not all the genes can be analyzed. We recently have designed a comprehensive approach by capture of all 1300 nuclear genes targeted to mitochondria plus mitochondrial genome, followed by massive parallel sequencing with deep coverage, such that point mutations and copy number changes in both mitochondrial and nuclear genomes can be detected simultaneously with the estimation of deletion breakpoints and mtDNA heteroplasmy. We used Agilent's eArray to tile coding exons of nuclear genes and the entire mitochondrial genome at an optimized ratio to a DNA array, followed by SureSelect method to pull down the targeted DNA templates, which were then sequenced at a deep coverage. Our results showed that point mutations or variants in both nuclear and mitochondrial genes were correctly identified. In-house developed algorithms similar to the analysis of oligonucleotide array CGH data were used for large deletions in both nuclear and mitochondrial genomes. Detection of mtDNA copy number changes (mtDNA depletion) requires more sophisticated analytical tools that are currently being developed. This approach will also lead to the discovery of new disease genes. In conclusion, we have developed a high throughput, one-step comprehensive molecular diagnostic approach that would allow the identification of the molecular defects in mitochondrial disorders at an affordable cost.

1669/W

Use of chromosomal microarrays in the diagnosis of primary neuromuscular diseases. *L. Medne[1,2], A.R. Foley[2,5], Y. Hu[2], Y. Zou[2], M. Yang[6], M. Leach[2], E.H. Zackai[1], J. Ganesh[1,4], K. Chatfield[1,4], M. Falk[1], E. Place[1], A.M. Neumeyer[8], L. Bliss[8], B.S. Tseng[8], R.E. Schnur[9], L. Coffee[9], J. Stone[9], T. Shaikh[7], L.G. Shaffer[10], T. Winder[11], S. Mulchandani[3], B.D. Thiel[3], L.K. Conlin[3], N.B. Spinner[1,3], C.G. Bönnemann[2].* 1) Div Hum Gen, Children's Hosp Philadelphia, Philadelphia, PA; 2) Division of Neurology, Children's Hospital of Philadelphia, Philadelphia, PA; 3) Division of Pathology, Children's Hospital of Philadelphia, Philadelphia, PA; 4) Section of Biochemical Genetics and Metabolic Disease, Children's Hospital of Philadelphia, Philadelphia, PA; 5) Dubowitz Neuromuscular Centre, UCL Institute of Child Health and Great Ormond Street Hospital for Children, London, UK; 6) Department of Neurology, The Children's Hospital, University of Colorado, Denver, CO; 7) Department of Pediatrics, University of Colorado - Denver, Aurora, CO; 8) Department of Neurology, Massachusets General Hospital, Harvard University, Botson, MA; 9) Division of Genetics, Cooper University Hospital, Camden, NJ; 10) Signature Genomics Laboratories, Spokane, WA; 11) Prevention Genetics Laboratory, Marshfield, WI.

We report 7 patients with four different primary neuromuscular disorders - Ullrich congenital muscular dystrophy (UCMD), congenital muscular dystrophy (CMD) due to alpha-dystroglycan glycosylation defect, myotubular myopathy and Duchenne muscular dystrophy (DMD) - in whom the use of genomic microarray analysis was crucial in establishing the diagnosis, identifying the mutational mechanisms and providing appropriate genetic counseling for the family. Some patients had findings limited to the primary neuromuscular disease while other had additional systemic involvement to suggest a contiguous gene deletion. Patients 1 and 2 have Ullrich congenital muscular dystrophy (UCMD) without additional findings. Patient 1 has a 69 Kb deletion at 21q22.3 encompassing *COL6A2* gene. Sequencing of the other allele revealed an intronic splice mutation (c.1970-9) with cDNA testing showing 7 bp insertion from the used of the novel splice acceptor site leading to a downstream frameshift (G656AfsX17). Patient 2 has heterozygous deletions detected by SNP array: 1.61 Mb deletion encompassing the entire *COL6A1* and *COL6A2* and other genes; and 47 Kb deletion encompassing *COL6A2* gene. Patient 3 has 76 Kb deletion at 22q12.3 containing part of *LARGE* gene, which lead to recognition of the CMD phenotype and identification of p.Glu509Lys mutation in the other allele. This is only the 3rd report of a patient with CMD due to *LARGE* gene mutations. Patient 4 had a 2.9 Mb deletion at Xq27.3-q28 encompassing *MTM1* and other genes, which accounted for his congenital myopathy with severe respiratory distress and additional findings of Pierre-Robin and genitourinary abnormalities. Patients 5, 6 (brothers) and 7 had Xp21 microdeletions encompassing dystrophin, *MAP3K71P3*, and *GK* genes. Patient 7 had a larger deletion accounting for the additional adrenal hypoplasia phenotype. In 4 of the 7 patients presented here microarray analysis was ordered as part of first-round diagnostic work-up without a suspicion for a specific neuromuscular disease. Results showed previously known disease entities like 'DMD plus' due to Xp21 microdeletions as well as novel mutational mechanisms like *COL6A1* and/or *COL6A2* gene deletions in autosomal recessive UCMD. These cases clearly illustrate that chromosomal microarrays have a place in the diagnostic repertoire for neuromuscular disorders as phenotypes may not be clearly apparent due to age or masked by more severe or additional systemic findings.

1670/W

***COL3A1* haploinsufficiency results in EDS type IV with delayed onset of complications and longer life span when compared to missense and splice-site mutations.** *D.F. Leistritz, M.G. Pepin, U. Schwarze, P.H. Byers.* University of Washington, Seattle, WA.

Ehlers-Danlos syndrome (EDS) type IV, the vascular type, results from mutations in *COL3A1*, the gene that encodes the proα1(III) chain of type III procollagen. We have identified heterozygous *COL3A1* mutations in 508 families, about 95% of which lead to the synthesis of an abnormal type III procollagen. Mutations that result in substitutions for glycine residues in the triple helical domain of the proα1(III) chain account for two-thirds of identified mutations in *COL3A1*, and splicing mutations comprise most of the remainder. We identified frameshift or premature termination codons that result in nonsense mediated mRNA decay of the *COL3A1* mRNA encoded by that allele ("null mutations") in 19 families (about 4% of the total); cultured cells from these individuals produced about half the normal amount of type III procollagen and no abnormal molecules, as expected. We reviewed the clinical and family histories and medical complications in 53 individuals with *COL3A1* null mutations. Compared to individuals with missense or exon-skipping mutations, we found that in the cohort with *COL3A1* null mutations mean life span was extended by close to 20 years, the age of first complication was delayed by almost 15 years, and major complications were limited to vascular events. The families were ascertained following a complication in a single individual but only 25% of relatives, some of whom had reached their 70s or 80s without incidents, had a complication and only 30% had any minor clinical features of EDS type IV. In families with osteogenesis imperfecta that results from mutations in the *COL1A1* gene, more than 50% of affected individuals have haploinsufficiency mutations and have OI type I, the mildest OI phenotype. Because null mutations in *COL3A1* are expected to be as common as those in *COL1A1*, our data suggest that they are far less penetrant than missense and splicing mutations yet may be causes of late onset arterial aneurysms. Since ascertainment on the basis of clinical findings alone has a low yield, genetic testing for *COL3A1* mutations should be offered to all first degree relatives of an individual identified with a *COL3A1* null mutation, and should be considered in those with compatible vascular complications at a young age and, potentially, in individuals with late onset arterial events.

1671/W

Expanding the Phenotypic Overlap Between CHARGE and Kallmann Syndromes due to CHD7 Mutations. *H. Feret[1], D.M. McDonald-McGinn[1], A. Santani[2], E.H. Zackai[1].* 1) Division of Human Genetics, Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, PA; 2) Dept of Pathology & Lab Medicine, Children's Hospital of Philadelphia, PA.

Kallmann syndrome (KS), characterized by hypogonadotropic hypogonadism and anosmia, is a genetically heterogeneous condition, with multiple modes of inheritance described. Approximately 6% of KS is caused by mutations in CHD7. CHD7 also causes CHARGE syndrome, an autosomal dominant condition with multisystemic involvement in which the majority of cases are the result of de novo mutations. In previous studies, the majority of patients with KS as a result of CHD7 mutations have had some findings consistent with CHARGE syndrome. Additionally, until more recently, anosmia and arrhinencephaly were not recognized as important diagnostic criteria suggesting a CHARGE syndrome diagnosis. Here we report 2 families in which the fathers, with signs consistent with Kallmann syndrome, had children with CHD7 mutations and a CHARGE syndrome phenotype, as well as a newborn with multiple congenital anomalies including arrhinencephaly with a novel CHD7 mutation. The proband of Family 1 is a 19 month old male with bilateral chorioretinal colobomas, dysplasia of the semicircular canals, and slightly thickened left helix. His 2 =BD month old sister has bilateral cleft lip and palate, bilateral retinal and optic nerve colobomas, and right microphthalmia. The father has anosmia, with absence of the olfactory tacks and bulbs. Physical exam of the father revealed no other findings suggestive of CHARGE syndrome. All three individuals were found to be heterozygous for a R2319C missense alteration in the CHD7 gene. The proband of Family 2 is a 7 year old male with unilateral cleft lip and palate, right facial palsy, developmental delay, short stature, and dysmorphic ears. His father was diagnosed with Kallmann Syndrome, requiring the use of a GnRH agonist pulsatile pump to conceive. CHD7 sequencing in the proband revealed a heterozygous missense mutation, H2096R. Parental testing is pending. Patient 3 is an ex-34 week old male with growth retardation, bilateral cleft lip and palate, congenital heart disease, arrhinencephaly, intestinal malrotation, microphallus and cryptorchidism. CHD7 sequencing revealed a novel deletion of two bases (delAA) from nucleotide 3572_3573, resulting in a frameshift and premature stop codon. These cases suggest CHD7 mutation analysis be considered in cases of isolated KS or anosmia, regardless of the presence of additional features of CHARGE syndrome, in order to provide appropriate diagnosis and genetic counseling.

1672/W

Presymptomatic testing in 1554 at risk persons for late onset inherited neurological diseases. *M. Gargiulo[1], A. Herson[1], A. Mallet[1], C. Cazeneuve[1], C. Depienne[1,2], J. Feingold[1,2], A. Durr[1,2].* 1) AP-HP, Hôpital de la Salpêtrière, Department of Genetics, Paris, France; 2) CR-icm, INSERM/UPMC, UMR_S975, Paris ,France.

Since 1992, 1554 at risk persons for late onset inherited neurological diseases entered a presymptomatic testing (PT) procedure in our centre, including multistep and multidisciplinary care with genetic counseling, psychological and social interviews before bloos sampling, molecular analysis, test disclosure and follow up. They were at risk for Huntington diseases (HD, n= 1329/85%), but also for autosomal dominant cerebellar ataxias (SCA1, 2, 3, 6, 7 n=179/11.5%), autosomal dominant spastic paraplegias (SPG, n=33/2.1%), Creutzfeld-Jacob disease and fronto-temporal dementias (CJD/FTD n=13/0.8%). Mean age at first contact (34.7 ±11.9 years) was similar in all groups, close to the mean onset of the disease. After a variable delay, only part of the testeees decided to take the test and asked for their result: 85% for CJD/FTD, 68% for HD, 53% for SCAs, but only 45% for SPG ($p<0.001$). This indicated that proportion of those taking the test increases in with greater disease severity. The most frequent motive for PT is the need to know (34.4%). Less frequent were reproductive decision making (16.9%), anticipation and preparation (10.9%), and information of children about their risk (9.8%). Even if all carriers considered themselves as being unaffected, perceived first subtle symptoms motivated 10.3% of the testees to take the test, specially in the SCA group (20.1%, $p<0.002$). 3% had no clear motivation (3.2%). Interestingly, the more motivations are mentioned by the testees, the more likely they continue until the result compared to those who decided not to take the test in the end: the need to know 37.6% versus 28.4% ($p<0.001$), reproductive decision making 18.7% versus 13.6% ($p=0.012$), inform their children about their risk 11.4% versus 6.9% ($p=0.005$) and the need to anticipate and prepare the future 12.7% versus 7.5% ($p=0.002$). 981 testees (63%) asked for result disclosure and were non carriers in 56% and 40.5% carriers for HD, 58.9% and 37.9% for SCA, 80% and 20% for SPG. During follow up, prenatal testing was option for at risk persons only in 16.5% for HD, 8% for SCA, 0% for SPG and CJD/DFT . In conclusion, PT for late onset inherited neurological diseases remains an overall rare request. Increased disease severity and detailed motivations seemed to promote the uptake of PT and prenatal diagnosis is clearly not a direct consequence of PT in carriers.

1673/W

Combining genotype and phenotype to enhance risk estimation of a common complex disease: The REVEAL Study. *D.M. Lautenbach[1], C.A. Chen[3], L.A. Cupples[4,5], J.S. Roberts[6], R.C. Petersen[7], R.C. Green[1,2,5], for the REVEAL Study Group.* 1) Neurology, Boston University School of Medicine, Boston, MA; 2) Medicine (Genetics Program), Boston University School of Medicine, Boston, MA; 3) Data Coordinating Center, Boston University School of Public Health, Boston, MA; 4) Biostatistics, Boston University School of Public Health, Boston, MA; 5) Epidemiology, Boston University School of Public Health, Boston, MA; 6) Health Behavior and Health Education, University of Michigan School of Public Health, Ann Arbor, MI; 7) Neurology and Health Sciences Research, Mayo Clinic College of Medicine, Rochester, MN.

Background: The presence of specific clinical symptoms or signs may enhance the predictive utility of genetic tests, but very few examples of this have been developed using risk genes for common complex diseases. Mild Cognitive Impairment (MCI) is a clinical syndrome in which patients have mild memory problems but not dementia, and is considered a risk factor for Alzheimer's disease (AD). *APOE* is a polymorphic genotype where one or two copies of the ε4 allele increase the risk of AD. We analyzed *APOE* genotype data along with AD conversion data collected in a clinical treatment trial to create risk curves demonstrating 3-year "imminent" risk of AD for individuals with MCI. **Methods:** Data were obtained from a medication trial involving 769 MCI patients ("The Memory Impairment Study" Petersen et al. 2005), which provided three-year risk data stratified by *APOE* genotype. Cox proportional hazards regression revealed a significant difference in AD risk by age and *APOE* ε4 status. The sample was divided into approximate tertiles to create age groups, and three-year risk curves were created for each age group and *APOE* ε4 category (presence vs. absence of ε4 allele). Using an average 1.5% annual AD conversion rate among individuals without MCI, a three-year general population risk curve was generated. **Results:** The three-year risks for each age-group were: 8.4% for *APOE* ε4 negative and 42.0% for *APOE* ε4 positive individuals (age group: 55-70 years), 20.5% for *APOE* ε4 negative and 47.4% for *APOE* ε4 positive (age group: 71-77 years), and 30.7% for *APOE* ε4 negative and 57.1% for *APOE* ε4 positive (age group 78 years or older). A risk communication protocol has been developed, which involves presenting three curves: 1) three-year general population AD risk 2) three-year AD risk based on MCI diagnosis alone and 3) three-year AD risk based on MCI diagnosis and *APOE* ε4 status. Pictographs have been created to aid in numerical risk communication. **Conclusion:** The resulting risk estimates and curves will be employed as part of a new randomized trial in the NIH-funded REVEAL Study. Estimating and communicating risk for a late-onset, common complex disease based on both genotype and phenotype information is a novel concept in genetic risk counseling, which may become more common in the genomic era. Data from the REVEAL Study will inform refinement of disease risk estimation and disclosure based on both genotype and phenotype information.

1674/W

Confined Placental Mosaicism For Tetrasomy - Be Careful Not To Falsely Reassure. *K. Bajaj, M. Simard, S. Klugman.* Reproductive Genetics, OB-GYN, Montefiore Medical Center, New York, NY.

BACKGROUND: Confined placental mosaicism (CPM), or a discrepancy between fetal karyotype and placental karyotype was first described in 1993 by Kalousek and Dill. When reported at the time of chorionic villus sampling, true fetal mosaicism is found in approximately 10%.of the cases of placental mosaicism. CPM is usually described with trisomies and marker chromosomes and to our knowledge has only been reported twice with tetrasomy. Tetrasomy for the short arm of chromosome 18 has been associated with facial dysmorphism, skeletal abnormalities, ataxia, seizures, and moderate to severe mental retardation. CASE: 36 year-old healthy female of Eastern-European descent with one prior normal female child underwent chorionic villus sampling for advanced maternal age at 12 weeks gestation. The karyotype result revealed mosaicism for tetrasomy of the short arm of chromosome 18 (47, XX, i(18)(p10)[10]/46, XX[10]). The patient was counseled regarding the possibility of a severe phenotype associated with i(18p) and that though it was uncertain whether this result represented confined placental mosaicism or true fetal mosaicism, the risk of true fetal mosaicism was 10% percent. The possible mechanisms for tetrasomy 18p were reviewed. Karyotypes performed on both the patient and her partner, as well as an early anatomic survey, were normal. Given these findings, the patient felt reassured. Unfortunately, an amniocentesis was performed at 16 weeks gestation; the result confirmed true fetal mosaicism 47, XX,+i(18)(p10)dn[11]/46,XX[12]. The patient opted for termination. CONCLUSION: Our patient was falsely reassured that it was unlikely that the true fetal karyotype was mosaic for tetrasomy 18p. The data on confined placental mosaicism do not include tetrasomies and thus genetics providers must be careful when counseling patients with this unique but important clinical scenario.

1675/W

Large scale population screening for SMN1 gene copy number, clinical implications. *S. Ben-Shachar[1], A. Orr-Urtreger[1,2], E. Bardugo[1], R. Shomrat[1], Y. Yaron[1,2].* 1) Gnetic Institute, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel; 2) Sackler Faculty of Medicine, tel Aviv University, Tel Aviv, Israel.

Background: Spinal muscular atrophy (SMA) is a common autosomal recessive disorder characterized by degeneration of motor neurons in the spinal cord, resulting in a progressive muscular atrophy. The majority of SMA cases are caused by homozygous deletion of exon 7 of the *SMN1* gene. The aim of our study was to determine the frequency of *SMN*1 deletion carriers in the Israeli population and to determine feasibility of population screening for SMA.

Methods: From March 2007 to August 2009, 6394 individuals with no family history of SMA underwent genetic screening for SMA at the Genetic Institute, Tel Aviv Medical Center. Analysis was performed using multiplex ligation-dependent probe amplification (MLPA, MRC-Holland), designed to detect *SMN*1 exon 7 and exon 8 copy number.

Results: A total of 159 individuals were found to carry a *SMN1* heterozygous exon 7 deletion, a carrier frequency of 1:40. In addition, 690 individuals (10.8%) were found to carry 3 or more *SMN1* exon 7 copies, confirming the observation that some individuals carry more than one *SMN1* copy per chromosome (*cis* configuration). This result implies that about 1:740 individuals detected with 2 copies of SMN1 exon 7, are in fact carriers of exon 7 deletion on one chromosome and have 2 copies on the other chromosome. These individuals cannot be detected by the current MLPA or similar quantitative methods, leading to false negative diagnosis.

Conclusions: Our initial experience from this large-scale population based SMA screening program confirms that SMA screening is effective and feasible. Due to the inability of this screen to detect SMA carriers with *cis* configuration, individuals with SMN1 sequence mutations, and *de novo* fetal mutations, it is estimated that only about 90% of SMA carriers will be detected. Detection rates may be further increased by testing exon 7 copy number in both parents and performing prenatal diagnosis even when only one parent is found to be a carrier. However, as a result of invasive testing, an increased risk of miscarriages may be expected.

1676/W

Prenatal Molecular Diagnosis; Experience from a Large Center in Israel. *E. Pras[1, 2], L. Peleg[1, 2], H. Reznik-Wolf[1], H. Lahat[1], H. Yonath[1, 2], M. Karpati[1], L. Ries-Levavi[1], M. Berkenstadt[1], B. Feldman[1], M. Frydman[1, 2].* 1) Institute of Human Genetics, Sheba Medical Center, Ramat Gan, Israel; 2) Sackler Faculty of Medicine, Tel Aviv University, Israel.

Parental tolerance for genetic diseases in Israel is extremely low. Prevention efforts begin with premarital or pre-gestational screening programs and continue with increasing attempts to perform molecular diagnosis when there is an affected child in the family. The overall result is an increasing demand for prenatal diagnosis of a growing number of genetic diseases. Over a two year period, between January 2008 to December 2009, we have performed 470 prenatal molecular diagnoses for 73 different Mendelian disorders. The prenatal procedure chosen, chorionic villus sampling (CVS) or amniocentesis was determined according to the risk of having an affected fetus. For a risk of 10% or more CVS was performed (dominant, recessive X-linked, fragile X with more then 70 repeats), otherwise amniocentesis was done. Each molecular diagnosis was performed by two different methods in order to minimize mistakes. Common disorders were diagnosed with commercial assays. For rare disorders we used direct sequencing combined with family haplotype analysis. In 4 cases (0.8%) the disease causing mutation was not known and therefore the diagnosis relied solely on family haplotype analysis. The most common problem encountered was maternal contamination (10 samples) which led to a repeated procedure. The most common disorders requiring prenatal diagnoses were fragile X (175 cases), followed by Tay-Sacks (75), cystic fibrosis (33), spinal muscular atrophy (20), Gaucher (16), skeletal dysplasias (15) and familial dysautonomia (9). Prenatal diagnosis was also offered for adult onset diseases such as Marfan syndrome (13), polycystic kidney disease (3), Huntington chorea (4), and also for potentially treatable disorders such as connexin 26/30 related hearing loss (15) and phenylketonutia (3). Sixty one affected fetuses were diagnosed, the vast majority of which were aborted. One case of misdiagnosis was encountered. This series represents one of the largest of its kind.

1677/W

The importance of psychoanalysis for dealing with genetic mutations. *J. Forbes[1,2], T. Genesini[1,2], C. Riolfi[1,2], M. Barbosa[1,2], R. Chiavassa[1,2], L. Lise[1,2], E. Macedo[1,2], J. Palma[1,2], D. Rudiger[1,2], M. Zatz[1].* 1) Human Genome Res Ctr, Univesity Sao Paulo (USP), São Paulo, Brazil; 2) Institute of Lacanian Psychoanalysis (IPLA), São Paulo, São Paulo, Brazil.

One important aspect of genetic disorders is that they often affect not only individuals but the entire family. We have hypothesized that a social virus that we have denominated RC (resignation from patients and compassion from relatives) has a negative impact on families with patients affected by genetic disorders. In progressive neurodegenerative disorders it may also accelerate the progression of the symptoms leading affected patients to exclude themselves from social life prematurely. From September 2006 to October 2009 we have interviewed 52 subjects belonging to families with affected members of whom 43 were submitted to psychoanalysis, 11 of them were normal relatives (not affected) and 32 were affected with: 8 spinocerebelar ataxia, 8 myotonic dystrophy, 6 limb-girdle muscular dystrophy, 4 facioscapulohumeral , 2 spinal muscular atrophy, 2 Duchenne, 1 Becker and 1 Freidreich ataxia. The patients were seen in a weekly basis by a trained team of supervised psychoanalysts. The treatment approach is based on obtaining the maximum separation between the subject and the prêt-à-porter feelings that are, at least hypothetically, caused by his degenerative condition. In order to increase the treatment effectiveness, the psychoanalysts did not fulfill the patients' expectation to receive the same amount of compassion that they were used to receiving from their relatives. Interestingly, although all religions preach compassion towards handicapped individuals, all the interviewed patients declared that they did not want people to feel compassionate for them. Every three months the patients were reexamined by the geneticist and by the psychoanalyst who is responsible to supervise the whole team (namely, Zatz and Forbes, respectively) and the treatment impact reassessed. The results showed that patients began to show a responsible and inventive way of dealing with the disease. Moreover, the family resumed their lives by creating a new relationship with the affected relative. The advances in human genome sequencing will result in an enormous amount of information and a trained team of genetic counselors to help individuals to interpret them. We anticipate that psychoanalysis will play a growing role helping individuals dealing not only with genetic disorders but also with the knowledge of their own genome. Supported by FAPESP/CEPID, ABDIM, IPLA.

1678/W

New and Improved Medical Genetics Resources at NCBI. *B.L. Kattman, D.R. Maglott.* National Center for Biotechnology Information - NCBI/NLM/NIH, Bethesda, MD.

The last decade has seen tremendous advances in our knowledge of the genetic and genomic factors involved in health and disease. NCBI has led the way in providing large quantities of data for a wide variety of users via databases, and the tools and viewers to access them. To improve service to the medical community, NCBI is enhancing current resources and creating new resources to facilitate exploration, interpretation, and evaluation of human variation.

With all the information being generated about human variation, processing these complex data is a service best supplied by a centralized database provider such as NCBI. dbSNP, dbVAR, and dbGaP provide essential roles in accessioning and processing reported variants. However, views aggregating information about the traits associated with each variant or set of variants, and the validity of any clinical interpretation of a variant or set of variants have not been readily available. Currently, users of NCBI resources can browse, search for and/or visualize data about variants maintained in dbSNP, dbVAR, dbGaP, OMIM, GeneReviews, GeneTests, LOVD at NCBI, PubMed, PubMedCentral, and the sequence databases, but not easily, nor in an integrated fashion. To bridge this gap and support expert review and curation, NCBI is developing two new resources:

• ClinVar, a public database to aggregate information relating variation to phenotype, (http://www.ncbi.nlm.nih.gov/clinvar) and

• The Genetic Testing Registry (GTR), a centralized, public resource that will provide information about the availability, scientific basis, and usefulness of genetic tests. http://www.ncbi.nlm.nih.gov/gtr

Here, we present a brief summary of our current tools and resources applicable to medical genetics, and then focus on the details of our exciting new projects, ClinVar and the GTR.

1679/W

Incorporating Genetic Risk Information for Type 2 Diabetes (T2D) Into Primary Care Clinics: Feasibility In Two Diverse Clinics - A Prototype Model? *M.L. Bembe, D.L. Baker, G.S. Ginsburg, A. Cho.* IGSP, Duke University, Durham, NC.

Genetic risk information for Type 2 Diabetes (T2D) is not typically utilized in primary care clinics. Is there synergism in a model that combines a standardized risk assessment (SRA) for T2D with genetic risk information? Duke University Institute for Genome Sciences & Policy (IGSP) partnered with 2 diverse clinics (an internal medicine primary care practice and a community and family medicine clinic) to test this. Outpatients were approached by research study coordinators, and if interested, enrolled that day. Four study visits over ~ 14 months included baseline, risk counsel visit (RCV), a remote post-RCV 3 months later, and final visit 12 months post-RCV. Baseline measures included blood work (fasting glucose and insulin), family history, metrics (BMI and waist circumference), medication use, lifestyle (self-report of diet and exercise) plus surveys. Genetic testing for 4 genes associated with increased diabetes risk, validated in other studies, was obtained by buccal swab. RCVs 1-2 months post baseline involved 30-40 minutes with a clinic provider; all subjects received education packets with individualized profiles for combined diabetes risk. Participants were randomized to receive genetic test results at RCV or at study completion. A remote 3 months post-RCV captured current weight and additional surveys. Final visit, 12 months post-RCV, included repeat fasting blood work, metrics and surveys. In 12 months, at two different clinics, over 1300 patients have been approached. A log of reasons for patient non-participation shows the most common reason for refusal to be no time that day. Over 350 of 400 enrolled subjects remain active. There have been 280 RCVs with 189 of 209 three-month surveys completed. Final twelve-month visits are scheduled to begin in two months. Study recruitment and follow-up participation indicate many primary care patients are receptive to genetic risk testing for T2D, especially when included as part of diabetes SRA and presented by a clinic provider. Subjects receive a personalized risk assessment of their combined diabetes risk, with genetic risk as only one component. This exemplifies a novel approach that demonstrates the feasibility of integrating T2D genetic risk research with diabetes standard risk assessment in diverse primary care clinics, perhaps a prototype for personalized, preventative medicine in the future.

1680/W

Lower numeracy is associated with lower recall of genetic risk information for Alzheimer's disease: The REVEAL Study. *A.G. Besser[1], C.A. Chen[2], J.S. Roberts[3], D.M. Lautenbach[4], L.A. Cupples[5,6], R.C. Green[4,6,7] for the REVEAL Study Group.* 1) Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, NY; 2) Data Coordinating Center, Boston University School of Public Health, Boston, MA; 3) Health Behavior and Health Education, University of Michigan School of Public Health, Ann Arbor, MI; 4) Neurology, Boston University School of Medicine, Boston, MA; 5) Biostatistics, Boston University School of Public Health, Boston, MA; 6) Epidemiology, Boston University School of Public Health, Boston, MA; 7) Medicine (Genetics Program), Boston University School of Medicine, Boston, MA.

Background: Numeracy, the ability to comprehend, use, and attach meaning to numbers, is essential for understanding risk information. Previous research has demonstrated an association between low numeracy and impaired risk comprehension, but this relationship has not been explored in the context of genetic susceptibility testing for common diseases. As risk recall is commonly used to measure risk understanding, we hypothesized that individuals with low numeracy will show lower levels of risk recall than individuals with higher numeracy. **Methods:** Data were obtained through the Risk Evaluation and Education for Alzheimer's Disease (REVEAL) Study, a multi-site randomized clinical trial, in which lifetime risk of developing Alzheimer's disease (AD) and *APOE* genotype (an AD susceptibility polymorphism) were disclosed to individuals seeking a risk assessment for AD. Six weeks after disclosure, participants were asked to recall their lifetime risk, *APOE* genotype, and the number of risk-increasing alleles they were found to possess. Multivariate logistic regression was performed to determine whether recall was associated with mathematical aptitude (objective numeracy) and self-perceived mathematical abilities (subjective numeracy), as measured by two validated numeracy scales. The regression model was adjusted for age, education, gender, and race, as well as levels of cognition, distress, and anxiety, as measured by validated psychological scales. **Results:** Data from 236 participants were analyzed (mean age = 58.3 years; 54.2% female, 45.8% male; 86.9% Caucasian, 13.1% African-American; 94.5% with college education or higher). Of these, 136 (57.6%) were able to correctly recall all three measures of risk, whereas 100 (42.4%) were unable to recall at least one of these measures. Lower subjective numeracy was found to be a significant predictor of poorer recall (p = 0.04). **Conclusions:** Individuals with lower subjective numeracy have lower recall of genetic risk information. Lower risk recall may indicate decreased risk understanding and have negative consequences for medical decision-making. Genetic counselors should be aware that low numeracy is an obstacle to risk understanding and should tailor risk assessments to individuals' numerical abilities in order to provide optimal care.

1681/W

Molecular characteristics of Multiple epiphyseal dysplasia in sixty-nine Korean patients. *S. Park[1], H. Park[1,2], O.H. Kim[3], T.J. Cho[4], S.I. Cho[1], I.K. Yeo[1], H.R. Song[5], G. Nishimura[6], S. Unger[7], A. Superti-Furga[8], I.H. Choi[4], W.J. Yoo[4], J.S. Shim[9], H.W. Kim[10], C.Y. Chung[11], C.W. Oh[12], C. Jeong[13], K.S. Song[14], S.Y. Kim[1], M.W. Seong[15], S.S. Park[1].* 1) Department of Laboratory Medicine, Seoul National University Hospital, Seoul, Korea; 2) Yeon Cheon Health Center and County Hospital, Yeon Cheon, Korea; 3) Department of Radiology, Ajou University Hospital, Suwon, Korea; 4) Department of Orthopaedic Surgery, Seoul National University Children's Hospital, Seoul, Korea; 5) Department of Orthopedic Surgery, Korea University Guro Hospital, Seoul, Korea; 6) Department of Radiology, Tokyo Metropolitan Kiyose Children's Hospital, Kiyose, Japan; 7) Institute for Human Genetics, Freiburg University Hospital, Freiburg, Germany; 8) Center of Pediatrics and Adolescent Medicine, Freiburg University Hospital, Freiburg, Germany; 9) Department of Orthopaedic Surgery, Sungkyunkwan University Samsung Medical Center, Seoul, Korea; 10) Department of Orthopaedic Surgery, Yonsei University Severance Children's Hospital, Seoul, Korea; 11) Department of Orthopaedic Surgery, Seoul National University Bundang Hospital, Seongnam, Korea; 12) Department of Orthopedic Surgery, Kyungpook National University Hospital, Daegu, Korea; 13) Department of Orthopedic Surgery, Catholic University Holy Family Hospital, Bucheon, Korea; 14) Department of Orthopaedic Surgery, Keimyung University Dongsan Medical Center, Daegu, Korea; 15) Department of Laboratory Medicine, National Cancer Center, Goyang, Korea.

Background: Multiple epiphyseal dysplasia (MED) is a clinically and genetically heterogeneous group of diseases characterized by mild short stature, limb deformity, arthralgia and early-onset of osteoarthritis mainly in the hip and knee joints. At least six genes are reported to be causative : *COMP*, *MATN3*, *COL9A1*, *COL9A2*, *COL9A3* and *SLC26A2*. In this study, we present the molecular genetic characteristics of MED in 69 Korean patients who were preselected under the impression of MED. Methods: We sequentially analyzed exon 1, 2 of *MATN3*, exon 8-19 of *COMP*, exon 8, 9 of *COL9A1*, exon 3, 4 of *COL9A2*, exon 3 of *COL9A3*, entire three exons of *SLC26A2* and their flanking regions by direct sequencing, until any mutation was detected. Results: In fifty-nine out of 69 patients (85.5%), sequence variations which had already been reported to or were presumed to be causative for MED phenotype were identified. Thirty-three patients had mutations in *MATN3* gene (55.9%, 33/59), twenty-five in *COMP* (42.4%, 25/59), one in *COL9A2* (1.7%, 1/59), and one in *SLC26A2* (1.7%, 1/59). Mutations in *MATN3* and *COMP* genes were concurrently found in one patient. The most common genotype in this series was *MATN3*: c.361C>T (p.R121W), which accounted for 35.6% (21/59) of MED patients and 63.6% (21/33) of the *MATN3* gene mutations. Conclusions: We identified causative mutation of *COMP*, *MATN3*, *COL9A2* or *SLC26A2* in 85.5% of MED patients diagnosed clinically. Pattern of genotypes in MED patients in Korean population was different from those in other ethnic groups. Based upon this data, we believe that sequential analysis of *MATN3* gene followed by *COMP* and other genes would be an efficient way for the molecular diagnosis of Korean MED patients.

1682/T

VIVO: Enabling National Networking of Scientists. *M.R. Tennant[1,2], K.L. Holmes[3,4], S. Henning[1], M. Linnemeier[5], K. Börner[5], V. Davis[6], S. Russell Gonzalez[6], N. Ma[5], C. Tank[5], M. Conlon[7], VIVO Collaboration.* 1) Health Science Center Libraries, University of Florida, Gainesville, FL; 2) UF Genetics Institute, University of Florida, Gainesville, FL; 3) The Bernard Becker Medical Library, Washington University School of Medicine in St. Louis; 4) Department of Genetics, Washington University School of Medicine in St. Louis; 5) Cyberinfrastructure for Network Science Center, Indiana University; 6) Marston Science Library, University of Florida; 7) Clinical and Translational Science Institute, University of Florida.

VIVO is an open source semantic web application that enables discovery of research and scholarship across an institution. The VIVO application was developed by Cornell University in 2003 and today serves as the basis for $12.2M award by the National Center for Research Resources to facilitate national networking of scientists. There are seven partner institutions involved in the project: University of Florida (project lead), Cornell University, Indiana University, Ponce School of Medicine, The Scripps Research Institute, Washington University School of Medicine, and Weill Cornell Medical College. VIVO is populated with information about researchers and allows them to highlight areas of expertise, display academic credentials, visualize academic and social networks and display information such as publications, grants, teaching, service, and more. Profile information can be automatically imported from authoritative data sources such as institutional records, and bibliographic and grant databases. VIVO can offer a way to stay on top of the literature and keep current on the efforts of colleagues and competitors, alike. VIVO can help identify potential collaborators, events, seminars, programs, and facilities on the local campus and beyond. VIVO allows students to showcase their research - essential when applying for fellowships and post doctoral and faculty positions. VIVO is a resource to attract new students and faculty and offers them a way to visualize the academic environment virtually. It can be difficult to grasp cutting edge trends and identify collaboration networks, as science is often evolving faster than it can be documented. VIVO provides network analysis and visualization tools to maximize the benefits afforded by the data available in VIVO. VIVO enables high quality data to be revealed about researchers, their collaborators, their funding sources, and more. This data can serve as the foundation for further network analyses and elegant visualizations of the research enterprise on the individual, local, and global levels. There are a number of ways that you can get involved with the VIVO project: adoption, data provision, application development, and data consumption. The open source VIVO software and ontology are available for download at http://vivoweb.org. Over the next year, there will be many updates to the software and ontology and we look forward to participating in events that support collaboration and discovery.

1683/T

Personalized Cancer Genetics Training for Personalized Medicine: Improving community-based health care through a genetically literate workforce. *K. Blazer[1], D. MacDonald[1], J. Culver[1], C. Huizenga[1], G. Uman[2], J. Weitzel[1].* 1) Clinical Cancer Genetics, City Hope Comprehensive Cancer Ctr, Duarte, CA; 2) Vital Research, Los Angeles, CA.

Context: Genetic cancer risk assessment (GCRA) is an established standard-of-care practice. Community healthcare providers are increasingly requested to provide cancer risk assessment and genetic testing, but many are inadequately prepared to provide these services. Objective: Develop and assess the efficacy of a multi-modal interdisciplinary course in GCRA for community-based practitioners that combines traditional and distance learning methods delivered in three phases to facilitate didactic learning, interdisciplinary training and continuing support for the integration of clinical GCRA skills and research participation into practice through CME Web-conference activities. Design and Participants: A prospective analysis of competitively-selected MDs, advanced-practice nurses (APNs)and genetic counselors (GCs), representing areas of need across the U.S.(selected based on geographic and/or underserved need) who participated in one of six sessions between 2006-2010. Outcome Measures: Assess the impact of the course on cancer genetics knowledge, cancer genetics risk assessment skills, professional self-efficacy, changes in practice and participation in research activities measured by multiple instruments at baseline, immediate- and 12-month-post course. Results: 131 participants(48 MDs,41 APNs,42 GCs) were included in the analysis. GCRA-related knowledge ($p<.001$; mean 28%;range 12.2%-38.5%) and skills ($p=.003$)increased significantly across all disciplines and cohorts. Subset analysis (n=49) showed significant increases in professional self-efficacy through 12-months post course. Significant increases reported in the number of alumni who provided GCRA services ($p=.018$), disseminated cancer prevention information ($p=.005$) and/or high-risk screening and prevention recommendations ($p=.004$), enrolled patients in a hereditary cancer registry ($p=.013$),and conducted educational outreach about GCRA ($p=.003$). Conclusions:Significant gains in GCRA-related knowledge,skills,self efficacy and practice change demonstrate the effectiveness of the multimodal, multiphase course. Sustained alumni participation in post-course professional development activities beyond the prescribed 12-month period has evolved into a dynamic distance-mediated Community of Practice in clinical cancer genetics. This innovative course models the goal of lifelong learning and improved patient care envisioned by the Institute of Medicine and other leading CME stakeholders.

1684/T

Baseline genetic knowledge among participants in the Coriell Personalized Medicine Collaborative. *M.A. Keller[1], E.S. Gordon[1], C.B. Stack[1], T.J. Schmidlen[1], M.F. Christman[1], M. Daly[2].* 1) Coriell Institute for Medical Research, Camden, NJ; 2) Fox Chase Cancer Center, Philadelphia, PA.

While historically physicians and genetic counselors were expected to interpret and understand genetic test results, the shift to direct-to-consumer testing in the area of genetics, increases the need for patients to have a baseline knowledge of genetics. The Coriell Personalized Medicine Collaborative (CPMC®) is a longitudinal observational study designed to investigate the utility of personalized genomic information in health management. Participants in the CPMC receive risk reports for a number of common complex diseases based on genetic and non-genetic risk factors and answer questions about what they did with the information. The CPMC enrolls adults into its “Community Cohort” directly through group informed consent sessions. These sessions are open to the public with additional sessions for employees of partner hospitals. In addition to required questionnaires related to medical and family history, participants were asked to complete a baseline genetic knowledge assessment consisting of 15 knowledge questions, and 5 genetic education background questions. As of December 2009, 2189 participants completed the genetic knowledge assessment. Sixty three percent of participants were female with an average age of 50 years. Respondents were predominantly Caucasian (93%) with a bachelor’s degree or higher (67%). Across all respondents, the mean score was 76% correct (11.4/15). Using an ANOVA model that adjusted for multiple participant characteristics, several demographic factors were found to be associated with the number of correct responses. Age was inversely associated with the number of correct responses, with younger individuals performing better than older individuals ($p<0.001$). Level of education (bachelors or graduate degree) was associated with an increased number of correct responses ($p<0.001$), as was employment in healthcare (as a physician, pharmacist or genetic counselor) or the life, physical, or social sciences ($p<0.001$). Males scored slightly higher than females (78% vs. 75% correct; $p<0.001$). There were no differences by income level ($p=0.74$). The fact that income did not contribute to differences in genetic knowledge suggests that there may be more equality in access to genetic education than previously thought. Further, the fact that on average, respondents with a high school diploma answered 67% correct suggests an overall basic understanding of genetics across the population.

1685/T

The concept of "Molecular Anatomy of the Cell" provides the link between molecular biology and human genetics. *J. Korabecny[1], M. Korabecna[2].* 1) FamilyCell, Pilsen, Czech Republic; 2) Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic.

We present "Molecular Anatomy of the Cell" as a new concept for teaching molecular biology and genetics during medical education. The users of the presented material (200 tables either in printed or electronic version) should be mainly undergraduate students not only at medical faculties -the material has been successfully applied also during education of biology teachers. The explanation of molecular biology is based on illustrations - icons - created by an experienced designer in cooperation with a teacher of biology and genetics. For creation of pictures of molecules, structures and their stereological relations, the programs allowing the 3D modeling used in designer practice were used (for example Rhinoceros). Molecular structures are depicted in agreement with the newest international textbooks of molecular biology with regard to the studies based on electron microscopy. The structures and events for presentations are selected to provide direct link to human genetics. The products of genes playing the known role in monogenic or complex diseases are especially mentioned together with the brief explanation of clinical problems connected the diagnosis with. For further reading, students can find OMIM numbers of the all in "Molecular Anatomy of the Cell" mentioned entries. This approach has been tested in practice at Faculty of Medicine in Pilsen (Charles University) and at the Faculty of Education (Westbohemian University) in Pilsen, it seems to stimulate the interest of students in further study and continuation in the field of human genetics having the logical link to basic principles of molecular biology. The work has been supported by grants of the Ministry of Education, Youth and Sport of the Czech Republic numbers C54-2008, C35-2009 and 14/89 - 2009.

1686/T

Social Networks as an strategy for Education in Genomic Medicine. *S. March, J. Bedolla, N. Alvarez, J. Avila, C. Davila, X. Soberon.* Education and Outreach, INMEGEN, Mexico, Mexico City, Mexico.

Conventional media such as tv, radio, printed ads or books, used to promote, diseminate or communicate ideas, products or information related to any topic are now in lower rate consumption. Actually, digital medias will increase the communications, and the way to keep in touch with people who wants to know about genomics. New generations are most of the time wired to various types of electronic devices such as internet. It is not rare social media to use as part of their daily lives. In developing countries such as Mexico, people between 12 and 25 years old are becoming more experience digital media users. Social media like Twitter, Facebook and YouTube are changing the way that many research centers contact their targets. The National Institute of Genomic Medicine (INMEGEN) started using social media as a tool for education in genomic medicine since 2008, using interactive or Multimedia CD (images, videos, sounds, animations) to give information to spanish speaker users. Real time web transmissions (RTWT) such as seminars, lectures, simposiums, among others, became one of the most important tools for the development of an educational platform in genomic medicine in Mexico. This strategy focus on scientific researchers, life sciences students, decision makers, politicians, the Mexican congress and general public. In 2009 we started to podcast information using Facebook (http://www.facebook. com/inmegen),Twitter (http://twitter.com/INMEGEN), Youtube (http://www.youtube.com/inmegen medios) purpose in order to attract talent into genomic medicine, as well as promote research and academic networks worlwide, and to get even closer to general public. In this first analysis from October 2009 until now, INMEGEN's podcasts have been downloaded for a total of 3541 times (http://www.inmegen.gob.mx/index.php?option=com_content&task=view&id=1071&Itemid=155&lang=en). The main users came from Mexico and China. RTWT have increased to 75 connected institutions for a 3750 audience in Mexico, USA and some Latin American countries. This educational effort contributed to support the national platform in the development of genomic medicine in Mexico, will allow Mexican society to promote the discussion of ethical and social challenges related to genomic medicine. In addition this communication strategy will lead to a better understanding of genomic medicine, its benefits for health care and the economic development of Mexico and Latin America.

1687/T

Building A Genomics Pathway. *M. Slifer.* HIHG, Univ Miami, Miami, FL.

Medical School Pathway Programs are designed to provide select medical students with additional education opportunities in areas of special interest relevant across medical specialties, as well as introduce these students to the potential of Physician Scientist research. Several medical schools have adopted the four year Pathway approach to enhance training in community-based and social medicine. In 2005, the University of Miami Miller School of Medicine, initiated a Pathway Program in Human Genetics and Genomics. The Pathway was built around a genetics and genomics lecture series and mentored Summer research projects. However, we experienced a 100% drop-out rate for what would have been the first graduating class. Student evaluations consistently expressed dissatisfaction with the emphasis on basic science research, and students struggled to appreciate its relevance to clinical medicine and Physician careers. Incorporating these concerns, the Pathway was revamped. For the lecture series, we developed a clinical case-based curriculum. Students were provided background materials to review prior to lectures. Then, expert lecturers presented cases and encouraged an open discussion of the relevant genetic bases, therapeutic options, and the family/social context impacting each case. Additionally, the summer research program was revised to provide more exposure to different facets within each student's research project. Among the changes, students are encouraged to attend clinics and clinical rounds in addition to their bench and/or informatics research. Satisfaction has improved (4.8 out of 5 on the Satisfaction scale) and Pathway retention is currently 24 of 26 medical students (8% drop-out). The two students who dropped, are pursuing other competing interests (student government, and a different research program) and neither student endorsed dissatisfaction with the present Genetics and Genomics Pathway. By aligning Pathway procedures with student preferences we are able to achieve Pathway educational goals and have increased participation, retention, and satisfaction with the Human Genetics and Genomics Pathway.

1688/T

Results from a survey investigating the background and training of pre- and post-doctoral fellows in statistical genetics and genetic epidemiology. *A. Wilson, M. Krishnan, The NIH Working Group on Statistical Geneticists Training Needs.* Genometrics Section, NIH/NHGRI, Baltimore, MD.

With increasing numbers of genome-wide association and sequencing studies being performed, the scientific community has been deluged with data that require scientists who can develop and perform statistical genetic analyses. There is concern that there are not enough scientists to perform these analyses. A survey was conducted in 2008-2009 to determine the number of pre- and post-doctoral trainees and their type of training and potential expertise. The survey also queried the background and expertise of their faculty level trainers, whether the number of training slots available was adequate and whether these positions were filled. Members of the International Genetic Epidemiology (IGES) were invited to participate through the IGES newsletters prior to the 2008 IGES annual meeting. A second notice went to the IGES membership at the end of January and to selected members of the American Society of Human Genetics. In February 2009, there were 391 responses, with 197 from faculty level trainers, and 96 and 100 responses from pre- and post-doctoral trainees, respectively. Sixty-one percent (105/172) of the Faculty level trainers had NIH funding, and 32% (55/171) had pre-doctoral students supported on NIH T32 training or F fellowship grants. The number of current pre-doctoral trainees per trainer was 2.4 (132 trainers) and the total number of pre-doctoral trainees "in the pipeline" was 318. Similarly, the average number of post-doctoral trainees per trainer was 1.2 (130 trainers) and the total number of post-doctoral trainees "in the pipeline" was 150. The most frequent response for the reason that training slots were unfilled was that there were not enough qualified domestic applicants. The distribution of pre-doctoral fellows (96) was roughly normally distributed with the largest number of pre-docs in their third year of training. At least 40% of the pre-doctoral trainees had not had a single formal course in the following disciplines: computational biology, computer science, genetics, mathematics, medical genetics, molecular biology, population genetics or quantitative genetics. The distribution of years of training for post-doctoral fellows was more uniform than that of pre-doctoral fellows, with 64% (51/80) having two or less years of training. Based on the distribution of length of post-doctoral fellowships, roughly 11 - 17 post-doctoral fellows complete training each year.

1689/T

Newborn Screening Clearinghouse: Access to Information for All. *N. Bonhomme[1], L. Simon[2], A. Harris[2], S.F. Terry[1].* 1) Genetic Alliance, Washington, DC; 2) Health Resources and Services Administration, Rockville, MD.

State newborn screening programs strive for quality and excellence in serving the newborns of our country, and they are constantly balancing these goals with limited resources and healthcare priorities. Individuals, families, and communities have an increased interest in health information, particularly in online and interactive forms. Despite the public's increased attention to health, parents are still generally unaware of the availability and need for newborn screening. Likewise, as increasing numbers of newborns are screened for more conditions, parents and healthcare providers have a growing need for centralized, easily accessible and accurate information. The Newborn Screening Clearinghouse (NBSC) is intended to connect parents and healthcare providers with information about the conditions screened, the programs screening and other available resources through a cooperative agreement between the Health Resources and Services Administration, Genetic Services Branch and Genetic Alliance. Genetic Alliance will partner with the National Newborn Screening and Genetics Resource Center, the Genetics and Newborn Screening Regional Collaborative Groups, March of Dimes, the Association of Public Health Laboratories and many other partners to build on resources already available. The NBSC will allow for extensive networking and resource sharing by improving activities already ongoing and creating new avenues for information exchange. It will create a central connection to informational resources and data on newborn screening quality indicators. The NBSC will be responsive to emerging technologies and the challenges that public health programs face. We will report on the status of the NBSC, its developing website, input from regional and national stakeholders, and future plans.

1690/T

Community genetics and health forums: a model community engagement program for leadership development. *L. Stark, H. Aiono.* Gen Sci Learning Ctr, Univ Utah, Salt Lake City, UT.

The Genetic Science Learning Center (GSLC) at the University of Utah used community-based education programs as a vehicle for collaboratively developing a Community Council. The programs engaged community leaders and members in learning about chronic diseases common in their community, the contributions of genetics and lifestyle choices to chronic disease risk, and the benefits of collecting a family health history. Leaders of diverse Salt Lake City, Utah communities were invited to apply for $5,000 mini-grants to support development and presentation of mini-forums on these topics in their communities. Based on their applications, grants were awarded to organizations representing the African American, African refugee, Chinese, Hispanic/Latino, Native American, and Tongan communities. The GSLC's Community Liaison worked with each community organization and its leaders to identify the chronic diseases of highest concern in their community, to design appropriate educational programs (mini-forums), and to identify suitable presenters. Family health history served as the "hook" to engage community members, since it personalized the information and made it relevant. Many programs used materials available on the GSLC's Learn.Genetics website (Connection Between Heredity and Health section at http://learn.genetics.utah.edu/content/begin/traits/activities/); culturally appropriate adaptations were developed for communities that requested them. The Community Leadership Team met monthly to collaboratively plan a multi-cultural forum involving all communities, which they titled Community Faces: A Genetics and Health Forum. These meetings supported the development of trusting relationships among community leaders and with participating University faculty/staff, enhanced members' leadership confidence and capacity, built their knowledge about genetics and health, and provided an opportunity to share and learn from each other's mini-forum experiences. The Forum provided a focus for the Team's work together and a common, successful sharing and learning experience. As a result of its experience with this project, the Community Leadership Team has decided to continue working together and is evolving into a Community Council. Funding for this project was provided by the Education and Community Involvement Branch of the NIH National Human Genome Research Institute with additional support from the University of Utah Center for Clinical and Translational Science.

1691/W

A MICRORNA PROFILE FOR TWO CELL MODELS COMMONLY USED IN GENETIC EXPRESSION GENERATED BY MIRDEEP. *V. Williamson[1,3], A. Kim[1,2], B. Xie[4], O. McMichael[1], Y. Gao[4], V. Vladimirov[1].* 1) Psychiatry, Virginia Institute for Psychiatric and Behavioral Genetics, Richmond, VA; 2) Department of Human Genetics, Virginia Commonwealth University, Richmond, Va; 3) Integrative Life Sciences, Virginia Commonwealth University, Richmond, Va; 4) Center for the Study of Biological Complexity, Virginia Commonwealth University, Richmond, Va.

The discovery of microRNAs (miRNA) reshaped our appreciation of gene regulation. Deep sequencing is ideal for the absolute quantification of known and discovery of new miRNAs because of its decided depth and coverage. Next generation machines can produce 32 gigabytes of sequence in a single run with an average read length of 32 bases, which is more than adequate to study small non-coding RNAs such as miRNAs. We describe miRNA profiles for two, neuroblastoma derived (BE2-C) and lymphoblastoid (LC) cell lines, using the MiRDeep algorithm. MiRDeep employs a probabilistic algorithm of miRNA biogenesis to discover known and novel miRNAs species in a deep sequencing run(1). To indicate prediction strength, MiRDeep generates a score based on posterior Bayes statistics. This score indexes the number of times in which a read sequence conforms to traditional miRNA biogenesis as well as its overall frequency in the dataset. MiRNA precursors have a specific hairpin structure, which play an important role in the processing of the miRNA precursor. MiRNA hairpin structure formation is sequence dependent and highly specific to the miRNA species, allowing us to distinguish real miRNA hairpins from pseudo hairpins(2). MiRDeep allows the user to permute the sequence of predicted miRNA,thus to assess the reliability of such predictions. Also, some miRNAs show high evolutionary conservation across species, whereas others more recently evolved, are likely to be species specific. To answer this question we examined the overall level of species preservation of the novel predictions determining what degree these novel miRNAs can be considered uniquely human. In order to truly understand the role miRNAs play in gene regulation, we must first have adequate knowledge of cell models in which they are tested. Therefore we examine total (known and novel) miRNA expression profiles in both cell lines. We evaluate expression differences using chi-square statistics as previously shown(3). We observed significant differences in the level of expression in hsa-mir-17 and hsa-let-7g between the two cell lines ($\chi 2 = 69.94$, df=1, $p<0.0001$). The differences concerning hsa-mir-17 may be of note because this miRNA has been recently implicated in Schizophrenia(4). 1. M. Friedländer et al., Nat. Biotech 26:407(2008). 2. Jiang,P. et al., Nucleic Acids Research 35: 339(2007). 3. P.L. Auer et al. Genetics 110.114983 (2010). 4. N.J. Beveridge et al., Cellular Signaling 21:1837(2009).

1692/W

A novel pathway analysis method to prioritize 'susceptibility pathways' in multigenic traits. *N. Akula[1], A. Baranova[2], D. Seto[3], J. Solka[3], M. Nalls[4], A. Singleton[4], L. Ferrucci[5], T. Tanaka[5], S. Bandinelli[6], Y.S. Cho[7], Y.J. Kim[7], J-Y. Lee[7], B-G. Han[7], BiGS. Consortium[8], F.J. McMahon[1].* 1) Mood & Anxiety Disorders, NIH, Bethesda, MD; 2) Molecular and Microbiology Department, George Mason University, Fairfax, VA; 3) Department of Bioinformatics and Computational Biology, George Mason University, Manassas, VA; 4) Molecular Genetics Section, Laboratory of Neurogenetics, National Institute on Aging, Bethesda, MD; 5) Longitudinal Studies Section, National Institute on Aging, National Institute of Health, Baltimore, MD; 6) Geriatric Unit, Azienda Sanitaria di Firenze, Florence, Italy; 7) Center for Genome Science, National Institute of Health, Seoul, Korea; 8) Scripps Genomic Medicine and Scripps Translational Science Institute and Dept of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA, USA; Dept of Psychiatry, Univ of Chicago, Chicago, IL, USA; Dept of Psychiatry, Portland VA.

Genome-wide association studies (GWAS) are a valuable approach to complex traits. However, single markers often fail to achieve levels of statistical significance required to withstand correction for multiple testing, and genetic association signals may fail to replicate. Alternative approaches that embrace the multi-genic basis of complex traits are needed. Here we introduce Network Interface Miner for Multigenic Interactions (NIMMI), a network-based method that combines GWAS signals with existing data from the human interactome. NIMMI builds biological networks using human protein-protein interactions (PPIs), weighted by connectivity estimated with a modification of the Google Page Rank algorithm. These weights are combined with genetic association p-values, thus producing what we call 'susceptibility networks'. There is no requirement for pre-selection of the most significant subset of SNPs, but users can specify SNP inclusion thresholds as desired. As a proof of principle, NIMMI was tested on 3 GWAS datasets previously analyzed for height, a classical polygenic trait. Despite the differences in sample size and ethnic background, NIMMI captured 100% of known height-associated genes within the top 20% of ranked networks and demonstrated its superiority to a single marker approach. A comparison of networks scored within the top 3% revealed significant enrichment in genes involved in the processes of development, signal transduction, transport, regulation of gene expression, nucleic acid, protein and zinc metabolism. All these networks were successfully replicated across all 3 GWAS datasets. NIMMI efficiently combines genetic association data with biological networks, thus prioritizing susceptibility pathways for further studies. NIMMI is a simple, user-friendly, open-source software tool which is available at http://mapgenetics.nimh.nih.gov/datashare.html.

1693/W

CIDRSeqSuite: Software Utilities for Automation of Next-Generation Sequencing Tasks. *M.W. Barnhart, S.M.L. Griffith, K.N. Hetrick, J.L. Goldstein, D. Mohr, B.A. Marosy, D.R. Leary, B.D. Craig, L. Watkins, Jr., K.F. Doheny.* IGM/CIDR, Johns Hopkins Univ/CIDR, Baltimore, MD.

The Center for Inherited Disease Research provides high quality genotyping services and statistical genetics consultation to investigators working to discover genes that contribute to common disease. In exploring next-generation sequencing technologies, CIDR found the need to automate a variety of bioinformatic workflows and analyses. Developed at CIDR to run on 64-bit Linux, CIDRSeqSuite is a highly configurable collection of Java 1.6 and Perl software tools that processes sequencing data generated by Illumina's GAII instrument. The suite also allows users to generate quality control reports, perform analyses, and annotate SNP data via SeattleSeq. By wrapping open source tools, CIDRSeqSuite automates workflows that would otherwise require several independent steps at the command line. For example, using a single command, a set of Illumina raw sequence output (qseq.txt files) is processed through to SNP list generation with QC metrics summary reports generated at various stages of the analysis. Available reports include capture efficiency (used for targeted resequencing projects), SNP transition/transversion ratio, allelic composition of all reads at a given genomic position, and summary coverage statistics for targeted regions. In addition, the software can compare SNP genotype calls between array data and sequencing data. Also available are graphical outputs such as quality-score box plots and nucleotide distributions. Command-line utilities allow conversion among various file formats, e.g., SAM to BAM and vice versa, and between the Sanger, Solexa, and Illumina FASTQ formats. Additionally, CIDRSeqSuite contains a workflow that estimates capture efficiency after the first read of a paired-end run has been completed, allowing CIDR to save instrument time and to reprioritize samples for resequencing in the event of a laboratory problem, such as an error during the target sequence enrichment process. Current work involves demultiplexing qseq.txt files for GAII indexed-sample runs in order to provide a workflow independent of vendor-supplied tools. Each user can customize CIDRSeqSuite to launch specific versions of the wrapped programs: Maq, BWA, Picard, SAMTools, Bowtie, and FASTX-Toolkit. CIDRSeqSuite can adapt dynamically to its runtime environment, making the best use of available computing resources. This software has enabled CIDR to assess next-generation sequencing data quality quickly while reducing human error.

1694/W

Genetic ME - A visualization application for merging and editing pedigrees for genetic studies. *D.K. Bui[1], M.C. Ortube, MD[2], D.E. Weeks, PhD[3], A. Martinez, MS, MS[2], Y. Conley, PhD[4], M.B. Gorin, MD, PhD[2]*. 1) David Geffen School of Medicine at UCLA, Los Angeles, CA; 2) Department of Retinal Disorders and Ophthalmic Genetics, Jules Stein Eye Institute, Los Angeles, CA; 3) Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA; 4) Department of Health Promotion & Development, School of Nursing at University of Pittsburgh, Pittsburgh, PA.

Purpose: In order to study the genetics of diseases more accurately and effectively, software is needed to facilitate the reconciliation of genetic information collected independently from multiple members of the same family. We identified a need for an application that would allow for the visual comparison, editing, and merging of overlapping pedigree structures given by related individuals to create a unified pedigree for family-based studies with a clear mechanism for tracking that reconciliation process.

Methods: Genetic ME is a Java application designed to meet the need mentioned above. It leverages the existing technology of Cranefoot, a powerful and flexible open source pedigree drawing tool. It provides a graphical user interface (GUI) that allows users to view two pedigrees and their corresponding familial, demographic, clinical, and genotyping data side by side for easy comparison. Mock family data was created and used to assess the capabilities of this new application.

Results: Using the GUI, users can edit current individuals' information, add new individuals, or remove existing individuals from the displayed data. Users can reconcile differences between the two displayed pedigrees by applying one of three merging algorithms (replacement of an individual or branch from one pedigree by the other, combination of data from one pedigree into another, and appending individuals and sublineages from one pedigree to the other). Individuals and data can be merged from pedigree #1 to pedigree #2 or vice versa. The application then displays the merged pedigree with the new changes for approval before any modifications are saved into a new composite file. The original and resulting text files are stored in tab-delimited format and the graphic files in Portable Network Graphics (PNG) format for future use. Tracking is implemented so that all of the changes made to the data set for the individuals and to create the merged pedigree can be tracked to their original source material.

Conclusion: Genetic ME is an open source Java application developed for the ease of viewing, editing and merging pedigree structures and for comparing and reconciling disparate clinical or genetic information of individuals from two or more sources. An executable file and source code will be available for download in fall 2010.

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1695/W

Phylogenetic analysis of the human microflora. *E.R. Chan, J. Hester, D. Serre*. Genomic Medicine Institute, Cleveland Clinic Foundation, Cleveland, OH.

Advances in DNA sequencing technology have expanded our ability to study the impact of the microflora on human health and disease. This impact is not trivial considering the microbiome contains over 100 times the genes in the human genome. A common method of capturing the diversity of the microflora is by sequencing of the 16S ribosomal RNA gene. This gene is required in all prokaryotic cells and has highly variable regions flanked by conserved regions which allows for 1) amplification using universal primers and 2) phylogenetic analysis and taxonomic identification. Deep sequencing of this gene provides a comprehensive view of the species that are contained in a particular microbial environment but requires novel methods to analyze the massive amounts of data that can now be generated. Previous taxonomic approaches compared individual sequences to a database of annotated 16S rRNA sequences which relies on the sequences (or closely related sequences) to be in the database, accuracy of the database, as well as the algorithm developed to match the sequence to the annotated entries. Alternatively, a phylogenetic approach has been developed to infer global differences in bacterial composition between microenvironments by examining a phylogenetic tree comprised of all the sequences captured. This method can distinguish whether there is an overall difference in the microflora between samples but provides no taxonomic information of the bacteria involved. Here we describe a novel method of analyzing deep sequencing data of 16S rRNA genes using a phylogenetic approach which incorporates a dynamic hierarchical tree cutting method to define groups of similar bacteria irrespective of their annotation (or lack thereof). The advantage of this approach is that taxonomic information can still be applied to these defined groups using existing 16S rRNA databases. We apply this method to examine the microflora composition in cases and controls for several pilot studies including a study on colorectal cancer in humans and the effects of bariatric surgery using a rat model. We also apply our method to a previously published data set involving TLR5 knock-out mice. We believe that the method described here will significantly improve our understanding of the role of the microflora on human disease by providing a comprehensive analysis of the microbiome and identifying communities whose proportion differs with regards to disease state without the need for previous annotations.

1696/W

Updated Tools for Genomic DNA Alignments. *D. Church, R. Agarwala, N. Bouk, C. Clausen, V. Schneider, A. Astashyn, D. Maglott, M. DiCuccio.* NCBI,NLM,NIH, Bldg 38A Rm 6S614K1, Bethesda, MD.

The decreased cost of sequencing has led to an increased cost of the analyses of these sequences. The increased burdens associated with sequence analysis can be seen in tasks ranging from read production to the analysis of assembled genomes. Many projects, including read analysis of sequences from second generation sequencing machines, alignment of assembled sequences to identify large-scale genomic variation and the alignment of individual de novo assemblies to each other require robust alignments to a reference assembly . NCBI has been working to provide a suite of tools to improve genomic analysis specifically in the area of genomic alignments. Additionally, tools to interrogate and visualize these alignments are being developed. We have focused our efforts in four areas: (1) producing alignment algorithms that take advantage of modern assemblies that represent allelic diversity(such as the human public reference assembly, GRCh37), (2) producing alignments that can better represent structural variation, (3) producing more robust assembly-assembly alignments and (4) providing better tools for interrogation and visualization of alignment data. We will demonstrate the use of these tools in numerous applications, including the alignment of second generation sequencing data to GRCh37 and the alignment of structurally variant BAC and fosmid clones to GRCh37. Additionally we will introduce a new tool called NCBI Remap (http://www.ncbi.nlm.nih.gov/genome/tools/remap), which allows users to project features from one genome assembly onto another. NCBI Remap produces mapping summaries, feature by feature reports, as well as remapped annotation data than can be loaded to most browsers. Additionally, the results, along with the underlying assembly-assembly alignments, are packaged in files that can be visualized using our client-side genome analysis tool, Genome Workbench (http://www.ncbi.nlm.nih.gov/projects/gbench). Finally, we will present a comparison of the NCBI Remap tool to the UCSC lift-over tool. Acknowledgments Work at NCBI is supported by the NIH Intramural Research Program and the National Library of Medicine.

1697/W

MutaREPORTER software to define, characterize and archive mutations leading to human genetic disease. *M. Devisscher[1], F. Decouttere[1], K. De Boulle[2], P.J. Willems[2].* 1) Genohm, Zwijnaarde, Belgium; 2) GENDIA, Antwerp, Belgium.

Only a small fraction of the mutations causing genetic disease have been published in the literature or listed in public mutation databases, Consequently, the clinical significance of many novel mutations remains undetermined. To address these problems we developed MutaDATABASE, a standardized, centralized, and free open access database, and MutaREPORTER, specific software, which is designed to define, characterize and archive variations in human disease genes. MutaREPORTER has bidirectional communication with MutaDATABASE as it both retrieves all existing information out of MutaDATABASE, and also allows easy entry of new data, including novel mutations into MutaDATABASE. This makes submission of novel variations to an integral part of the diagnostic workflow. All new information sent by MutaREPORTER to MutaDATABASE is verified by an assigned MutaCURATOR before entry into MutaDATABASE. MutaREPORTER also contains an integrated organized communication tool, allowing groups of genetic labs that offer diagnostic testing for the same gene (MutaCIRCLES) to discuss novel gene variations, additions to the phenotypic spectrum and unusual findings related to that specific gene with all members of that MutaCIRCLE. MutaREPORTER is a Flex-based Web 2.0 application with a user friendly interface that specifically targets genetic labs. The performance of the visualization engine is assured by semantic zooming, view porting and a caching mechanism. The underlying architecture makes the framework easily extensible with future tools and functionalities. MutaREPORTER currently provides: • Access to all info in MutaDATABASE • A genome browser with comprehensive, user-customizable, track-based views of the genomic sequence, gene structure, protein sequence, and conservation. • A variation checker that checks whether the HGVS nomenclature has been issued to describe the variation • Facilitated access to prediction tools that are important in the assessment of the pathogenicity of variants (Splice site prediction, SIFT, POLYPHEN) • Possibility to automatically import variants from in-house databases (requires some software that interacts with the lab's LIMS) • Possibility to easily submit variants into MutaDATABASE, both graphically as by using HGVS nomenclature • Possibility to communicate with other genetic labs that offer diagnostic testing for the same gene (MutaCIRCLES).

1698/W

From candidate to modifier genes, non-coding RNA in genetic diseases with miRiFix database. *M. Girard[1], S. Bandiera[1], C. Mugnier[2], M. Le Merrer[1], A. Munnich[1], S. Lyonnet[1], A. Henrion Caude[1].* 1) Inserm U781, Department of Genetics, Hopital Necker-Enfants Malades, University Paris Descartes, Paris, France; 2) University Paris Descartes, Paris, France.

Identification and analysis of microRNAs (miRs) enhance our understanding of the important roles that non-coding RNAs play in complex regulatory networks. However, there are still few data supporting the involvement of miRs in Mendelian disease inheritance other than cancer. MiRs may be regarded either as candidate gene within a disease locus, or as putative modifier gene, which can regulate the expression of a given disease-causing gene. At this latter level, single nucleotide polymorphisms (SNPs) within the target gene add a supplemental layer of complexity. Herein, we present a comprehensive resource, aimed at linking small and long non-coding RNAs with hereditary diseases : mirifix.com. MiRiFix is an easy-to-use, web-accessible framework of tool and data integration. Our model crosses up-to-date information on human miRs and the Genatlas database, which provides integrated data on gene mapping and genetic diseases. MiRiFix enables to systematically explore the computational involvement of miRs in the pathogenesis of diseases, and retrieves : (i) miR as a candidate gene from a locus, using the updated compendium of human miRs and their mapping information, (ii) a set of miRs predicted to regulate a disease-causing gene, in both its 3'-UTR and coding sequence, using distinct algorithms, and finally (iii) a set of SNPs predicted to be functional in terms of miR regulation. We will present the efficiency of miRiFix in predicting previously established links, but also in retrieving novel data on mapped diseases orphan of identified genes. Our web resource provides a unique integrated way to assess computational roles of non-coding RNAs in hereditary disease.

1699/W

The Functionality of Missense Mutations is Dependent on both the Algorithm and Multiple Sequence Alignment. *S. Hicks[1], S.E. Plon[2,3], D.A. Wheeler[2], M. Kimmel[1].* 1) Department of Statistics, Rice University, Houston, Texas; 2) Human Genome Sequencing Center, Baylor College of Medicine, Houston, Texas; 3) Texas Children's Cancer Center, Department of Pediatrics, Baylor College of Medicine, Houston, Texas.

Large-scale sequencing projects yield missense mutations with unknown biological significance. Several computational algorithms have been developed to predict the impact of missense mutations on protein structure and function. Informative classification is highly dependent on the algorithm of choice and the parameters used in the algorithm. The goal of this study is to investigate the variability in predictions of missense mutations from three commonly used algorithms with four different multiple sequence alignments, each having their own bias towards a particular algorithm. The three methods, SIFT, Align-GVGD, PolyPhen-2 were evaluated using both a small number of common polymorphisms (n=4) and a large number (n=140) of cancer associated missense mutations from the TP53 mutation IARC database (http://www-p53.iarc.fr/). We also evaluated a more recent method Xvar (http://xvar.org) and will include it in the analysis as the ability to accept a users alignment becomes available. We compared the results from each algorithm providing the following alignments: (1) a small highly curated alignment with long evolutionary depth ideal for Align-GVGD, (2) an uncurated alignment automatically generated in Uniprot using the built in ClustalW feature with sequences included based on a criteria of 90% or 50% identity, (3) an automatically generated alignment from SIFT, and (4) an automatically generated alignment from PolyPhen-2 with a wide breadth of sequences. Using manually curated alignments the results show PolyPhen-2 had a slightly higher specificity than Align-GVGD and SIFT. In contrast, all of the programs dramatically change their predictions using uncurated alignments. As the number of sequences in the alignment increases, Align-GVGD dramatically overcalls variants as neutral for example it miscalls all 140 deleterious variants as neutral when using the PolyPhen-2 or 50% identity alignment. In contrast, Polyphen-2 cautiously predicts variants as neutral when there are a limited number of sequences in the uncurated alignment, but as the number of sequences increases the algorithm is able to make a more informed prediction. Similar results are seen using a larger set of missense mutations with sufficient evidence of clinical neutrality from the BRCA1 and BRCA2 mutation database (BIC). Thus, informative classification is dependent on the algorithm used, the number of sequences in the alignment, and whether or not the alignment has been manually curated.

1700/W

Identifying differential expressed genes and detecting full length transcripts with mRNA-seq data. *W. Huang, L. Li.* NIEHS, NIH, Research Triangle Park, NC.

Next-Gen sequencing-based mRNA-seq has been increasingly used for identifying novel transcripts and differential expressed genes. The lack of computational methods and tools for analyzing mRNA-seq data, however, limits its broad adoption for biomedical research. The widely used mRNA-seq applications include identification of differentially expressed genes in comparative studies, and detection of full length transcripts. To facilitate mRNA-seq data analysis from these applications, here we describe a new statistical framework for identifying transcripts with different abundance levels among different samples. In addition, we present a computational strategy for locating full length transcripts using paired-end read data. We also introduce a new efficient tool, EpiCenter, for analyzing mRNA-seq data. Some key features of our methods are: 1) multiple traditional and novel data normalization procedures adapted for different scenarios, 2) accounting for large variability of read depth of coverage to reduce false discovery rate, and 3) computationally efficient. To test effectiveness of our methods, we applied our methods and tools to two real whole genome mRNA-seq data for mouse and *Schizosaccharomyces* pombe, respectively. We then compared the results with those from microarray data analysis from the same samples. While the results from mRNA-seq data and microarray data are largely consistent, the comparison suggests mRNA-seq is more powerful than microarray in detecting true differentially expressed genes.

1701/W

CEP290*base*: A Unique Overview of *CEP290* Mutations and Their Associated Phenotypes. *S. Lefever, F. Coppieters, E. De Baere.* Center for Medical Genetics, Ghent University, Ghent, Belgium.

Mutations in *CEP290* may cause a wide spectrum of partially overlapping ciliopathies, ranging from isolated Leber Congenital Amaurosis to the lethal Meckel-Grüber syndrome. Although some mutations seem to be phenotype-specific, others segregate with distinct diseases. In addition, the phenotype is highly variable, probably due to the presence of modifier alleles in other members of the ciliary proteome. The goal of this study was to design a locus-specific database for mutations in the *CEP290* gene, containing both genotypic and phenotypic information. The database was created using MySQL. PHP and JavaScript were used to develop the web-based interface. Mutation nomenclature is according to the HGVS guidelines. CEP290*base*(http://medgen.ugent.be/CEP290base) contains all mutations in *CEP290* published so far, including detailed information on their pathogenic potential. The database links to dbSNP, UniProt and OMIM and automatically fills in queries for PolyPhen and SIFT. In addition, a comprehensive description of phenotypic features is provided for each patient in different systems (ocular, renal and neurological). The database lists all variants in both an overview and graphical representation, and offers easy access to patients and variants through several query possibilities. For patients with *CEP290* mutations, variants in other genes can also be included, thereby providing the opportunity to link modifiers to associated clinical manifestations. Last but not least, users can submit novel or known variants they have identified. In conclusion, CEP290*base* offers the first comprehensive locus-specific mutation database of all mutations identified in *CEP290* to date, and their associated phenotypes. The database contributes to the establishment of genotype-phenotype correlations, and may assist in providing prognostic information for a patient with *CEP290*-related pathology.

1702/W

On the identification of condition specific sub-networks from microarray gene expression profiles. *H. Ma[1], E. Schadt[2], L. Kaplan[3], H. Zhao[4].* 1) Program in Computational Biology and Bioinformatics,Yale University, New Haven, CT; 2) Pacific Biosciences, Menlo Park, CA; 3) Gastrointestinal Unit and MGH Weight Center, Massachusetts General Hospital and Department of Medicine, Harvard Medical School, Boston, MA; 4) Department of Epidemiology, Public Health and Genetics, Yale University, New Haven, Connecticut.

[Background] The identification of condition specific sub-networks has important biological applications such as selecting disease-associated biomarkers as well as discovering alterations in regulatory or signaling pathways across different phenotype groups. In this paper, we propose a new method of extracting condition specific sub-networks from microarray gene expression data, COSINE (COndition SpecIfic sub-NEtwork). COSINE uses a scoring function that jointly considers the differential expression of single genes and the differential co-expression patterns of gene pairs, and that employs the genetic algorithm to search for the sub-network that maximizes the scoring function among sets of different conditions. [Results] We compare COSINE with other methods on both simulated data with various patterns of expression alterations, and real data from cross population comparisons among HapMap samples. The results demonstrate that COSINE is more powerful in terms of identifying the truly significant sub-networks of appropriate size and meaningful biological interpretation. Additionally, the weight parameter λ in the scoring function allows for fine tuning the relative emphasis on single gene differential expression versus gene pair differential co-expression, thereby providing more flexibility in the structural feature of the selected sub-networks. Pathway enrichment analysis of the identified networks from the HapMap dataset shows that a number of immune related pathways play a leading role in differentiating the expression profiles across human populations, an observation that may not be identified using traditional analytical methods. [Conclusions] Our new method COSINE has a number of advantages over existing methods to identify meaningful responsive sub-networks from several gene expression profiles. The network perspective and global optimization approach can be very helpful in understanding the conditional changes of important biological processes.

1703/W

Tertiary Analysis For Variant Detection In Next-Generation Datasets. *D. Mohr[1], B. Craig[2], B. Marosy[2], K. Hetrick[2], M. Barnhart[2], S. Griffith[2], J. Goldstein[2], A.F. Scott[1], K.F. Doheny[2].* 1) Genetic Resources Core Facility (GRCF) High Throughput Sequencing Center, Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Center for Inherited Disease Research (CIDR), Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD.

As next generation sequencing becomes more readily available, more and more investigators that lack access to dedicated bioinformaticians are finding themselves overwhelmed with analysis of large datasets. Many facilities will provide primary and secondary analysis (basecalls in fastq format and initial alignment to reference in SAM or vendor format), but few open access facilities have the capacity to offer tertiary analysis (variants, indels, etc), and if they do, the investigator is left to mine these lists for biologically meaningful information. Many commercial solutions are available, but are often cost prohibitive and/or lacking features essential for variant detection. At the GRCF High Throughput Sequencing Center, using CIDRSeqSuite, Illumina base calls are converted to fastq format, aligned to reference, converted to pileup format, duplicates are marked and filtered to produce variant lists, and subsequently submitted to and annotated by the SeattleSeq SNP annotation server. Here we present a tertiary workflow using readily available open source tools, including Sequence Variant Analyzer (SVA), Integrated Genomics Viewer (IGV), Polymorphism Phenotyping (PolyPhen), and SeattleSeq to mine potential variants generated by high throughput sequencing.

1704/W

Exome sequencing allows for rapid gene identification in a Charcot-Marie-Tooth disease family. *G. Montenegro[1], E. Powell[1], J. Huang[1], Y. Edwards[1], G. Beecham[1], W. Hulme[1], C. Siskind[2], M. Shy[2], J. Vance[1], S. Zuchner[1].* 1) John P. Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL; 2) Department of Neurology and Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, MI.

Charcot-Marie-Tooth (CMT) disease comprises a large number of genetically distinct forms of inherited peripheral neuropathies. The relative uniform phenotypes in many patients with CMT make it difficult to decide which of the over 35 known CMT genes are affected in a given patient. Genetic testing decision trees are therefore broadly based on a small number of major subtypes (e.g. CMT1, CMT2) and the observed mutation frequency for CMT genes. Since conventional genetic testing is expensive many rare genes are often not being tested at all. As a novel and alternative approach whole exome sequencing has recently been introduced. This method is capable of re-sequencing the near complete set of coding exons of an individual. We performed whole exome sequencing in an undiagnosed family with CMT. Across 40 neuropathy related genes screened by this approach we identified a non-synonymous GJB1 (Cx32) mutation, V95M. This variant had been reported previously as pathogenic in X-linked CMT families. Sanger sequencing confirmed complete co-segregation in seven affected individuals. Affected individuals had a marked early involvement of the upper distal extremities and displayed a mild reduction of nerve conduction velocities. We have shown for the first time in a genetically highly heterogeneous dominant disease that exome sequencing is a valuable method for comprehensive medical genetic diagnoses. Further improvements of exon capture design, next-generation sequencing accuracy, and a constant price decline will soon lead to the adoption of genomic approaches in gene testing of Mendelian disease.

1705/W

The Gentrepid candidate gene prediction web server. *M. Oti[1], J.Y. Liu[1], S. Ballouz[1,2], D. Fatkin[1,2], M. Wouters[1,2].* 1) Victor Chang Cardiac Research Institute, Sydney, NSW, Australia; 2) University of New South Wales, Sydney, NSW, Australia.

Gentrepid (https://www.gentrepid.org/) is a web resource which predicts and prioritizes candidate disease genes for both Mendelian and complex diseases. It uses two approaches to prioritize candidate disease genes, Common Pathway Scanning (CPS) and Common Module Profiling (CMP). CPS assumes specific phenotypes are associated with dysfunction in proteins that participate in the same complex or pathway and applies network data derived from protein-protein interaction and pathway databases to identify relationships between genes. CMP is based on the hypothesis that disruption of genes of similar function will lead to the same phenotype and identifies likely candidates using a domain-based sequence similarity approach. In a benchmark using Mendelian diseases, the two methods had a combined sensitivity of 0.52 and specificity of 0.97, reducing the candidate list by 13-fold. Another benchmark against highly significant Type II Diabetes GWAS SNP data resulted in a sensitivity of 0.18 and a specificity of 0.96 while reducing the candidate list by 19-fold, indicating its applicability to complex diseases as well. These approaches are also applicable to candidate gene prediction in other species. Here I describe the Gentrepid system and its web interface.

1706/W

MutationTaster - Rapid evaluation of the disease-potential of sequence alterations. *J.M. Schwarz[1,2], C. Rödelsperger[3,4], M. Schuelke[1,2], D. Seelow[1,2].* 1) Neuropaediatrics, Charité - Universitätsmedizin Berlin, Berlin, Germany; 2) NeuroCure Clinical Research Center, Charité - Universitätsmedizin Berlin, Berlin, Germany; 3) Institute for Genetics, Charité - Universitätsmedizin Berlin, Berlin, Germany; 4) Max Planck Institute for Molecular Genetics, Berlin, Germany.

With the advent of Next Generation Sequencing, the search for disease mutations is currently shifting from a single gene approach to sequencing the whole exome, complete linkage regions or large numbers of candidate genes simultaneously. These approaches inevitably reveal numerous exonic or intronic alterations (recent studies suggest more than 1,000 alterations per Mbp in one individual). Testing all of them for their disease potential is obviously not feasible, so there is a growing need for an automated pre-evaluation. However, currently available prediction tools only analyse single amino acid substitutions and are usually not able to process hundreds or thousands of queries in a reasonable time.

We have therefore developed MutationTaster, a web-based application aimed at the prediction of the disease potential of DNA sequence alterations in humans. MutationTaster incorporates many different biological analyses such as amino acid exchanges, evolutionary conservation, the loss of protein features or of splice-sites and hence allows a better distinction between harmless and disease causing alterations. Moreover, it can also analyze intronic or silent alterations.

The prediction is generated by a naïve Bayes classifier which was trained with more than 500,000 SNPs validated by HapMap, 8,000 InDel polymorphisms, and 55,000 known disease mutations, leading to a ratio of correct predictions above 90%. The evaluation of a single alteration usually takes less than 0.3 seconds, making MutationTaster an ideal tool for the analysis of the high numbers of alterations found by Next Generation Sequencing. For this task, we additionally offer alignment software which handles raw data from all common deep sequencing platforms as well as scripts for batch analysis of the alterations found. Besides, we designed an intuitive web interface for the query of single alterations. The software is freely available at http://www.mutationtaster.org.

To our knowledge, MutationTaster is the first comprehensive application covering the whole process from raw Next Generation data to the prediction of the disease potential of the detected alterations.

1707/W

The GeneCascade - a website for the identification of disease genes and the evaluation of sequence alterations. *D. Seelow[1,2], J.M. Schwarz[1,2], M. Schuelke[1,2].* 1) Neuropaediatrics, Charité - Universitätsmedizin Berlin, Berlin, Germany; 2) NeuroCure Clinical Research Center, Charité - Universitätsmedizin Berlin, Berlin, Germany.

We have developed a comprehensive software suite for identification of disease genes and prediction of the disease potential of sequence alterations. Our suite consists of 3 intuitive tools, is completely web-based and freely accessible at http://neurocore.charite.de/. **HomozygosityMapper** (http://www.homozygositymapper.org) allows the rapid identification of disease-linked regions in individuals with an inbreeding background. It is based on a model-free algorithm, robust against genotyping errors and numbers of magnitudes faster than conventional linkage analysis. HomozygosityMapper detects candidate regions and displays the underlying genotypes graphically. For each candidate region, it provides a direct link to GeneDistiller. Researchers can at any point decide to share their results with co-workers or to make them publicly accessible on our website; HomozygosityMapper can hence be used as a public repository. **GeneDistiller** (http://www.genedistiller.org) is our candidate gene search engine which integrates data from various biomedical databases. It displays all genes within a genomic region or from a list of candidate genes with user-selected gene-specific data such as molecular function or known corresponding phenotypes. GeneDistiller can highlight interesting features and score similarities or interactions with known disease genes. Users can sort and filter genes by any of their properties. Besides, GeneDistiller offers a user-driven ranking of the genes placing the most likely candidates on top of the list. This process remains transparent and researchers can interactively adjust prioritisation settings on behalf of their own background knowledge and expectations. The last step in identifying disease-causing mutations is the sequence analysis of candidate genes or of whole linkage intervals and the evaluation of every single alteration detected. Our tool, **MutationTaster** (http://www.mutationtaster.org), scores the disease potential of exonic as well as of intronic sequence alterations by various tests for different protein and gene properties. It outperforms most similar applications in terms of accuracy (>90% correct predictions) and speed (<0.3s). It also provides an interface to automatically analyse the vast number of alterations found by *Next Generation Sequencing* and to manage the results.

1708/W

Methods for analyzing next-gen whole exome sequence variation in small pedigrees with unknown diseases. *M. Sincan[1,2], TC. Markello[1,2], DA. Adams[1,2], K. Fuentes-Fajardo[2], H. Carlson-Donohoe[1,2], C. Toro[2], C. Tifft[2], PF. Cherukuri[3], JK. Teer[3], P. Cruz[3], N. Hansen[3], JC. Mullikin[3,4], WA. Gahl[1,2].* 1) Medical Genetics Branch, NIH/NHGRI, Bethesda, MD; 2) NIH Undiagnosed Diseases Program, NIH, Bethesda, MD; 3) Genome Technology Branch, NHGRI, NIH Bethesda MD; 4) NIH Intramural Sequencing Center, NIH, Bethesda MD.

Next generation sequencing technologies are becoming more available as a tool to diagnose genetic causes of diseases. We have sequenced 28 individuals from 8 families with undiagnosed diseases. Our families include both affected and unaffected individuals. Each family has three or four individuals with one or two affected individuals. The power of next gen sequencing gives us a very long list of potentially disease causing variations that are shared among various members of the families. For our families, the number of variants is between 79,710 and 107,817. Using conserved domain based analysis to determine the potential deleteriousness of these variants can reduce the number of variants to between 6,051 and 7,613. This is still a very big number to analyze. To this end, we have applied Mendelian filters to check if genotypes of family members are concordant with affected and unaffected status and various Mendelian inheritance models that are not in conflict with the current pedigree. The nature of our problem, i.e. dealing with undiagnosed diseases, forces us to apply different possible inheritance models. After we remove the positions that are not compatible with our filter, we still end up with a list of variants numbering in the hundreds for a single family. We also applied filters based on other families and SNPs that have frequency information. We assume that our cases are most likely rare diseases, if we find the same variants in other families and/or in dbSNP with a reasonable frequency, we flag them as potentially less likely to cause the disease in question. We found that it is a complex task to search for disease causing changes in the human genome, but data available to meta tag the variome is becoming increasingly available and tools we develop let us cross match our patients' data to this growing body of information. We illustrate the tools we have developed to make these analyses.

1709/W

Prioritizing loci by gene category analysis implicates SNPs of moderate effect on serum lipid levels. *A.V. Smith[1,2], E. Losievskaja[1], G. Eiriksdottir[1], T. Aspelund[1,2], L. Launer[3], T. Harris[3], V. Gudnason[1,2].* 1) Icelandic Heart Association, Kopavogur, Iceland; 2) University of Iceland, Reykjavik, Iceland; 3) National Institute on Aging, National Institutes of Health, Bethesda, MD, USA.

A major issue in the understanding and interpretation of genome-wide association studies is missing heritability. One hypothesis is that additional variants not reaching stringent levels of significance contribute to additional heritability; notable recent work on schizophrenia has demonstrated more inclusive sets of SNP increase explained variation (International Schizophrenia Consortium Nature 2009). While this type of approach suggests many additional hits of moderate effect, it does not provide any mechanism for prioritizing particular regions or genes of biological interest to a disease. Since numerous published association scans have observed clear enrichment of specific gene categories, we hypothesized that SNPs at moderate p-value could be prioritized on a genome-wide basis using gene categories to more efficiently reveal SNPs of moderate effect than sets chosen solely via p-value threshold. We have tested this hypothesis using association scans on serum lipid levels in the AGES-Reykjavik study. Leveraging a novel in-house pipeline which identifies gene categories significantly enriched in a genome scan, we first identified Gene Ontology Biological Process categories which are significantly enriched in association scans of LDL cholesterol, HDL cholesterol and TG levels in two publicly available studies (Willer et al. Nature Genetics 2008; Kathiresan et al. Nature Genetics 2009). A number of lipid metabolic processes are clearly enriched in these scans (including, for example, "Triglyceride Metabolic Process" and "High-density Lipoprotein Particle Remodeling"). We subsequently examined whether SNPs from additional loci in the same categories not reaching genome-wide significance themselves are significantly associated beyond expectations in the independent AGES-Reykjavik cohort. We have observed that these prioritized, category-based SNP sets significantly increase explained variation in the AGES-Reykjavik cohort, and to a greater level than any observed from comparable sets of SNPs prioritized solely using p-value thresholds in the discovery sets. By comparing the seed gene sets from the two starting scans, this increased predictability extends beyond effects attributable to new hits from increased sample size. These results suggest that prioritizing loci by gene categories associated with top hits could speed the discovery of additional variants with disease at more moderate levels.

1710/W

One-way cryptographic hashes to detect overlapping individuals across samples. *M.C. Turchin[1,2], J.N. Hirschhorn[1,2,3].* 1) Program in Genomics and Divisions of Genetics and Endocrinology, Children's Hospital, Boston, Massachusetts, United States of America; 2) Program in Medical and Population Genetics, Broad Institute of Harvard and Massachusetts Institute of Technology, Cambridge, Massachusetts, United States of America; 3) Department of Genetics, Harvard Medical School, Boston, Massachusetts, United States of America.

To achieve sufficient power for discovery, genome-wide association studies often involve consortia in which investigators share summary association statistics but not individual level data due to IRB and/or consent-related restrictions. Because studies sometimes recruit in overlapping areas, there may be undetected duplication of individuals across studies, which could lead to inflation of meta-analysis association statistics. We have developed a tool to search for identical individuals across different samples by comparing one-way-encrypted hashes derived from individual-level data that is only shared by investigators in this strongly encrypted format.

Cryptographic hash functions are algorithms that produce a unique output for every unique input, and make it extremely difficult to reconstruct inputs from the outputs . By splitting individual-level data into many small groups of SNP genotypes, and creating hashes for each group of genotypes, one can quickly compare lists of encrypted values to identify duplicated individuals. Ideally, at genotype accuracies of 100%, duplicate individuals should produce identical sets of cryptographic hashes. In practice, because of missing data and genotype errors, the majority of hashes will match, and given enough sets of genotypes, the level of matching will rise well above that expected by chance.

We developed a software package, gEncrypt, which implements this cryptographic hash procedure. We applied gEncrypt to the GoKinD data set available from dbGAP. This data set contains 5 individuals who, based on near perfect genetic identity, were either genotyped twice or have identical twins in the study. We split the dataset into two halves, with one of each of the duplicate pairs residing in each half. The two resulting datasets were encrypted using gEncrypt, and the resulting lists of cryptographic hashes were compared against one another. The resulting data identified all 5 pairs of duplicate individuals with >80% identity of the hash outputs. gEncrypt could enable investigators from different studies to compare genomic datasets across studies for the presence of identical (and perhaps closely related) individuals, with minimal risks to individual-level privacy.

1711/W

Preterm Delivery: A Novel Bioinformatics and Genomics Approach. *A. Uzun[1,2,4], S. Sharma[1], G.D. Papandonatos[3], S. Istrail[3], J.F. Padbury[1,4].* 1) Pediatrics, Women and Infants Hospital, Providence, RI; 2) Center for Computational Molecular Biology, Brown University; 3) Center for Statistical Sciences, Brown University; 4) Brown Alpert Medical School.

Statement of purpose Preterm birth (PTB) in the United States is now 12.7 %. Premature infants have an increased risk of neonatal mortality as well as serious health problems. The governing paradigm is that term and preterm births involve parallel mechanisms. Labor occurs via similar final common pathway(s). Clinical care and investigations into the etiology of PTB have relied heavily on the assessment of these pathways. Nonetheless, the pathogenesis has remained elusive. New tools are needed to interrogate this enigmatic disease. **Methods** We have developed a web-based aggregation tool to create a database for the genes, genetic variations and pathways involved in preterm birth, the *Database for Preterm Birth* (dbPTB). dbPTB was implemented using a MySQL database running on a Linux server with PERL and PHP scripts used for all data retrieval and output. dbPTB uses *SciMiner* to extract the gene and protein information from published articles specific to PTB. All articles deposited to dbPTB were reviewed by a team of curators consisting of researchers and students trained in the molecular and cell biology of PTB. Genes identified from public databases, archives from expression arrays and genomic regions from linkage analyses are aggregated with genes curated from literature. Lastly, pathway analysis is used to impute genes from pathways identified in the curations. **Summary of results**Our aim is to have the complete set of putative genes involved in PTB from these in silico sources. Computational analysis will be used to identify minimally informative set of SNPs and/or haplotype blocks to compare the genetic architecture in a case controlled comparison preterm vs. term births. It is also important to recognize that the approach we have developed is generalizable to other diseases. To date, dbPTB identified 898 putative genes which are supported by 817 journal articles, drawn from a total of 18 million records in PubMed, 5053 unique MeSH terms, and 28,712 unique entries in HGNC. The pathways and articles which carry information about PTB and related links to bioinformatics sources in dbPTB include UCSC Genome bioinformatics, OMIM, NCBI Entrez Genes, and HGNC. Development of new tools and databases allow researchers to visualize problems in broad extensions. Building biological databases is an approach that will enable biomedical informatics to contribute new insights into disease pathogenesis and novel treatments.

1712/W

MARVEL: genome-scale variant annotation and prioritization for human genetic studies. *O. Valladares[1,2], M.R. Han[1,2], L.D. Hwang[3], E. Clevac[1,2], K. Cao[1,2], B.B. Gregory[4,5], G.B. Schellenberg[1,6], L.S. Wang[1,2,5,6].* 1) Department of Pathology and Laboratory Medicine, University of Pennslyvania, Philadelphia, PA; 2) Penn Center for Bioinformatics,University of Pennslyvania, Philadelphia, PA; 3) Bioengineering Graduate Program; 4) Master of Biotechnology Graduate Program; 5) Penn Genome Frontiers Institute; 6) Institute on Aging, University of Pennsylvania.

The latest wave of high-throughput genotyping and sequencing technologies has brought enormous success but also challenges of analyzing data with unprecedented size both in numbers of sample and loci. To address the finding that simple association tests such as GWAS only explain part of the observed heritability, new strategies using functional knowledge and rare variants and mutations with strong effects are necessary. These approaches require flexible and comprehensive bioinformatic solutions that incorporate functional genomic data in an automatic fashion. We report MARVEL (Multiple Annotation of Relevant Variants, Elements, and Loci), a LAMP application that annotates and prioritizes genetic variants using public functional genomic databases. MARVEL currently accepts SNPs from next generation DNA sequencing and genome-wide SNP arrays. MARVEL carries out two types of annotations: (1) location-wise annotation identifies affected genes, transcribed regions, and other functional elements such as eSNPs; (2) sequence-dependent annotation predicts the strength of impact on the function and structure of protein by amino-acid substitutions. MARVEL supports password-protected login and provides a web-based interface that allows users to filter and rank variants, link to external databases, and view on the UCSC genome browser directly. We tested MARVEL on a whole-exome targeted Solexa sequencing dataset on a Linux desktop with 2.4Ghz Intel Core2 CPU and 2GB RAM. MARVEL annotated 89,753 SNPs in 11 seconds and identified 6,029 synonymous and 10,161 non-synonymous exonic SNPs on 5,960 genes, and 430 published eSNPs. A beta version of MARVEL is currently available as a web service (wanglab.pcbi.upenn.edu/marvel) and as a standalone download in September 2010. We plan to add support for indels by sequencing and CNVs from SNP and aCGH arrays, and tissue/disease-specific annotation capabilities.

1713/W

KARMA: K-tuple Alignment with Rapid Matching Algorithm. *X. Zhan, P. Anderson, Y. Li, G. Abecasis.* Center for Statistical Genetics, Biostatics, University of Michigan, Ann Arbor, MI 48109.

Rapidly evolving sequencing platforms are transforming the analysis of DNA sequence data, with many studies generating whole genome sequence data for hundreds or thousands of individuals. Accurately handling these data poses many computational challenges. One of these concerns the accurate alignment of short DNA sequence reads. An accurate aligner should be able to adapt to reads of varying quality, gracefully handle repeated regions of the genome, use base quality information, effectively use computational resources, and allow for polymorphism in the reference. With these goals in mind, we are developing KARMA, a hash-table based aligner, that handles Illumina and ABI SOLiD short read sequence data and can handle ~1 Gigabase of sequence per CPU per hour. We will describe performance metrics for our aligner, including accuracy of alignments, and provide several illustrative use cases. We will also illustrate several of the features currently under active development.

1714/W

Pathway Analysis for Multiple Traits in Genome-Wide Association Studies. *J.G. Zhang[1], J. Li[1], H.W. Deng[1,2,3].* 1) Dept Basic Med Sci, Univ Missouri, Kansas, Kansas City, MO; 2) College of Life Sciences & Bioengineering, Beijing Jiaotong University, Beijing, P. R. China; 3) Systematic Biomedicine Research Center, University of Shanghai for Science and Technology, Shanghai, P.R. China.

Pleiotropy, a phenomenon that single genes contribute to multiple phenotypes, is commonly observed in studies of human complex diseases. The availability of genome-wide association study (GWAS) provides an opportunity for systematically analyzing pleiotropic genes and deciphering the genetic architecture of complex diseases. Although a set of correlated phenotype data related to complex diseases of interest are usually collected for GWAS, these phenotypes tend to be analyzed separately in a single-trait analysis framework. This strategy ignored potential genetic correlations among phenotypes and hence may miss pleiotropic genes that can play crucial roles in the pathogenesis of diseases. In this study, we develop a method to identify potential pleiotropic pathways and genes by incorporating multivariate analysis and gene set analysis. We also elaborate the reduction of irrelevant SNPs and the extraction of a core set of candidate pleiotropic SNPs, which contribute to the statistical variation of phenotypes of interest. The utility of our method is demonstrated through analyses on empirical GWA data. The results show that our method can detect pleiotropic pathways and genes responsible for affecting hip bone mineral density and body mass index. In summary, our method offers a powerful tool for researchers to examine the pleiotropic pathways and genes associated with complex human diseases, and can be a useful complement to classical GWAS analyses in a single-trait analysis framework.

1715/W

Managing multi-platform next generation sequencing data - a unified approach. *X. Zhang[1,2], Q. Chen[1,2], M. Yeager[1,2], K. Jacobs[1,2].* 1) Core Genotyping Facility, SAIC-Frederick Inc., NCI-Frederick, Gaithersburg, MD; 2) Division of Cancer Epidemiology and Genetics, NCI,NIH, Bethesda, MD 20892, USA.

The complexity and volume of the data produced by Next Generation platforms such as Roche's 454 FLX, ABI's SOLiD, Illumina's GA, HiScan, and HiSeq, and Pacific BioScience's SMRT sequencers pose many data management challenges. The NCI's Core Genotyping Facility has developed architecture and an automated data management pipeline to support multiple sequencing platforms to standardize the laboratory data management and make data processing automatic, transparent, efficient, and reproducible. The data architecture accommodates the need for capturing all of the important information required or generated during a workflow for a sequencing research project. The automated pipeline takes a project manifest and raw image data produced by sequencing platforms as input, and execute the tasks that will: perform image and signal analysis to produce base calls, sequencing reads, and pre-alignment QC metrics; align sequencing reads to a reference genome; generate standard SAM/BAM files with required genomic annotations; perform post-alignment QC, and filter out failed reads/alignments, samples and sequencing runs; generate QC and executive reports including summary statistics and granular coverage plots; call SNPs and structural variations; manage project storage space by implementing a data retention policy that serves as a guide for compression and automated data archiving. The data architecture and pipeline has been applied in several exome and targeted sequencing projects, and has significantly reduced turnaround time for sequencing data generation, QC, analysis, and delivery.

1716/W

GeneDistiller - the interactive search and prioritization of candidate disease genes. *M. Schuelke[1,2], J.M. Schwarz[1,2], D. Seelow[1,2].* 1) Neuropaediatrics, Charité - Universitätsmedizin Berlin, Berlin, Germany; 2) NeuroCure Clinical Research Center, Charité - Universitätsmedizin Berlin, Berlin, Germany.

Linkage studies often yield intervals containing several hundred positional candidate genes. Different manual or automatic approaches exist for the determination of the gene most likely to cause the disease. While the manual search is very flexible and takes advantage of the researchers' background knowledge and intuition, it may be very cumbersome to collect and study the relevant data from a plethora of public databases. Automatic solutions on the other hand usually focus on certain models and algorithms ranging from protein interactions to text mining approaches, but remain 'black boxes' and only offer a very limited degree of flexibility. The main disadvantage of these approaches is the neglect of the researchers' previous knowledge or their concept of the character of a certain disease.

We have therefore developed a freely available web-based application that combines the advantages of both approaches. Information from various data sources such as gene-phenotype associations, organ/tissue-specific gene expression patterns and protein-protein interactions was integrated into a central database. Researchers can define a list of genes of interest by specifying a candidate interval or by directly entering candidate genes. They can select which information shall be displayed and taken into account for the candidate gene search. Genes can thus interactively be filtered, sorted and prioritized according to criteria derived from the background knowledge and preconception of the researcher.

GeneDistiller provides knowledge-driven, fully interactive and intuitive access to multiple data sources. It displays maximum relevant information, while saving the user from drowning in the flood of data. A typical query takes less than two seconds, thus allowing an interactive and explorative approach to the hunt for the candidate gene. The results of the search can be printed or saved as HTML pages or as Excel-files. GeneDistiller can be freely accessed at http://www.genedistiller.org.

1717/W

Autosomal chromosomal abnormalities in genome wide association studies. A report from the Mayo electronic MEdical Records and GEnomics Network (eMERGE) Study. *H. Jouni[1], K. Shameer[1], Y.W. Asmann[2], M. de Andrade[2], C.G. Chute[2], I.J. Kullo[1].* 1) Division of Cardiovascular Diseases, Mayo Clinic, Rochester, MN; 2) Division of Biomedical Statistics and Informatics, Mayo Clinic, Rochester, MN.

Background: The phenotypic correlates of autosomal chromosomal abnormalities detected in genome wide association studies (GWAS) are unknown. **Methods:** As a part of the eMERGE consortium, 3,412 patients were recruited for a GWAS of peripheral arterial disease (PAD-1,687 cases and 1,725 controls). Genotyping was performed using the Illumina Human 660W-QuadV1 platform (561,490 SNPs/95,876 intensity-only probes). Three Illumina algorithms (cnvPartition1.2.1, LOH detector, and ChromoZone) were run using Log R ratio and B-allele frequency data to identify regions with copy number variation, loss of heterozygosity (LOH), and mitotic events. A chromosomal abnormality was defined as having an aberration exceeding 20% of a chromosome's total length. The electronic medical record (EMR) of these patients was reviewed to ascertain associated disease states. **Findings:** Intensity plots were abnormal in 25 patients: LOH (8), mitotic events (8), deletions (del=6), and amplifications (3). We focused on 5 patients who had interstitial deletions of 20q, of whom 4 had a myeloproliferative disorder (MPD) [polycythemia vera (2), essential thrombocythemia (1), myeloproliferative/myelodysplastic syndrome, unclassified (1)]. All 3 men in this group had PAD and prostate cancer (Ca) in addition to MPD. Bioinformatic analysis of del20q showed a common deleted region (38511024 bp® 48638502 bp) encompassing ~10 Mb. When compared to human reference genome (GRCh37/hg19), the common deleted region was found to include ~118 candidate genes that could be implicated in MPD/ PAD/ prostate Ca. We also implemented a novel data mining technique using hierarchical clustering of overlapping International Classification of Disease codes which successfully detected the 3 men with MPD, PAD, and prostate Ca.

Characteristics of the 5 Patients with 20q Deletions

Age	Gender	Atherosclerotic Disease	Hematologic Disorder	Other Significant Medical History
75	M	PAD	Polycythemia vera	Prostate cancer and squamous cell carcinoma
84	M	CAD/PAD	Myelodysplastic/Myeloproliferative disorder, unclassified	Prostate cancer and basal cell carcinoma
87	M	CAD/PAD	Essential thrombocythemia	Prostate cancer and squamous cell carcinoma
70	F	None	Polycythemia vera	Pulmonary arterial hypertension, multiple sclerosis, and pyoderma gangrenosum
72	F	PAD	persistently elevated ESR of unknown etiology	Premature atherosclerosis: PAD diagnosed at age 62

CAD: coronary artery disease - PAD: peripheral arterial disease.

Conclusion: In a GWAS of 3,412 adults, we detected autosomal chromosomal abnormalities in 25 patients. Mining of the EMR in these patients can yield novel insight into disease pathophysiology. We identified a subset of 5 patients with del20q, of whom MPD was present in 4, and all 3 men had prostate Ca and PAD. We plan further correlation studies of autosomal chromosomal abnormalities with disease states leveraging the eMERGE consortium of ~18,000 adults with high density genotyping data linked to the EMR.

1718/W

PedHunter 2.0 and the Anabaptist Genealogy Database (AGDB). *W.-J. Lee[1], T.I. Pollin[2], J.R. O'Connell[2,3], R. Agarwala[1], A.A. Schaffer[1].* 1) National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, DHHS, 8600 Rockville Pike, Bethesda, Maryland 20894, USA; 2) Department of Medicine, Division of Endocrinology, Diabetes and Nutrition, University of Maryland School of Medicine, 660 W. Redwood Street, Baltimore, Maryland 21201, USA; 3) Animal Improvement Programs Laboratory, Agricultural Research Service, United States Department of Agriculture, 10300 Baltimore Avenue, Beltsville, Maryland 20705, USA.

Version 1.0 of the software PedHunter was released in 1998 to solve optimal pedigree connection problems and other problems related to pedigree construction, verification, and analysis. PedHunter 2.0 released in 2010 supports 50 queries in four categories: 1) testing a relationship; 2) finding all individuals satisfying a certain relationship; 3) printing information; and 4) complex queries. Additionally, PedHunter 2.0 provides seven utility programs including pedigree trimming and pedigree renumbering. PedHunter was initially designed to search the Anabaptist Genealogy Database (AGDB), which is described below. PedHunter has also been used to construct genealogies for linkage and haplotype analyses on Hutterite families, an Icelandic population, a Southern Italy population, and a Northern Italy population. PedHunter is freely available and can be downloaded from http://www.ncbi.nlm.nih.gov/CBBresearch/Schaffer/pedhunter.html. There have been several versions of AGDB based on three genealogy sources and multiple updates. AGDB version 4.0 (AGDB4), containing 417,789 individuals and 102,341 marriages, was created in 2004. The numbers of individuals from the three sources in AGDB4 are 30,821, 66,014 and 376,152, among which only 1,122 individuals are commonly found in all three sources. Usage of the AGDB requires an Institutional Review Board (IRB)-approved protocol to study an Amish or Mennonite population. Several groups have used AGDB in their research on rare and common diseases. Although AGDB contains no explicit phenotype data, lifespan can be inferred when both birth and death dates are available. Several studies on lifespan using AGDB have established that lifespan is heritable, that lifespan may be associated with other implicit traits, and that lifespan can be associated with experimentally measured traits. Using newly available updates to two of the AGDB genealogy sources, we are preparing AGDB version 5 (AGDB5) and expect it to contain at least 100,000 more individuals than AGDB4. Using PedHunter 2.0 to query AGDB4, we quantified the extent to which the Old Order Amish (OOA) are a closed population ideal for elucidating the role of genetic variation. Specifically, we quantified the number of founders and their genetic contribution to ~34,000 presumed living Lancaster County, Pennsylvania OOA individuals born in 1930-2000. The completion of AGDB5 will enable us to update OOA studies to include individuals born in 2000-2009.

1719/W

The Genome-Wide Annotation Repository (GWAR). *B. Winfrey, J. Giles, W. Bush.* Ctr Human Genetics, Vanderbilt Univ Medical Ctr, Nashville, TN.

The explosion of high-throughput data collection approaches for biological sciences and the analysis of their results has dramatically expanded the amount of information available for annotation of existing result sets and the generation of new hypotheses. Often, the results of an individual analysis are published in a stand-alone database or are simply released as flat-files, which limits their utility in other analyses. To address these issues, we constructed the Genome-Wide Annotation Repository (GWAR) to centrally house and inter-relate analysis results and genomic annotations from multiple sources. These include SNP-based annotations, such as expression QTLs (eQTLs) and sites of selection, and gene-based annotations, such as KEGG or Reactome pathway membership or protein structure families. Currently, information within the repository can be used in a variety of ways to conduct analysis of genome-wide association data, including enrichment analysis using PARIS, and epistasis analysis using Biofilter and ATHENA. As new data sources are added to the repository, they become available for downstream analysis tools allowing novel re-analysis of existing GWAS data. Possible analyses include enrichment by protein family or transcription factor binding site rather than traditional pathways, or epistasis analysis among eQTLs for genes in a relevant biological process from the Gene Ontology (GO).

1720/W

TE-scape of the Human Regulome. *W. Makalowski[1], A. Pande[1], I. Makalowska[2].* 1) Institute of Bioinformatics, University of Muenster, Muenster, Germany; 2) Laboratory of Evolutionary Genomics, Adam Mickiewicz University, Poznan, Poland.

At least half of of the human genome originated in transposable elements (TEs). It is not surprising then that TE-originated genome fragments affect many cellular processes. Using publicly available data and computational tools we investigated their role in the human genome both as dynamic collaborators to robust controllers. This prompted us to look for the TEs as performers contributing to bi-directional promoters regions, cis regulatory modules (CRMs) and insulators. Bidirectional gene organization is defined as two genes arranged head to head on opposite strands with less than 1,000 bp between their transcription start sites. It was suggested that bidirectional organization may provide stronger resistance to invasion by transposable elements, which is possibly one of the reason why many important genes, e.g. 30% of housekeeping genes, are arranged in this way. Interestingly, 67% of the analyzed human biderctional genes contain TEs in their promoter regions. Cis regulatory modules are at the heart of differential gene regulation for generation of patterns and the diversity of cell types in a multicellular organisms. These are genomic regions that are bound by transcription factors (TFs) that control spatio-temporal gene expression in developmental networks. Our analysis of the human CRMs derived from the TiGER database revealed that TEs occupy 48% of these “rewireable” networks, regulating different facets of developmental dynamics. Moreover, 36% of the tissue specific enhancer regions in the human genome originated in TEs. Probing into the diversity of differential gene expression leading to diversity of cell types took us to the insulator regions within the human genome. These zinc-finger protein binding sites are involved in several transcriptional mechanisms, such as gene repression and enhancer blocking. Our analysis shows that a significant fraction of the CTCF binding sites derived out of TEs. This leads credence to the contribution of TEs to expression and robust control of the nucleosome and genome dynamics through regulation and control by virtue of “action-at-a-distance”. Undeniably, TEs act as catalysts in functional resilience for the transcriptional networks in the human genome. A resonating presence within promoters, insulators, enhancers, and the cis regulatory networks reflects their modulatory propensity through evolution.

1721/W

Genomic Reconstruction of the Evolutionary History of DUF1220 Domains. *M. O'Bleness[1], L. Dumas[1], H. Kehrer-Sawatzki[2], J. Sikela[1].* 1) Human Medical Genetics and Neuroscience Programs, Department of Pharmacology, University of Colorado School of Medicine, Aurora, CO 80045 USA; 2) Institute of Human Genetics, University of Ulm Albert-Einstein-Allee 11 89081 Ulm, Germany.

One of the most evolutionarily striking copy number variations (CNVs) that has been identified to date is the dramatic human lineage-specific amplification of a 65 amino acid protein domain of unknown function, DUF1220. Sequences encoding DUF1220 domains are increasingly amplified generally as a function of a species evolutionary proximity to humans, where the greatest number of copies is found (218). DUF1220 domains are virtually all primate specific and are typically found within the NBPF (neuroblastoma break point factor) gene family. Recently 1q21.1 CNVs that either encompass or flank DUF1220 domains have been implicated in a number of human diseases, including microcephaly, macrocephaly, autism, schizophrenia, mental retardation and heart disease, suggesting a link between DUF1220 amplification and the etiology of these disorders. The recent availability of numerous mammalian genome sequences presents an opportunity to investigate the evolutionary history of DUF1220 domain amplifications in unprecedented detail. To accomplish this, available mammalian genome sequences were interrogated and all DUF1220 domains and the genes in which they are found were identified. Interestingly previously unidentified NBPF genes were found in several non-primate mammalian genomes (only recently made available and heretofore not known to contain NBPF genes), and a newly identified non-primate mammalian conserved promoter region, upstream of several NBPF genes, was also identified. These new findings represent the most complete picture of DUF1220 evolution so far reported and lend further support to the view that there have been strong selection pressures that favored the rapid copy number expansion (~16 copies added/Myr in human lineage since the homo/pan split) of these domains over recent evolutionary time.

1722/W

SequenceVariantAnalyzer: Software for the annotation, visualization, and analysis of variants emerging from whole-genome and whole-exome sequencing. *D. Ge[1,2], E.K. Ruzzo[1], M. He[1], D.B. Goldstein[1].* 1) Center for Human Genome Variation, Duke Univ, Durham, NC; 2) Department of Biostatistics and Bioinformatics, Duke Univ, Durham, NC.

SequenceVariantAnalyzer (SVA) is a user-friendly software tool designed for the interrogation and interpretation of human genetic variants identified by next-generation sequencing studies. This software environment allows the simultaneous analysis of multiple genomes, each of which can be stratified by basic phenotypic information (case or control status). SVA takes as input a set of genetic variants (differences from a reference genome) from one or more genomes and allows users to visualize these variants in their genomic contexts using a built-in genome browser. The “annotation module” is the preliminary routine within SVA which functions to give each genetic variant a genomic context based on the SVA interface with publicly available databases, such as dbSNP and Ensembl. The annotation module also classifies each identified variant based on its predicted biological function into major “functional categories”, such as “premature stop” or “nonsynonymous coding”. This annotation also enables the user to filter the entire list of identified variants based on many important properties, including: genotype, presence in case or control genomes, functional category, or genomic location. In addition to this detailed characterization of each identified variant, SVA can also carry out a variety of association tests based on phenotypic designations for all variants or for select groups of variants. These tests comprise the “analysis module” and are used to prioritize the genetic variants by their observed association with, or contribution to, the human disease or biological trait of interest. Both the annotation and analysis modules within SVA allow for user-customization based on hypotheses about the biological trait of interest. The computational performance of SVA (maximum memory usage and time requirements) has been thoroughly evaluated using both whole-genome and whole-exome sequencing data. The performance of SVA exhibits its feasibility for use in large next-generation sequencing datasets, especially for large whole-exome studies where the computational demands are nominal. The biological utility of SVA has been demonstrated through its application to two datasets where the genetic causes of two human diseases were readily identified from whole-genome sequencing data. SVA is available at: http://www.svaproject.org/.

1723/W

Differential analysis to identify genome-wide DNA methylation markers using enrichment-based sequencing data. *Y. Xu[1,2], A. Ting[1], H. Hu[1,2].* 1) Quantitative Hlth Sci, Cleveland Clinic, Cleveland, OH; 2) Statistics, Case Western Reserve University, Cleveland, OH.

DNA methylation is an epigenetic modification of human and mammalian genomes, involved in both normal developmental processes and disease states through the modulation of gene expression and the maintenance of genomic structure. Study of whole genome DNA methylation profiles may hold substantial promise for identifying mechanisms through which genetic and environmental factors jointly contribute to disease risk. Next-generation sequencing technologies have emerged as powerful tools for whole-genome profiling of epigenetic modifications, and there is great interest in strategies for analyzing genome-wide/whole-genome DNA methylation data using high-throughput next-generation sequencer. We propose an integrated statistical and computational approach that conducts the differential analyses of methylation profiles in a multiple comparative study design. Our method is particularly suitable to the clinical data from large patient populations that need to correlate with patient groups and clinical parameters. We applied our approach to a colon cancer study with whole-genome methylation profile data generated using MBD-isolated Genome Sequencing (MiGS) with the Illumina Genome Analyzer II. The results are validated using the conventional bisulfite sequencing.

1724/W

A high-throughput analytical pipeline for genome-wide association scans. *X. Deng[1,2], Z. Wang[1,2], Q. Chen[1,2], M. Yeager[1,2], G.S. Tobias[2], M.A. Tucker[2], S.J. Chanock[2], K.B. Jacobs[1,2].* 1) Core Genotyping Facility, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD 21702, USA; 2) Division of Cancer Epidemiology and Genetics, NCI, NIH, Bethesda, MD 20892, USA.

The NCI's Core Genotyping Facility (CGF) has built a robust and high-throughput analytical pipeline that has been used to perform over 12 Genome-wide Association Studies (GWAS) on multiple cancer sites including: prostate, breast, pancreas, lung, kidney, bladder, testes, gastric, and brain. These scans included subjects collected from many large cohorts and case/control studies. Our data infrastructure facilitates creation of datasets for quality control, population characterization, and association analysis, drawing genotypes from multiple projects that often combine genotypes across genotyping platforms, assays panel, laboratory processing methods, and genotyping centers. Genome-wide SNP assays from Illumina (317K, 240S, 370K, 550K, 610K, 660K and 1M, Omni1, and OmniExpress) and Affymetrix (500k and 6.0) are harmonized across various assay products and versions, as the different chip content is non-uniform and often uses conflicting identifiers and annotation, e.g. different dbSNP and human genome builds. The genotype calling process is designed to reduce differential misclassification that often occurs when combining data from samples collected, prepared, or genotyped heterogeneously. Phenotype information from various sources are harmonized and stored according to a common schema, which facilitates downstream analyses. The data infrastructure is highly automated and all analysis and data deliveries are versioned and tracked via a formal build process. Once a GWAS genotype/phenotype dataset has been built, the pipeline performs standard analyses, which include comprehensive genotype QC, duplicate detection/verification, population admixture analysis (model based and PCA), reference panel and missing SNP imputation, genotype-phenotype association analysis, CNV data export and segmentation. The pipeline proves highly efficient and robust particularly for conducting GWAS on data merged across multiple Illumina chip types. Funded by NCI Contract No. HHSN261200800001E.

1725/W

Identifying Sets of Susceptibility SNPs Using Generalized Ridge Logistic Regression. *Z. Liu[1], Y. Shen[1], J. Ott[1,2].* 1) Beijing Institute of Genomics, Chinese Academy of Sciences, No.7 Bei Tu Cheng West Road, Beijing 100029, China; 2) Rockefeller University, 1230 York Avenue, New York, NY 10065.

In genome-wide case-control association studies, association scan statistic is a powerful approach to detect susceptibility genomic regions and map disease-causing genes located on them. However, this method suffers from several drawbacks: (1) It is not always easy to determine the size of each sliding window, where different sizes may lead to different results; (2) linkage disequilibrium (LD) within local genomic regions is seldom taken into account; (3) consecutive genetic makers are simultaneously selected, which can lead to much "noise" and thus reduce power. In order to overcome these disadvantages of association scan statistics, we propose a novel method for identifying sets of susceptibility single-nucleotide polymorphisms (SNPs) based on a forward model selection procedure, using generalized ridge logistic regression (GRLR) for stepwise fitness. GRLR imposes a proper penalty on each SNP in the genomic region of interest based on its distance from the region center. Our method automatically combines information from local chromosomal regions and selects sets of genetic markers that are not necessarily adjacent to each other and whose associated significance levels are evaluated in randomization samples. In simulation studies, we compare the performance of our method with that of five other methods in current use, including standard logistic regression, Fisher product method, truncated product method, Lasso logistic regression and the elastic net method. Results show that in most scenarios our method outperforms the other methods. We also present a published whole-genome dataset on age-related macular degeneration with approximately 100 cases and 100 controls and 10,000 SNPs, where our method successfully detects a set of two SNPs whose associated empirical p-value is less than 0.05.

1726/W

Systematic Removal of Outliers Reduces Heterogeneity in Case-Control Association Studies. *Y. Shen[1], Z. Liu[1], J. Ott[1,2].* 1) Beijing Institute of Genomics, Chinese Academy of Sciences, No.7 Bei Tu Cheng West Road, Beijing, China 100029; 2) Rockefeller University, 1230 York Avenue, New York, NY 10065.

In human case-control association studies, population heterogeneity is often present and can lead to increased false positive results. Various methods have been proposed to remove its deleterious effects, including genomic control (GC), principal component analysis (PCA), and multidimensional scaling (MDS). We assume that heterogeneity is due to a relatively small number of individuals (outliers) whose allele or genotype frequencies differ from those of the remainder of the sample. For this situation, we propose a novel method of handling heterogeneity as follows: In a coordinate system of the largest four principal components in MDS, we systematically remove one after another of the most extreme outlying individuals and each time compute the largest association test statistic and its associated p-value. Generally this leads to a gradual decrease of the resulting p-values (increase of significance) even though individuals are removed from the dataset. We stop when the p-values begin to increase again and take the smallest p-value as our overall test statistic, whose associated significance level is assessed in randomization samples. In power simulations of our methods and three other approaches in current use, averaged over various scenarios, our method is often more powerful than genomic control and standard logistic regression although the overall best method turned out to be the logistic regression analysis with MDS components as covariates. We also apply our method and the other approaches discussed above to two published datasets: One is a genome-wide dataset with 541 case and control individuals and approximately 408,000 SNPs, where our method has better performance than other methods; the other is a genome-wide dataset with 96 cases and 50 controls and approximately 104,000 SNPs, where our method validates the discoveries in previous investigations. While our method is competitive with other method for handling heterogeneity (although not generally superior to them), the main advantage of our approach is that it represents an adaptive and statistically rigorous procedure to remove outliers.

1727/W

The NCBI dbGaP database of genotypes and phenotypes provides resources for genome-wide association studies and medical resequencing. *S. Sherry, M. Feolo, Y. Jin, M. Kimura, K. Tryka, R. Bagoutdinov, J. Paschall, L. Hao, A. Sturcke, L. Phan, M. Quintos, N. Popova, S. Pretel, L. Ziyabari, M. Lee, Y. Shao, Z. Wang, M. Xu, M. Kholodov, G. Godynskiy, N. Sharopova, A. Pshenichnov, J. Ostell.* National Center for Biotechnology Information, National Institutes of Health, Bethesda, MD.

The dbGaP database of Genotypes and Phenotypes (www.ncbi.nlm.nih.gov/dbgap) archives and redistributes comprehensive data sets from genome-scale studies, thus providing summary reports, documents, data browsers and connections to other information resources. For authorized investigators, dbGaP distributes de-identified, individual-level phenotype and genetic data, and aggregate-level association results. 78 dbGaP studies provide individual-level data for 188,466 study participants including 69,376 phenotype measures, 132 billion genotypes, and 10 terabases of sequence data.

Any Principal Investigator (PI) registered in the NIH Federated Commons (eRA) system may request access to individual level data through the dbGaP controlled access system. Completed applications are automatically routed to the PI's institutional signing official (SO) for approval, and then forwarded for review and an approval decision by an NIH Data Access Committee. Submission of the data access request constitutes agreement and acknowledgment to the terms of use for the requested data sets. These terms are detailed in the "Data Use Certification" documents and generally include agreements to adhere to indicated limitations of use; not redistribute the data; not attempt to identify or contact study participants; acknowledge intellectual property principles; and adhere to publication embargo policies.

Public data in dbGaP includes study descriptions, documents, variable summaries, descriptions of controlled-access components and limited aggregate data. NCBI provides a growing collection of tools to **browse** catalogs of published association results, and **examine** multiple GWAS association results simultaneously in genome coordinates. dbGaP content is connected when possible to other public information resources, including NHGRI's Genome Wide Association Catalog, PubMed, Gene, SRA and dbSNP.

The dbGaP FTP site provides all public content. There you will find study release notes, documents in XML markup and PDF formats, a report describing all of the components available through controlled access, and phenotype variable summary data.

1728/W

Genotype Library and Utilities (GLU): a suite of tools for performing genome-wide association scans. *Z. Wang[1,2], C. Liu[1,2], Q. Chen[1,2], M. Yeager[1,2], S.J. Chanock[2], K.B. Jacobs[1,2].* 1) Core Genotyping Facility, SAIC-F/NCI, Bethesda, MD; 2) Division of Cancer Epidemiology and Genetics, NCI, NIH, Bethesda, MD.

GLU is a software package for managing and analyzing large scale genotype data that was initially developed to support the NCI Cancer Genetic Marker of Susceptibility (CGEMS) projects. It was designed around key novel conceptual approaches, including efficient binary genotype representations and storage, a data streaming model, and an extensible and interoperable modular framework. GLU is a data management tool: handling many different genotype format transformation including high-performance native text and binary formats, PLINK text (PED) and binary formats, and WTCCC formats; filtering genotype data based on inclusion or exclusion criteria; sample renaming and locus remapping; file utilities for table joining and in-memory queries on relational sample meta data files. GLU is also a genotype quality control tool: summary genotype statistics for locus and sample; genotype concordance and duplicate check; deviations from Hardy-Weinberg proportions; IBD estimation and relatedness check. In addition, GLU provides analytic tools such as: linear and logistic regression models for high-throughput association testing; LD estimation, filtering, coverage and tagging; an embedded SNP and gene annotation database. GLU also includes features to characterize population structure such as population admixture estimation and principal component analysis on genetic correlations. Overall, GLU has the essential features to perform GWAS analysis, from parsing the raw genotype data, conducting quality control filtering, population structure analysis, to association analyses. It is the primary tool used by the GWAS pipeline at the NCI's Core Genotyping Facility and has supported all major GWAS discoveries and publications since 2006. GLU is a free and open source project that is steadily improving and accessible to the scientific community from http://code.google.com/p/glu-genetics. Funded by NCI Contract No. HHSN261200800001E.

1729/W

Integrating genetic and gene expression evidence into genome-wide association analysis of gene sets. *Q. Xiong[1], N. Ancona[2], ER. Hauser[3], S. Mukherjee[1,4,5,6], TS. Furey[1,5,7].* 1) Institute for Genome Sciences and Policy, Duke University, Durham, NC 27708; 2) Institute of Intelligent Systems for Automation National Research Council, Bari IT 70126; 3) Department of Medicine, Center for Human Genetics, Duke University Medical Center, Durham, NC 27710; 4) Departments of Statistical Science, Duke University, Durham, NC 27708; 5) Department of Computer Science, Duke University, Durham, NC 27708; 6) Department of Mathematics, Duke University, Durham, NC 27708; 7) Department of Biostatistics and Bioinformatics, Duke University, Durham, NC 27708.

Single variant or single gene analyses generally account for only a small proportion of phenotypic variation in complex traits and often result in high rates of false positive associations. As a result, a variety of statistical or computational methods have been developed to identify associations between gene expression or genetic variation and phenotypic variation in the context of gene sets/pathways. However, a computational platform for joint analysis of multiple types of genomic data in a single statistical framework is currently not available. We propose a novel methodology, Gene Set Association Analysis (GSAA), which integrates gene expression analysis with genome-wide association studies. GSAA employs a priori defined gene sets to infer sets of genes enriched for differential expression across two conditions and/or trait-associated genetic markers. The joint analysis in GSAA has important advantages compared with pathway analyses based on a single genomic data source or based on eQTLs. Compared with gene expression analysis alone or purely genotypic analysis, the data integration can reduce the effects of inherent noise in gene expression data and population stratification in SNP data that often lead to high rates of false positives. In addition, unlike eQTL-based analysis that only analyzes those SNPs responsible for gene expression variation, GSAA analyzes information from all genes and from all SNPs in the data sets simultaneously and integrates these two types of information as overall evidence of association. This procedure can make gene set association analysis more robust and comprehensive. Simulation studies illustrate that joint analyses of genomic data increase the power to detect real associations when compared to gene set methods that use only one genomic data source or a two-step regression method. We applied GSAA to glioblastoma data identifying p53 signaling as the most significant pathway along with others related to cell-cycle checkpoint response and transition between phases. Analysis of Crohn's disease data found that top-ranked pathways are most associated with NF-κB signaling and consequent molecular events caused by the deregulation of this pathway. These results are consistent with the theoretical framework elucidating the etiology established by experimental studies demonstrating that GSAA accurately identifies molecular pathways deregulated in disease. Software is freely available at http://gsaa.genome.duke.edu.

1730/W

Ontology derived systems biology approach to infer polygenic models for GWA Studies. *W. Zheng[1], L. Tsoi[1], T. Qin[1], E. Kistner-Griffin[2,3], A. Lawson[2].* 1) Biochemistry and Molecular Biology, Med Univ South Carolina, Charleston, SC; 2) Division of Biostatistics & Epidemiology, Department of Medicine, Medical University of South Carolina, Charleston, SC; 3) Hollings Cancer Center, Medical University of South Carolina, Charleston, SC.

Many evidences show that human diseases are caused by cumulative effect of multiple genetic variants. This polygenic model poses a significant challenge to current Genome-wide Association (GWA) study, as variants in a polygenetic model do not show strong association with the disease phenotype individually. A systems biology approach can be used to identify polygenic model from genes that are marginally associated with disease phenotypes from GWA study. We have developed the concept of ontology fingerprints (OntoFing), a list of ontology terms overrepresented in the PubMed abstracts linked to a gene or a disease along with their corresponding enrichment p-value, to characterize genes and diseases. By using OntoFing, we are also able to quantify relationships between genes to construct OntoFing-derived network. In this network, genes are nodes and the similarity scores between genes are weighted edges. We further developed a Bayesian hierarchical model to use existing pathway information to infer biologically relevant threshold for the weighted edges in the network. By examining the biological coherency between genes in the network, we are able to identify gene modules that show strong biological relevance to LDL, HDL and TG using data from a GWA study on dyslipidemia. We ranked the identified modules based on the similarity with the phenotype, and showed that the high ranking modules have higher proportion of genes previously identified to have polygenic effects on dyslipidemia. One module contains LPL and ABCA1, consistent with their polygenic effect on lipid concentration supported by other study. Additional genes in the same module point to a potential multi-gene model that influences lipid concentration. Such network modules have significant implication for the genetic bases of the polygenic disease model. Our methodology provides a novel way to analyze GWA study to infer novel polygenic model that may contribute to human disease. We are also applying the OntoFing approach to result from a GWA study investigating genetic variants associated with nephropathy among Type I diabetes patients. While no statistically significant genetic variants were identified to be associated with the phenotype after multiple testing correction, the prior information provided by OntoFing could potentially provide additional knowledge to increase the power for identifying genes involved in polygenic effect of nephropathy.

1731/W

Identifying unique sequences directly from the human genome. *J.C. Patrick[1], B.C. Shirley[1], P.K. Rogan[1,2].* 1) Computer Science, University of Western Ontario, London, Ontario, Canada; 2) Biochemistry.

We implement an *ab initio* method for finding locations of unique sequence intervals in the genome that obviates the requirement for comparison with a database of consensus repetitive sequence family members. The method exploits a state space search stategy similar to a depth-first search algorithm. It determines copy number of seed subsequences of a larger input sequence (eg. a complete chromosome) which are then compared at low stringency with a sequenced genome in parallel on a cluster computer. To define the boundaries of single copy segments, the above steps are recursively run on branched subsequences of repeat-containing intervals that occur adjacent to single copy segments discovered in the previous step. In addition to finding known repeat sequence families, ab initio is capable of detecting repeat elements that have not been previously recognized. To measure sensitivity, specificity, and accuracy of our *ab initio* implementation, 10 two million nucleotide long regions were selected for analysis, representative of a range of repeat content densities. Between 6 and 15 recursions were required to define single copy sequences in these target regions, corresponding to seed lengths ranging from 300 to 1500 nt. The algorithm achieves high specificity and sensitivity for detection of single copy sequences and resolves the boundaries between single copy and cataloged repetitive elements to within 20 nt resolution.

1732/W

Nonlinear Geographical Mapping for Genetic Structures. *W. Yang[1], E. Eskin[2].* 1) Bioinformatics Program, University of California, Los Angeles; 2) Computer Science Dept, Human Genetics Dept, University of California, Los Angeles.

Understanding the genetic structure of human populations plays a key role in medical, genetic, and anthropological researches. Previously, Principle Component Analysis (PCA) has been widely used to analyze the genetic data to reveal the population structure. However, using PCA for geographical mapping in two dimensions might not be able to accurately reveal the intrinsic genetic structure of population. There are two main reasons: (1) PCA is a linear method designed to model linear variability, but population variance is very likely to be nonlinear manifold in high dimensional space; (2) PCA is not a customized method for geographical mapping, as it computes a ranking list of principle directions but no guarantee that using only two of them could attain the original structure. Therefore, it is more suitable to use nonlinear dimension reduction method for genetic data analysis, especially, for geographical mapping. These methods generally aim at preserving local neighbor information while projecting to lower dimensional space, which coincides with the basic biological intuition that genetic similarity leads to locational similarity. The nonlinear dimension reduction techniques generally work as follows: 1. Identify k nearest neighbor samples for each genetic sample, 2. Compute the reconstructing weights using these neighbors for each sample, 3. Project the samples to a geographical location while preserving the reconstructing weights. The above procedure usually can be solved efficiently by spectral decomposition, thus is scalable for large data set. We apply this method to a single nucleotide polymorphism (SNP) data from European populations and observe better geographical mapping than the PCA method.

1733/W

CAGI: The Critical Assessment of Genome Interpretation, a community experiment to evaluate phenotype prediction. *S.E. Brenner[1], R.K. Hart[2], J. Moult[3].* 1) 461A Koshland Hall #3102, University of California, Berkeley, CA 94720-3102; 2) 324G Stanley Hall, University of California, Berkeley, CA 94720; 3) IBBR, University of Maryland, Rockville, MD 20850.

The Critical Assessment of Genome Interpretation (CAGI, kā'-jē) is a community experiment to evaluate computational methods for predicting the phenotypic impacts of genomic variation. Participants will be given unpublished genetic variants and will make predictions of resulting phenotype. These predictions will be objectively assessed against experimental characterizations. The long-term goal for CAGI is to improve the accuracy of phenotype and disease predictions of rare variants in clinical settings.

CAGI, which is based on the framework of the long-running Critical Assessment of Structure Prediction (CASP), will entail four phases:

- Unpublished associations of genotypes with molecular, cellular, or organismal phenotypes will be collected by the organizers from experimental and clinical labs
- Participants will make computational predictions of phenotypes from provided genotypes
- Experimental and clinical scientists will assess predictions
- A community workshop will be held to disseminate results and evaluate our collective ability to make accurate and meaningful predictions.

From this experiment, we expect to understand the diversity of mechanisms of genome variation, identify bottlenecks in genome interpretation, inform critical areas of future research, and connect researchers from diverse disciplines whose expertise is essential to methods for genome interpretation. Preliminary data sets we have be offered include enzymatic activity of human metabolic enzymes, segregation of rare variants identified in from resequencing in cancer cases and controls, efficiency of transcription for variants of a checkpoint protein variants, pathogenicity of mitochondrial variants, clinical phenotypes associated with complete genomes, and molecular mechanisms underlying GWAS disease associations. For more information, see http://genomecommons.org/cagi/.

1734/W

GSD: a web-based data management system for large-scale genetic studies. *F. Kuo[1,2], C. Huang[1], S. Diehl[1,2].* 1) Center for Pharmacogenomics & Complex Disease Research, UMDNJ-New Jersey Dental School, Newark, NJ; 2) Biomedical Informatics, UMDNJ-School of Health Related Professions, Newark, NJ.

Mapping genes for complex diseases requires large numbers of samples and genetic markers. With current genotyping technologies, family linkage or genome-wide association studies can accumulate billions of genotypes. In addition, as the study data dynamically grow from beginning to end, flexibility of managing and manipulating the information (with an audit trail) is essential. Here we describe a secure web-based Genetic Studies Data (GSD) management system for high-throughput population and family based genetic studies. GSD is a platform independent web-based DBMS system supporting of HTTP protocols over an encrypted Secure Sockets Layer (SSL)/Transport Layer Security (TLS) transport mechanism. It can accommodate genotypes generated by various genotyping platforms from an in-house laboratory or from collaborators' laboratories. In addition to marker genotypes, it can also manage multiple clinical and demographic/risk factor variables. Pedigree drawing is another useful functionality, and the software allows users to visualize pedigree structures along with affection status, genotypes and phenotypes displayed. Together with comprehensive user account management and study data access control, GSD is designed to comply with the requirements of IRBs in large-scale and/or multi-site collaborations for genetic or pharmacogenomic studies. The underlying database of GSD is an Oracle relational database, offering exceptional flexibility, facilitating complex data table and query designs, and expediting data quality control. The front end user interface is built on an Apache web server with SSL/TLS encryption by Perl CGI programming. Therefore, any modern web browser with encryption capability can act as a client. GSD aims to ease the burden of managing multiple on-going studies and integrating and managing data from fieldwork and the laboratory for these studies. Data import can be readily done by text copy/paste or data file uploads through the interface. For genetic association studies, GSD provides a number of statistical tests including Chi-Square, Fisher-Exact, and Trend Tests as well as linear and logistic regression analyses with categorical or continuous covariates. GSD's data export functionality includes formats used by most publicly available statistical analysis programs including LINKAGE, GENEHUNTER, MERLIN and PLINK. This research was supported by grants NIDCR DE13102 and DE016057, DE017900, NIEHS ES015864 and the Foundation of UMDNJ.

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ExprTarget: an integrative approach to predicting human microRNA targets. *W. Zhang[1,2], E.R. Gamazon[3], H.K. Im[4], S. Duan[5], Y.A. Lussier[3], N.J. Cox[3,6], M.E. Dolan[3]*. 1) Institute for Human Genetics, University of Illinois at Chicago, Chicago, IL; 2) Dept. of Pediatrics, University of Illinois at Chicago, Chicago, IL; 3) Dept. of Medicine, University of Chicago, Chicago, IL; 4) Dept. of Health Studies, University of Chicago, Chicago, IL; 5) Singapore Institute for Clinical Sciences, A-STAR, Singapore; 6) Dept. of Human Genetics, University of Chicago, Chicago, IL.

Variation in gene expression has been observed in natural populations and associated with complex traits or phenotypes such as risks for common diseases and differences in chemotherapy outcomes. Gene expression itself is a complex phenotype controlled by various genetic and non-genetic factors. The binding of a class of small RNA molecules, microRNAs (miRNAs), to the mRNAs has recently been demonstrated as an important mechanism of gene regulation. The regulation of miRNAs in gene expression has also been found to be associated with complex diseases including certain cancers. Because individual miRNAs may regulate the expression of multiple gene targets by binding to their mRNA transcripts, a comprehensive and reliable catalogue of miRNA-regulated targets will be critical to understanding the complex network of gene regulation and human diseases. Though experimental approaches have been used to identify many miRNA targets, considering cost and efficiency, miRNA target identification still relies largely on computational algorithms that aim to take advantage of different biochemical/thermodynamic properties of the sequences of miRNAs and their gene targets. Though successful to some extent, the prediction results of these computational methods often lack support by each other and experimental evidence. A novel approach (based on weighted logistic regression), therefore, was proposed to integrate some of the most common miRNA target prediction methods (miRanda, PicTar, TargetScan) and using the miRNA expression (together with the mRNA baseline expression) data (~220 expressed miRNAs) generated by our laboratory on 58 unrelated HapMap CEU (Caucasians from Utah, USA) samples and the TarBase (an archive of the miRNA targets validated by experiments) to represent positive predictions. Though with some limitations, our approach improved the miRNA target prediction relative to individual algorithms in terms of sensitivity and specificity. A database of human miRNA targets (ExprTarget, http://www.scandb.org/newinterface/download/microrna/) predicted by this approach was built to house this data and to facilitate future studies pertaining to gene regulation by miRNAs. ExprTarget will be integrated into www.PharmGKB.org. This work was funded through the Pharmacogenetics of Anticancer Agents Research Group grant U01GM61393, CA136765, P50CA125183, and the UC Cancer Center Pilot Project Program. Data deposits are supported by the PharmGKB grant U01GM61374.

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Phenome-wide association study on 40 loci replicates 22 known associations and proposes new disease-SNP associations. *J.C. Denny[1], L.A. Bastarache[1], M.A. Basford[1], M.D. Ritchie[1], J.M. Pulley[1], E. Bowton[1], C. McCarty[2], P. Peissig[2], L. Rasmussen[2], D.R. Masys[1], D.C. Crawford[1], D.M. Roden[1]*. 1) Dept of Biomedical Informatics, Vanderbilt University, Nashville, TN; 2) Biomedical Informatics Research Center, Marshfield Clinic, Marshfield, WI.

Genome-wide association studies (GWAS) have elucidated genetic associations for many diseases and phenotypes. Genetic data coupled to longitudinal electronic medical records (EMR) offers the possibility of the inverse experiment, phenome-wide association scans (PheWAS), to search for conditions associated with specific genetic variants. We performed a PheWAS in 40 single nucleotide polymorphisms (SNPs) with 52 previously known associations for 25 diseases. We used extant genotype data for 8,865 European Americans derived from BioVU, the Vanderbilt DNA Databank, which currently contains >86,000 DNA samples linked to de-identified EMR data. The genetic data consisted of targeted genotyping and GWAS data (Illumina's 660-Quad), the latter derived as part of the eMERGE network. Diseases were identified using International Classification of Disease, 9th edition, codes accrued from normal clinical billing processes, and then mapped to 776 custom-defined code groupings that combined similar diseases together (e.g., codes 401.X-404.X are all mapped to "hypertension"). Control populations were defined for each disease by excluding patients with the target disease and related conditions. New associations were subject to replication in a population of 4,164 individuals from the Marshfield Clinic Personalized Medicine Research Project. The Vanderbilt PheWAS replicated 22 of 52 previously known associations with p values from $9.7x10^{-11}$ to 0.047, median p-value $7.4x10^{-3}$. Diseases with significant known associations included multiple sclerosis, rheumatoid arthritis, type 2 diabetes, gout, atrial fibrillation, macular degeneration, hemochromatosis, schizophrenia, bipolar disease, coronary disease, obesity, Crohn's disease, and breast and ovarian cancer. In addition, PheWAS methods revealed ten new gene-disease associations with $p<0.01$ that replicated in the Marshfield data set ($p<0.05$). Examples of possible new associations included histoplasmosis with rs3135388 ($p=3x10^{-4}$, OR 4.2), lymphoid leukemia with rs2476601 ($p=7.9x10^{-3}$, OR 1.97), rs17696736 and acquired hypothyroidism ($p=5.0x10^{-3}$, OR 1.16). This study further validates EMR-based PheWAS as a method to discover SNP-disease associations by reusing existing genetic data, and posits several new associations in heretofore-unassociated genes. The PheWAS code and code translations software are freely available at http://knowledgemap.mc.vanderbilt.edu/research.

1737/W

Defining Optimal Experimental Design and Analytical Strategies for RNA Seq experiments. *J. Hester, J. Schweitzer, E. Sehayek, D. Serre.* Cleveland Clinic, Cleveland, OH.

RNA-Seq, sequencing a sample's transcriptome using next-generation sequencing, is a rapidly expanding technique that may replace gene expression microarrays in the next few years. However the bioinformatic analysis of these datasets is still in the early stages and strategies to design and analyze the wealth of data generated by these experiments remain to be optimized. To define key parameters in RNA-Seq design and specifically assess the cost-effectiveness of various read length and paired-end sequencing, we simulated ~30 million 36 bp and 51 bp reads, both single and paired-end, from human RefSeq transcripts. We also introduced random mutations in the sequences to assess the effect of sequencing errors on the results. We then used the TopHat algorithm to map the reads to the Human reference genome and identify splice junctions. Our simulations i) clearly indicate that different sequencing designs should be used to quantify transcript levels and to identify alternative splicing, and ii) provide analytical strategies to reduce the number of false positives. Applying of these strategies to a CACO-2 cell line found that by using proper filtering criteria even a single lane of 51bp paired reads of the Illumina GAIIx sequencer can provide a tremendous amount of information on transcript level, isoform structure, novel transcripts, coding polymorphisms or large chromosomal rearrangements.

1738/W

A Bayesian measurement error model approach to two-channel cell-based RNAi data with replicates. *I. Chang[1], C. Chen[2], W. Su[3], C. Chen[2], J. Huang[3], F. Tsai[2], W. Wang[2], C. Hsiung[2], J. Jeng[3].* 1) National Institute of Cancer Reasearch, National Health Research Institutes, Miaoli, Taiwan; 2) Division of Biostatistics and Bioinformatics, National Health Research Institutes, Miaoli, Taiwan; 3) Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan.

RNA interference (RNAi) is an endogenous cellular process in which small double-stranded RNAs lead to the destruction of mRNAs with complementary nucleoside sequence. With the production of RNAi libraries, large-scale RNAi screening in human cells can be conducted to identify unknown genes involved in a biological pathway. One challenge in these studies is to deal with the multiple testing issue and the related false positive rate (FDR) and false negative rate (FNR). This paper proposes a Bayesian measurement error model for the analysis of data from a two-channel RNAi high-throughput experiment with replicates, in which both the activity of a particular biological pathway and cell viability are monitored and the goal is to identify shRNAs that affect the pathway activity without affecting cell activity. Simulation studies indicate the excellent numerical performance of this method and provide insight into the effects of prior distributions and the number of replicates on FDR and FNR. This method is illustrated in analyzing the data from a RNAi high-throughput screening that searches for cellular factors affecting HCV replication without affecting cell viability; comparisons of the results from this HCV study and some of those reported in the literature are included.

1739/W

Integrated Visualization Tools for SNP Genotyping Quality Control. *C. Huang[1], F. Kuo[1,2], S. Diehl[1,2].* 1) Center for Pharmacogenomics and Complex Disease Research, UMDNJ-New Jersey Dental School, Newark, NJ; 2) Health Informatics, UMDNJ-School of Health Related Professions, Newark, NJ.

The accuracy of Single Nucleotide Polymorphism (SNP) genotype calls has a potentially great impact on statistical results of gene association studies. Most available software systems provide only a basic two dimension cluster calling algorithm to make genotype calls, and don't integrate the overall laboratory design to maximize quality control. Ideally, both positive and negative controls need to be included and tracked in DNA plate layouts, and used downstream to assess the quality of genotype calls. A false genotype call may be caused by many reasons including DNA and oligonucleotide quality, lab processing errors, contamination, failed instrument readings, etc. When there are many failed calls on a plate, it can be very helpful for troubleshooting to view where the failures on a plate layout most consistently occur to identify the possible causes. It is very helpful to have a tool that allows laboratory scientists to visualize signal intensities and genotype calls and performance of DNA samples across multiple assay plates. These features can help reduce genotyping costs and errors dramatically. It is also very valuable that the software can automatically perform quality checking and evaluate potential problems such as edge effects and consistency of positive controls and quickly generate a quality report based on wells, samples, SNPs, plates, studies, etc., so when laboratory problems occur they are rapidly detected and corrected so that damage to a genetic research project is minimized. Here, we present our SNPtyper software program that has been developed to provide these essential visualization and quality control capabilities. The program can automatically make genotype calls, perform quality controls, check for consistency among duplicated DNA samples and positive controls across multiple assay plates, and rapidly generate reports for both lab staff and bioinformatics personnel. Our software can also rapidly provide genotype call quality score based on fit to Hardy Weinberg expected frequencies in different races or ethnic groups intermingled on the same assay plate. It has the ability to display multiple genotype plate plots and layouts to compare the results of same DNA source plate. Examples of how the tool has helped identify the genotyping errors, plate miss-arrangements will be presented. This research was supported by grants NIDCR DE13102, DE016057 and DE017900, NIEHS ES015864 and the Foundation of UMDNJ.

1740/W

NCBI database for genetic associations with high-throughput molecular phenotypes. *J. Paschall[1], D. Hoffman[1], N. Sharopova[1], W. Gan[2], M. Feolo[1], S. Sherry[1], J. Ostell[1], JP. Struewing[2].* 1) National Center for Biotechnology Information, Bethesda, MD; 2) Natiional Human Genome Research Institute,Bethesda MD.

The success of genome wide association studies (GWAS) has identified hundreds of robust genetic associations across a range of disease phenotypes. In most instances, however, the causative variants and thus the molecular pathways linking a genomic variant to disease susceptibility remain unknown when the associated SNPs do not map to exons. It is critical for post-GWAS studies to identify which of these may influence gene regulation. An important approach to bridging this gap is testing for association between genomic variants and molecular level phenotypes such as gene expression (eQTL), epigenomic, and proteomic data. The NCBI has developed a searchable database focused on archiving and visualizing genetic associations with high throughput molecular phenotype data: (http://www.ncbi.nlm.nih.gov/gtex). This effort originated with the eQTL data from the NIH Common Fund Genotype-Tissue Expression (GTEx) project http://nihroadmap.nih.gov/GTEx/ which will assess the genetic component of gene expression variation across many different human tissues. The scope of this database extends to all existing and future high-throughput molecular association datasets. Current datasets include those derived from both microarray and RNA-seq technology. Association results are searchable by expressed gene, genomic variant, and tissue-type, and are visualized graphically as histograms and as tracks within a genome browser. Links will be computed and automatically updated between SNPs found to be associated with molecular phenotypes, and SNPs found to have disease phenotype associations as recorded in dbGaP and the NHGRI GWAS catalog (www.genome.gov/gwastudies). The goal is to allow the results of a new disease phenotype study to be quickly queried against all available molecular phenotype association data. This will provide additional biological support in interpreting those results, and allow for background processes to re-scan both disease and molecular phenotype associations over time in a continuous data-mining effort.

1741/W

Tbx20 gene targets and regulatory networks in the adult mouse heart. *N. Sakabe[1], I. Aneas[1], T. Shen[2], S. Evans[2], M. Nobrega[1].* 1) Human Gen Dept, Univ Chicago, Chicago, IL; 2) Skaggs School of Pharmacy and Pharmaceutical Sciences, UCSD, San Diego, CA.

Tbx20 is a transcription factor (TF) important for heart development. To assess its role in the adult heart, we created a heart Tbx20 knockdown mouse on which we observed a lethal phenotype involving severe cardiac abnormalities. To better understand the role of Tbx20 in the adult heart, we sought to identify regulatory regions, gene targets and pathways that could explain the knockdown phenotype. To this end, we used chromatin immunoprecipitation (ChIP) of Tbx20 to locate Tbx20 binding sites in the genome of 6 weeks whole heart mouse tissue. At 9% FDR, we identified 4,012 peaks. To our surprise, the canonical Tbx20 motif was not strikingly overrepresented, but instead, we discovered a highly overrepresented motif that resembles a T-box in ~65% of the peaks (2-fold enrichment). Peaks with and without the newly found Tbx20 motif presented conservation around the peak centers above expectation, which supports functionality. We called putative Tbx20 gene targets by assigning peaks to genes where they lie or to the flanking genes (~22% of the peaks were located within +- 6kb of the transcription start site). To assess pathways that were directly regulated by Tbx20, we analysed Gene Ontology (GO) enrichment of the putative gene targets. GO terms related to heart contraction, calcium transport and energy metabolism were among the highly enriched which hints for biological pathways that are regulated by Tbx20. We then searched Tbx20 peaks for other overrepresented motifs to identify potential partners for this TF and to try to identify TF combinations that would be important in adult heart function. Among many heart related motifs were MEF2A, CREB1, TEAD1 and ESRRb which are independently known to play a role in ion transport in the heart. We mutagenized individual high-scoring instances of these motifs and using a zebrafish reporter system, we observed ablation of enhancer function of selected Tbx20 ChIP peaks near ion channel genes. When we selected only genes near peaks containing the 4 motifs, we found that they are particularly assigned to ion transport genes. The GO and motif data, together with physiological observations in the knockdown mouse indicate that Tbx20 plays a major role in ion transport regulation in the heart. We will pursue more analysis similar to this one to discover other pathways that might be regulated by Tbx20 and that could explain the phenotype of the knockdown mouse at the molecular level.

1742/W

Intron gain in mammalian retrogenes. *I. Makalowska, M. SzczeÅ niak, J. Ciomborowska.* Faculty of Biology, Adam Mickiewicz University, Poznan, Poland.

For a long time intron gain phenomenon in mammals has been elusive and, despite intensive genome wide analyses, only few instances of 'intronisation' in mammals are known. Reported previously intron gains in mammalian genomes arised in retrogenes and are associated with either fusion with host genes or recruitment of new exons. We performed comparative analysis of human, chimpanzee and mouse genomes in order to identify 'intronisation' cases in retrogenes. We were mostly, although not only, interested in cases where the exon was split in result of new sequence insertion or as a consequence of mutations and emergence of splicing signals. Using BLAST searches and homology annotations we identified a number of novel retrogenes that underwent 'intronisation'. Many of them, beside the intron gain, show additional interesting features, including exaptation of repetitive elements, overlapping with other genes and alternative splicing. The latter is especially interesting, as it was not seen previously in retrogenes. Splicing forms, which we proved experimentally for RNF113B gene in human, show tissue-specific expression profiles. It also appears that they arose relatively recently and often are species specific. Results of our studies demonstrate new functions of retrogenes in the transcriptome diversification and show that retrogenes may be responsible for a wealth of species-specific features including species-specfic introns and splice variants.

1743/W

NSIT: Novel Sequence Identification Tool. *B. Pupacdi[1], A. Javed[2], M.J. Zaki[3].* 1) Chulabhorn Research Institute, Bangkok, Thailand; 2) IBM Thomas J. Watson Research Center, Yorktown Heights, NY; 3) Computer Science Department, Rensselaer Polytechnic Institute, NY.

Identification of novel sequences in a *de novo* human genome assembly is an important task. Recently Li *et al.* report that such assembly may contain as high as 5Mbps not present in the extant reference genome. Here we introduce **NSIT (Novel Sequence Identification Tool)** which quickly aligns a *de novo* assembly against a reference genome and accurately identifies all novel sequences. The challenge lies in the fact that a *de novo* assembly typically consists of a few hundred thousand scaffolds and contigs whose sizes vary greatly and respective chromosomal positions are unknown post-assembled. Querying each one against the reference sequence index is computationally daunting, even with fast alignment programs like BLAT and LASTZ. Unlike those algorithms, NSIT takes advantage of the fact that two human genomes are highly similar and only focuses on the main diagonal alignment as opposed to all chains of high-scoring segment pairs. As a result, its search space for identifying novel sequences greatly reduces. It indexes all possible k-mers per chromosome of the reference sequence, then coarsely maps each scaffold and contig onto a unique chromosome. Fine-scale alignment onto the assigned chromosomes follows. The coarse alignment only uses k-mers from every n-th position (n<50), and the detailed alignment uses all. Consecutive non-overlapping k-mers of the query are streamed against its respective index to find maximal unique matches, which become alignment anchors. No scoring matrices are used. Experiments show that NSIT aligned all but only 45Mbps of the *de novo* YH genome to the Build 36 genome within 1.5hrs using <2GB of RAM on a commodity desktop. All of the YH novel sequences were contained in these 45Mbps. Li *et al.* used BLAT and LASTZ as the first two steps and subsequently BLAST to align the remaining unmapped regions. NSIT offers a faster alternative step prior to using BLAST. Although the paper did not report their timings of BLAT and LASTZ, we suspect them to take much longer due to the larger search space. (Running BLAT on the YH assembly against just Chr 1 Build 36 lasted >12hrs and did not finish on our computer.) NSIT took 1.5hrs and BLAST took <15hrs on the remaining 45Mbps region. Our results contained exactly the ~5Mbps novel sequences reported in their paper. In conclusion, NSIT is a fast and accurate tool to identify novel sequences in a *de novo* genome assembly and will be a very useful tool as human genome sequencing becomes more routine.

1744/W

CYP1B1 Sequence Variations in a Cohort of Patients with Sturge-Weber syndrome from the U.S. *T.L. Yanovitch[1], S.F. Freedman[1], K.N. Tran-Viet[2], R. Metlapally[2], W. Call[2], E. Burner[1], T.L. Young[1,2].* 1) Ophthalmology, Duke Eye Center, Durham, NC; 2) Human Genetics, Duke University, Durham, NC.

Purpose: Sturge-Weber syndrome (SWS) is a rare, sporadic, neurocutaneous disorder characterized by ipsilateral facial port-wine staining in the ophthalmic distribution of the trigeminal nerve, glaucoma with associated vascular eye abnormalities, and occipital leptomeningeal hemangioma. The genetic and environmental factors resulting in this disorder are unknown. A recent report from India describes CYP1B1 mutations in patients with SWS that have buphthalmos and gyral calcifications. The purpose of this study was to screen patients with SWS from the U.S. for possible causative CYP1B1 sequence variants. Methods: After approval from the Duke University Institutional Review Board, informed consent was obtained from SWS patients and/or their parents. Direct genomic sequencing of the CYP1B1 gene was performed on patient DNA that had been extracted from blood or saliva. Base pair comparisons were made with known reference sequences to identify possible sequence variants. Array comparative genomic hybridization (CGH) was also performed to determine copy number variations. Results: A total of 11 patients were ascertained. About one-half (45%) of the patients were male. The median age was 12 years (range 3-51). In terms of ethnicity, 8 (73%) were Caucasian, 1 (9%) was African American, 1 (9%) was African, and 1 (9%) was of mixed race. The location of the facial port-wine stain was on the right side in 5 patients, the left side in 1 patient, and bilateral in 5 patients. All of the patients had glaucoma, with 4 (36%) having bilateral disease. Glaucoma was diagnosed before 1 year of age in 9 patients (81%). The remaining 2 patients presented with glaucoma as children. 10 (91%) patients had choroidal hemangiomas, and 3 (27%) had exudative retinal detachments. 4 (37%) patients had homonymous hemianopia visual field defects. Seizure disorder was present in 7 (64%) patients. Neuroimaging results were not available on all of these patients, but 1 patient had gyral calcifications. There were no disease-causing sequence variants (11 subjects) or copy number variant changes (3 subjects) found in CYP1B1. Conclusion: In our cohort of patients with SWS and associated infantile or juvenile-onset glaucoma, none were found to have CYP1B1 mutations. Array CGH also failed to identify variants in the region of CYP1B1. The absence of sequence variant findings suggests that other genes and factors are responsible for SWS associated glaucoma in the U.S. population.

1745/W

Construction of 330K Japanese BAC Library, YAMATO, and BAC DNA sequencing by SOliD3 in comparison to 3730 capillary sequencer. *T. Hirano[1,2], M. Tsukahara[2], I. Kikuzato[2], K. Morita[1], Y. Terabayashi[1], K. Fujimori[1,2], M. Machida[1,2], M. Nezuo[2], O. Asato[2], H. Irifukuhama[2], Y. Sato[2], N. Matsumoto[3].* 1) AIST, Tsukuba, Japan; 2) OCGP, Okinawa Cutting-Genome Project, Okinawa, Japan; 3) Graduate School of Medicine, Yokohama City University, Yokohama, Japan.

An original 330K BAC (bacterial artificial chromosome) library, named YAMATO, was constructed from cord blood cells of a single male, established as Japanese based on genetic analysis in HLA region. The average size of BAC was about 130K, which indicates 14.2 times coverage of human genome by just one YAMATO library About the half of the whole library was amplified, corresponding BAC DNA was purified, and the both terminal sequence was analyzed by capillary sequencer, resulting in more than 90% coverage for whole human genome based on NCBI human genome data. One BAC DNA located in HLA region, was shot gun sequenced by 3730, and total 171,735 bases were established by using sequencher. Simultaneously the same BAC DNA was analyzed by SOliD3, and mapped on either own capillary sequenced data or the corresponding NCBI data as reference sequence. Through the comparison of the sequence data obtained by 3730 and SOLiD3, the accuracy of SOLiD3 was estimated to be more than 99.99%. The Japanese BAC DNA sequence established was compared with the corresponding sequence of NCBI human genome data. The comparison revealed 4 large scale deletions, 4 major insertions and 2 translocations, as well as new 14,072 SNP candidates (8.2% of entire BAC sequence), most of them (96%) were not listed in DBSNP. Even just one BAC DNA analysis, these results clearly indicate the difference between Japanese and NCBI Caucasian genome. Such precise analysis based on BAC DNA is indispensable to establish the ultimate difference and diversity in human genome.

1746/W

Collecting gene sequence variants in all Mendelian disease genes from resequenced personal genomes in LSDBs. *P.E.M Taschner, I.F.A.C. Fokkema, J. Celli, J.T. den Dunnen, the Gen2Phen Consortium (http://www.gen2phen.org).* Dept Human Genetics, Leiden Univ Medical Ctr, Leiden, Netherlands.

Since the late eighties, starting with monogenic disorders, we have successfully linked sequence variants in specific genes to specific diseases. Now we approach the time that we can explore not a single gene but the entire genome for sequence variants and start to link these to complete individual phenotypes, disease-related, healthy or even prognostic. For the interpretation of all these variants, powerful tools are needed to efficiently sift through all variants found, link them to existing knowledge and prioritizing those that need further attention, especially those related to an individuals health. To support DNA diagnostics of monogenic diseases, gene-specific collections have been generated listing all variants identified world-wide, so called gene variant databases (Locus-Specific DataBases, LSDBs). Although generally accepted as essential in supporting an accurate and fast clinical diagnosis based on the latest findings, effective collection of these variants has been shown to be difficult. Many reasons for this exist, one of them being the lack of a central web-based gene-based repository in a format familiar to clinical geneticists. In the setting of the EU-funded Gen2-Phen project we have now established a gene variant database (LSDB) for all genes accepting all gene variant data available, including those obtained from complete exome or genome resequencing (see www.LOVD.nl). However, to make this resource most useful, we need the help of guardians for these database, who will curate incoming information and thereby ensure data quality. Therefore, we invite clinicians and researchers working on genetic disorders world-wide to become the guardian of their gene(s) of interest. As an incentive for the submission of new variants, tools linked to LOVD enable automatic conversion of chromosomal positions to gene-related positions used by LSDBs as well as links to available variant information. This approach would use the database as a reference to allow identification of variants occurring with low frequency, which are likely to be involved in genetic disorders and should be prioritized for further investigation. Funded by the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n=BA 200754 - the GEN2PHEN project.

1747/W

Comparative Analysis of Mitochondria Genome: Clinical Approaches. *L. Chou[1], K. Mallempati[1], R. Mao[1,2].* 1) ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, Utah; 2) Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, Utah.

Background: Human mitochondria genome is a double stranded circular DNA and mostly maternally inherited. It has no introns and 80%-93% of the sequences are coding regions. Common mutations in the mitochondria DNA cause diseases including Leber hereditary optic neuropathy (LHON), Myoclonic epilepsy with ragged red fibers (MERRF), and Neuropathy, ataxia, retinitis pigmentosa (NARP). Due to lack of histone and DNA mismatch repair mechanism, mitochondria has high mutation rate. During cell division, the proportion of mutant mitochondria DNA in daughter cells can shift. This level of heteroplasmy may differ between cells and tissues thus making accurate diagnosis of mitochondria related diseases more difficult. Methods: Fifteen previously genotyped samples (by DHPLC plus targeted Sanger sequencing), four normal samples, and nine Coriell samples were used to initially validate the chip-based re-sequencing microarray (Affymetrix). The original re-sequencing microarray protocol was modified and stream-lined to fit in a clinical workflow. The assay accuracy and reproducibility were accessed and any additional sequence variants detected were confirmed by a second method, Sanger sequencing. The next generation sequencing (NGS) approach was also evaluated. In this high-throughput approach, up to three different samples were barcoded and ran through the same lane in the GAII system (Illumina) to be more cost-efficient in comparison to the gold standard Sanger sequencing. The GAII data was extracted and analyzed by customized NextGENe software (SoftGenetics) for codon usage of mitochondria. Results and Discussions: Our modified re-sequencing microarray method showed 100% concordance when compared to the genotyped results, with an overall call rate (entire chip) between 97%-99%. Additional sequence variants (non-pathogenic) detected by the re-sequencing microarray but not reported by the DHPLC-Sanger method were re-amplified by in-house designed primers and Sanger sequenced for final confirmation. Although this modified microarray protocol fits in a clinical workflow very well, however, different levels of heteroplasmy can not be determined. The NGS with a barcode system is a potential approach for analyzing the mitochondria genome. In our study, cost per sample sequenced was decreased, the low level heteroplasmy (below 5%) was determined, and the potential of visualizing large deletions was accessed by high sequencing coverage.

1748/W

A matter of life and death: How microsatellites emerge and disappear from the human genome. *Y.D. Kelkar[2,1], F. Chiaromonte[3,1], K.D. Makova[2,1].* 1) Center for Medical Genomics, The Pennsylvania State University, State College, PA; 2) Department of Biology, The Pennsylvania State University, State College, PA; 3) Department of Statistics, The Pennsylvania State University, State College, PA.

Microsatellites - tandem repeats of short motifs - are abundant in human genome, and have high mutation rates. Their instability is implicated in 40 known genetic diseases; however, a systematic study of the molecular processes involved in microsatellite emergence and disappearance in the genome has been lacking. Microsatellite loci are hypothesized to follow a *life cycle*, wherein microsatellite are born (the *birth phase*), expand leading to the *adulthood phase*, until interruptions or large deletions cause their degradation (the *death phase*). Here we carried out a large-scale comparative genomic study to identify microsatellite births/deaths in three primate species, determined the causal mutational processes, and found the local genomic features that may drive the births/deaths. Using publicly available genome alignments, we identified a genome-wide set of *de novo* microsatellite birth/death events in human, chimpanzee, and orangutan, using macaque and marmoset as outgroups. Using maximum parsimony, we determined the causal mutational steps (substitutions, insertions and deletions) for the majority of the identified birth/death events. We mapped the events to transposable elements and genes in the human genome, and discerned the effect of these environments on microsatellite birth/death propensities. To identify the drivers of birth/death events among many regional genomic features, we implemented a multiple regression approach. We came to the following conclusions. First, although substitutions were the predominant cause for births/deaths of small microsatellites (under 12 bp), insertions and deletions were increasingly important for births/deaths of larger ones. Second, LINE and younger SINE elements showed higher and lower propensity for birth/death events, respectively. There was a dearth of births/deaths in the proximity of genic regions, even in introns, indicating that such events have long-term functional consequences. Supporting these observations, multiple regressions indicated that regions surrounding birth/death loci are marked by high substitution rates, skewed nucleotide composition, and high and low density of LINE and SINE elements, respectively. Using this, we could estimate the probability of functionally important regions to bear microsatellite genesis and loss. This has important applications, given that, while many extant microsatellites play important regulatory roles, some microsatellites' instability has major health consequences.

1749/W

eQTLs and sQTLs in Human Cerebellum and Parietal Cortex. *C. Liu, L. Cheng, F. Pibiri, J. Badner, C. Chen, C. Zhang, K. Grennan, E. Gershon.* Dept Psychiatry, Knapp Res Ctr, Univ Chicago, Chicago, IL.

About 150 Human cerebellum and parietal cortex samples from the Stanley Medical Research Institute were profiled for gene expression using the Affymetrix Human Gene 1.0 ST array. With SNP genotype data obtained from the Affymetrix 5.0 array, we performed expression quantitative trait locus (eQTL) and splicing quantitative trait locus (sQTL) mapping. After controlling batch effects and covariates, cis- and trans- associations have been detected in both brain regions. eQTLs and sQTLs shared by the two brain regions, and brain-region specific transcripts and splicing isoforms were identified with their corresponding SNPs.

1750/W

High Coverage Gene Expression Profiling on the Applied Biosystems 3500xL Genetic Analyzer. *A. Pradhan[1], C. Davidson[1], M. Kondo[2], A. Felton[1], R. Araki[3], S. Ando[3], M. Abe[3].* 1) Applied Biosystems, Foster City, CA; 2) Applied Biosystems Japan, Tokyo, Japan; 3) 3Transcriptome Profiling Group, National Institute of Radiological Sciences, Chiba, Japan.

Whole transcriptome expression profiling is typically performed using hybridization-based microarray methodologies. However, there are a number of limitations to microarray-based approaches such as low sensitivity and specificity, poor dynamic range, and, importantly, microarray expression profiling results are restricted to specific sequence annotations and content. The AFLP-based HiCEP (High Coverage gene Expression Profiling) method was developed to address the above shortcomings in gene expression profiling and provide a sensitive method for detecting a large proportion of transcripts in both known and unknown genes, with low false positive rate. Here we demonstrate the use of the new Applied Biosystems 3500xL Genetic Analyzer for the detection of known transcripts unregulated by ionizing radiation (IR). mRNA samples were prepared from mouse embryonic fibroblasts (MEFs) at 0, 3, 6 and 24 hours after IR exposure. The expression of *p21*, *CyclinG1*, *Gadd45a* was assessed to demonstrate the in-lane normalization functionality of the 3500xL system. Further, the expression changes detected by capillary electrophoresis (CE) were compared to TaqMan® Gene Expression Assays for the above transcripts. When the same sample is analyzed by CE, a certain amount of variation in signal strength may be observed within a single instrument among different capillaries; or among different injections from the same capillary. For applications such as HiCEP, minimal signal variation is desired and data analysis can be aided by reducing signal variation. Here we demonstrate that the normalization functionality incorporated into the 3500 Series of Genetic Analyzers is useful for the analysis of HiCEP gene expression data. Normalization increased the precision and accuracy of peak height determinations, which are particularly important for the detection of slight expression changes. An improved understanding of the differences in HiCEP sample replicates was possible following the application of 3500 calculated normalization. Normalization of HiCEP data also assists in statistically differentiating between peak height group means for *p21*, *CyclinG1*, and *Gadd45a* at various time points. Taken together, these analyses indicate that normalization improves consistency of HiCEP data and facilitates the comparison of replicate samples (both mRNA preparation and HiCEP reaction replicates) between injections and between capillaries within the same injection.

1751/W

Systems Genetics Analysis of Gene-Environment Interactions in Macrophage Inflammatory Responses. *L.D. Orozco[1], B.J. Bennett[1], C.R. Farber[2], A.J. Lusis[1].* 1) Human Genetics, University of California, Los Angeles, Los Angeles, CA; 2) School of Medicine, University of Virginia, Charlottesville, VA.

Genetic variation in naturally occurring populations can have a tremendous effect on how individuals respond to environmental stimuli. Recent genome-wide association studies in humans and mice have demonstrated the contribution of genetic variation to complex phenotypes such as atherosclerosis, as well as gene expression traits in various tissues. However, the contribution of gene-by-environment interactions (GxE) in gene expression traits or clinical phenotypes remains largely unexplored. Macrophages play critical roles in innate immunity, inflammatory responses, and complex traits such as atherosclerosis. We sought to understand the contribution of natural genetic variation, environmental stimuli and GxE interactions to genome-wide expression levels of primary macrophages. In order to examine the effect of genetic and environmental responses, we have developed a resource, called the Hybrid Mouse Diversity Panel (HMDP) consisting of 100 classical inbred as well as recombinant inbred strains. We obtained primary peritoneal macrophages and exposed them to control media, media containing bacterial lipopolysaccharide (LPS), or to oxidized phospholipids (oxPAPC) as inflammatory and oxidative stimuli. We then examined genome-wide gene expression levels using microarray analysis and integrated the data as a function of genetics. We identified loci mediating macrophage inflammatory responses and evidence of GxE interactions in thousands of genes. Interestingly, we identified several gene expression "hotspots" in LPS-treated macrophages, but not in oxPAPC-treated cells, suggesting 1) the presence of master regulators controlling the expression of hundreds of genes, and 2) that global effects of genetics and GxE interactions are decidedly context specific. In addition, because the HMDP consists of permanent inbred strains, we propose that the data generated through the use of the HDMP constitute a cumulative resource that can be used for the integration of genetic, phenotype and gene expression data, ideal for systems genetics.

1752/W

Strand-specific RNA sequencing of HepG2 cells identifies genes that are differentially expressed and alternatively spliced in response to TGF-beta. *C. Wadelius[1], S. Enroth[2], O. Wallerman[1], B. Touch[3], C. Barbacioru[3], M. Bysani[1], R. Andersson[2], S. Termén[4], A. Moustakas[4], C.-H. Heldin[4], S. Stanley[3], J. Gu[5], S. Kuersten[5], M. Barker[3], J. Komorowski[2, 6], K. McKernan[7], F. de la Vega[3].* 1) Dept Gen & Pathology, Uppsala Univ, Uppsala, Sweden; 2) The Linnaeus Centre for Bioinformatics, Uppsala University, Sweden; 3) Life Technologies, Foster City, CA, USA; 4) Ludwig Institute for Cancer Research, Uppsala, Sweden; 5) Life Technologies, Austin, TX, USA; 6) Interdisciplinary Centre for Mathematical and Computer Modeling, Warsaw University, Poland; 7) Life Technologies, Beverly, MA, USA.

Transforming growth factor-beta (TGF-beta) controls many complex behaviors of normal and transformed cells. In early stage adenomas TGF-beta acts as a tumor suppressor, whereas in advanced carcinomas it promotes tumor cell invasiveness and metastasis. HepG2 cells provide a good model for advanced tumor cells. RNA sequencing (RNA-Seq) can provide a detailed view of the dynamic transcriptional landscape of cells experiencing environmental perturbation and was therefore applied here to study TGF-beta response. We serum starved HepG2 cells for 24 hours and then treated for 1 hour with 2 ng/ml TGF-beta1 or with vehicle control. Total RNA was isolated, depleted of rRNA, fragmented and then used to create strand-specific cDNA libraries with the SOLiD™ Small RNA Expression Kit. Approximately one billion 50 bp reads were sequenced with the SOLiD™ System, over half of which could be uniquely aligned to the human genome. The aligned transcriptomes cover roughly 4% of each strand of the genome and more than half of the reads align within known transcribed regions. The expression level of each gene was defined and hundreds of genes were determined to be differentially expressed between the two conditions. The 50 bp length of the sequenced fragments permitted us to accurately align reads to splice junctions. In all, ~5 million reads aligned to ~90,000 known and putative splice junctions in both the control and stimulated conditions, which allowed us to determine the effects of TGF-beta on alternative splicing. The data suggests that many genes change splice pattern after stimulation. Furthermore, we have determined positions of nucleosome and critical histone modifications to see how they are related to the transcriptional output.

1753/W

Linkage mapping of eQTL acting in brain and blood. *A.J. Jasinska[1], S. Service[1], M.K. Lin[1], O-C. Choi[1], J. DeYoung[1], O. GrujiC[1], S-Y. Kong[1], M. Jorgensen[2], J. Bailey[1], S. Breidenthal[1], L.A. Fairbanks[1], R.P. Woods[3], J.D. Jentsch[4], N.B. Freimer[1].* 1) Center for Neurobehavioral Genetics, University of California, Los Angeles, CA; 2) Department of Pathology, Section on Comparative Medicine, Wake Forest University Health Sciences, Winston-Salem, NC; 3) Departments of Neurology and Psychiatry and Biobehavioral Sciences, David Geffen School of Medicine, University of California, Los Angeles, CA;; 4) Department of Psychology, University of California, Los Angeles, CA.

The expression of a substantial fraction of the brain transcriptome is reflected in the expression of peripheral blood which allows for efficient mapping of brain-acting eQTL using blood as a surrogate for brain tissue. To test this hypothesis, we used as a model organism the vervet monkey (*Chlorocebus aethiops sabaeus*). Using vervets housed in uniform rearing conditions, we were able to obtain high quality RNA from brain tissues collected according to a stringent protocol, reducing possible environmental confounders. We used an Illumina HumanRef-8 v2 chip to measure transcript levels in blood from 347 individuals from an extended inbred vervet pedigree from the Vervet Research Colony (VRC). Linkage eQTL mapping was performed with SOLAR using genome-wide genotypes from 261 STR markers typed in the VRC pedigree. We focused on transcripts for which duplicate measurements in blood demonstrated relatively stable levels over time allowing for efficient eQTL mapping in this tissue. Here we present results of eQTL mapping for 29 heritable transcripts for which expression levels between brain and blood are well correlated, and which displayed greater inter-individual variation than intra-individual variation between these tissues. Among 29 candidate transcripts for mapping brain eQTL in peripheral blood, 10 showed significant *cis* eQTL and 2 transcripts showed significant *trans* eQTL (LOD>3). Vervet sequence analysis showed that a SNP polymorphism in the probe interacting region occurred in 37.5 % of probes. To interpret eQTL mapping results and exclude possible false positive findings, we performed Q-RT PCR validation for 9 identified eQTL. Microarray results were validated for 6/9 eQTL transcripts (TMEM14C, B3GALTL, STOM, SMOX, TMED3 and CDNKA1A) and not validated for 3/9 (TUBA1B, TSPAN14, TMEM111) transcripts. All tested monomorphic probes and 2/3 of polymorphic probes were validated. This result indicates that the presence of microarray probe sequence polymorphism does not necessarily invalidate eQTL linkage mapping results. Our findings demonstrate that it is possible to successfully map eQTL from inaccessible tissue (such as brain) using readily available tissue (such as blood). This approach is possible only for the methodically-defined fraction of the transcriptome which is comparable between the two tissues.

1754/W

Global transcriptome analysis in an online multimodal atlas of the human brain. *S. Sunkin, D. Bertagnolli, M. Chakravarty, C. Dang, C. Lau, A. Guillozet-Bongaarts, C. Lee, E. Lein, M. Hawrylycz, J. Hohmann, L. Ng, C. Overly, Z. Riley, K. Smith, P. Wohnoutka, E. Shen, A. Jones.* Allen Institute for Brain Science, Seattle, WA.

The Allen Human Brain Atlas is an online multimodal atlas of human brain that integrates anatomic and genomic information coupled with a suite of visualization and mining tools. A key component of the resource is an 'all genes, all structures' dataset of microarray-based gene expression profiles from normal non-diseased postmortem brain. To create the Atlas, a process for collection of multiple data types from each brain and sampling of specific anatomic structures for microarray analysis was created such that the various data modalities could be mapped back into three-dimensional space. The process involved collection of T1- and T2-weighted magnetic resonance (MR) and diffusion tensor (DT) data, followed by multiple brain and tissue subdivision steps with increased resolution of histological data at each step, ultimately resulting in delineation and dissection of small tissue samples for microarray analysis. Quality control steps were inserted throughout the process to ensure data integrity and quality. Microarray data were generated using custom-designed Agilent 8x60K arrays. All data were normalized across the dataset to ensure brain-wide comparability of expression levels. Basic data analyses revealed variation in gene expression levels throughout the brain with differential expression detected across major anatomic divisions as well as between anatomic subdivisions within a structure. To enable unified navigation and visualization for users, spatial correspondences between histology data and MR images from the sampled brain were obtained via a series of assisted registration processes. MR images provide navigation through the anatomic sampling and gene expression data, with anatomy and histology data annotated using an ontology developed for this resource. Normalized gene expression data can be viewed in heatmap format across multiple structures and multiple gene probes. Gene expression profiles and MR and DT images are also downloadable from this site. Launched in May 2010 with expression profiles for over 700 anatomically distinct cortical and subcortical samples from one brain, more data and more sophisticated application features will be added throughout the remainder of this 5-year project. The Atlas can be accessed via the Allen Brain Atlas data portal at www.brain-map.org.

1755/W

A nanoliter scale method for high throughput, real-time PCR gene expression profiling in oncology and miRNA gene sets using the SmartChipTM System from WaferGen Biosystems. *T. Dodge, R. Bohenzky, S. Anandakrishnan, T. Boone, D. Dickens, D. Gelfand, P. Lin, S. Husain, H. Hubschle, S. Prasad, J. Sandhu, S. Taffler, M. Tan, H. Veereshlingam, S. Wei.* WaferGen Biosystems, Fremont, CA.

The SmartChipTM Real-Time System from WaferGen Biosystems is a platform for performing massively parallel real-time PCR assays for multiple applications, including gene expression studies. This system combines the benefits of real-time PCR, including sensitivity, precision and dynamic range, with the low cost per sample and high throughput profiling power of hybridization arrays. The system consists of three components; a SmartChipTM, comprising 5184 nanowells preprogrammed with gene-specific reaction content, a SmartChipTM Nanodispenser for applying sample and reaction mix to the SmartChipsTM, and a SmartChipTM Cycler for performing and collecting data from the real-time PCR assays. Using cDNA as an input for this system, one can generate thousands of data points which can comprise a thorough portrait of relative gene expression in a sample.

The SmartChipTM system has been extensively tested with two expression profiling panels: Human Oncology Expression Panel and the Human miRNA Expression Panel. These panels of 969 oncology related genes and over 800 miRNA's respectively show strong intra-gene precision and inter-panel reproducibility. Various controls build into the SmartChipTM allow for better interpretation of the results. The SmartChipTM Panels were also used to test cDNA from a variety of samples, including commercially available RNA tissue extracts, RNA from cells and RNA from clinical samples for research.

The data generated on the SmartChipTM system demonstrates that you can achieve the simplicity and robustness of real-time PCR with the high throughput and cost effectiveness of microarrays.

1756/W

Differential gene expression profiles in steroid treatment early response from children with acute lymphoblastic leukemia. *O. Perez-Gonzalez Sr, R. Cardenas-Cardos Mrs, R. Rivera-Luna, R. Coronel-Moran, N. Carranza-Rodriguez.* Experimental Oncology Laboratory, Experimental Medicine Dept, National Institute of Pediatrics, Mexico City, Mexico.

Purpose: The steroid initial treatment response of Acute Lymphoblastic Leukemia (ALL) patients is an important component of the classification criteria, designating high risk for those cases that do not present a suitable response. Nevertheless, these classification criteria do not have enough precision to assign the treatment according to the neoplastic behavior in all the cases. With the aim of identifying the associated patterns of genomic expression related to steroid initial treatment response, we propose a prospective, comparative and cross-sectional study using Affymetrix microarray platform with the HG-U133 2.0 Plus array. **Method**: We included 50 pediatric patients with confirmed diagnosis of Acute Lymphoblastic Leukemia treated at the National Institute of Pediatrics, Mexico. Once we obtained the consent, we performed bone marrow aspirate by usual methodologies for diagnosis confirmation and lymphoblast sampling for microarray hybridization. The diagnosis, treatment and response evaluation were realized according to the conventional procedures of the National Institute of Pediatrics. The statistic analysis was performed with Affymetrix Expression Console v1.1 and Partek's Genomics Suite for quality assessment (with RMA, Robust Multi-Chip Analysis) and gene expression profiles detection. **Results**: RMA algorithm showed a correlation between all the included microarrays from 0.83 to 1.0, considering all the microarray data adequate for the statistical analysis. We found a list of genes differentially expressed according to the steroid treatment response, and to the B or T immunophenotype, and then we integrated the metabolic pathways apparently related to differentially expressed genes with the Database for Annotation, Visualization and Integrated Discovery (DAVID Bioinformatics Resources 6.7) free-online software. Details of thes results will be presented at the meeting. **Conclusion**: These results show that gene expression profiles may clearly differentiate ALL patients according to their steroid treatment response including differences in the immunophenotype B or T. Differential gene expression profiles in ALL are being analyzed as a diagnostic tool to provide a more accurate allocation criteria for antineoplastic treatments in patients with this disease and allow us to understand the neoplastic behavior on different treatment response scenarios.

1757/W

Combinatory Pearson correlation coefficient and Kolmogorov-Smirnov distance metrics for identification of disease-specific biomarker genes. *Z. Zhao[1,2,3], H.C. Huang[1,2], S. Zheng[1,2].* 1) Biomedical Informatics, Vanderbilt Univ, Nashville, TN; 2) Bioinformatics Resource Center, Vanderbilt Univ, Nashville, TN; 3) Vanderbilt-Ingram Cancer Center (VICC), Vanderbilt Univ, Nashville, TN.

Gene expression microarrays have been widely used in disease studies. Disease-specific biomarker genes likely share gene expression profiles (GEPs) that are distinct in disease samples as compared with normal samples. The similarity of the GEPs may be evaluated by Pearson Correlation Coefficient (PCC) and the distinctness of GEPs may be assessed by Kolmogorov-Smirnov distance (KSD). In this study, we applied PCC and KSD metrics for GEPs to identify disease-specific biomarkers. We first analyzed and compared GEPs using microarray datasets for smoking and lung cancer. We found that the number of genes with highly different GEPs between comparing groups in smoking dataset was much larger than that in lung cancer dataset; this observation was further verified when we compared GEPs in smoking dataset with prostate cancer datasets. Moreover, our Gene Ontology analysis revealed that the top ranked biomarker candidate genes for prostate cancer were highly enriched in molecular function categories such as 'cytoskeletal protein binding' and biological process categories such as 'muscle contraction'. Furthermore, we picked top 20 significantly up-regulated and top 20 down-regulated genes based on PCC and KSD sorting. We found gene pairs comprising one up-regulated and another down-regulated had always best prediction performance. Finally, we used the top two ranked genes, *ACTA1* (encoding an actin subunit) and *HPN* (encoding hepsin), to explore the feasibility of diagnosing and monitoring prostate cancer using the expression intensity histograms of marker genes. In summary, our results suggested that this approach might prove promising and powerful for diagnosing and monitoring the patients who come to the clinic for screening or evaluation of a disease state including cancer.

1758/W

Analysis of genes that are co-expressed in mouse mesenchymal cell types reveals candidate genes for connective tissue diseases. *K. Summers.* Roslin Inst, Univ Edinburgh, Roslin, United Kingdom.

Connective tissue is composed of cells of mesenchymal origin, such as adipocytes, osteoblasts, chondrocytes, smooth muscle cells and fibroblasts. To identify genes which are specific to connective tissue, the tool Biolayout *Express*^{3D} was employed to cluster sets of genes with shared expression patterns across mouse cell types using microarray data. Fifteen clusters containing at least 15 transcripts showed expression restricted to cell types of mesenchymal origin. The largest of these clusters consisted of 220 transcripts which had high expression in osteoblasts and some expression in chondrocytes. These included *Adam12*, where knock out mice have abnormal muscle and adipose development and *Zic1*, associated with Dandy Walker malformation in mice. A smaller cluster (27 transcripts) shared high expression in chondrocytes and lower expression in osteoblasts. This group included *Fbn2*, the gene responsible congenital contractural arachnodactyly, which affects bones and ligaments. A cluster of 86 transcripts had expression restricted to chondrocytes. These genes included cartilage specific collagens (*Col9a1* and *Col10a1*) and *Halpn1* (cartilage link protein) as well as *Agc1*, encoding a protein associated with skeletal dysplasias in humans and *Bmp5*, encoding bone morphogenic protein 5 (associated with skeletal abnormalities in mutant mice). Another cluster of 29 transcripts expressed only in osteoblasts included many genes traditionally associated with muscle phenotypes, such as *Myog* and genes for myosin light and heavy chains. A cluster of 75 transcripts expressed at high level in all mesenchyme cell types contained *Fbn1* (Marfan syndrome) and other genes affecting a broad range of connective tissue phenotypes. The clusters contained structural protein genes, genes for the proteins which process the structural proteins in the endoplasmic reticulum, transmembrane receptors, transporter proteins and transcription factors which are likely to coordinate expression of other genes in the cluster. More than 15% of the genes had limited annotation. In different clusters these genes included putative transcription factors of unknown target and other genes with no homology to any known protein domain. Knowledge of the cluster in which they appeared provides a functional annotation. In addition, co-expressed genes are likely to be involved in the same function and therefore this novel clustering approach identifies candidates for diseases of similar phenotype.

1759/W

The Cartagene Genomics Project: Systems Biology of Human Functional Variation. *Y. Idaghdour[1,2], J. Hussin[1,2], C. Boileau[2], E. Gbeha[1], P. Awadalla[1,2].* 1) Sainte-Justine Research Center, University of Montreal, Montreal, Quebec, Canada; 2) CARTaGENE, Montreal, H3V 1A2, Canada.

An area of fundamental biomedical research that merges population and quantitative genomics is the identification of transcriptional and other intermediate biomarkers for disease susceptibility and disease status. These biomarkers are quantitative traits whose architecture is modulated through mechanisms that can incorporate genetic and environmental cues. But what are the relative magnitudes of these effects, and how gene expression profiles correlate with biomedical endophenotypes? To address these questions, we generated gene expression profiles from peripheral blood samples from a random aging cohort in Québec, Canada, coming from a number of different geographic locations and lifestyles and for which clinical material and medical information spanning a wide range of medically relevant phenotypes from a well characterized and extended pedigree have been collected. We documented latent structure in gene expression profiles and identified genes and networks of genes that best correlate with haematological and cardiovascular phenotypes. The analysis is being extended to incorporate genotypic data in order to identify the genetic control points of the transcriptional and clinical traits studied.

1760/W

Transcriptome characterization of a human embryonic stem cell line and an induced germ stem cell line. *H. Jiao[1,2], L. Vesterlund[1], V. Töhönen[1], P. Unneberg[1], J. Inzunza[1], A. Feki[4,5], O. Hovatta[3], J. Kere[1,2].* 1) Department of Biosciences and Nutrition, Karolinska Institutet, SE-141 57 Stockholm, Sweden; 2) Clinical Research Centre, Karolinska University Hospital, SE-141 57 Stockholm, Sweden; 3) Department of Clinical Science, Intervention and Technology, Karolinska Institute, Karolinska University Hospital Huddinge, Stockholm, Sweden; 4) Laboratory of Stem Cell Research, and Department of Obstetrics and Gynecology, Geneva University Hospitals, Geneva, Switzerland; 5) Department of Obstetrics and Gynecology, Geneva University Hospitals, Geneva, Switzerland.

Pluripotent embryonic stem cells (ES) isolated from in vitro culture of preimplantation embryos may be used in variety of studies to understand molecular basis in early development in human. Recently the development of induced pluripotent stem cells (iPS) artificially derived from non-pluripotent somatic cells by introducing the expression of certain reprogramming genes attracts even more attention because of their potentially therapeutic uses. However, the full potential of iPS cells and their relation to natural pluripotent stem cells is still being under investigation. The transduced cell lines are not always fully pluripotent, and such partially re-programmed lines offer us particularly interesting information about regulation of gene function. Transcriptome analysis has been a key area of biological research to understand the variety of gene functions in a cell. Next-generation sequencing technologies have revolutionized transcriptomics by providing opportunities for multidimensional examinations of cellular transcriptomes with manifold increased sensitivity. In order to characterize gene expression profiles of ES and a partially re-programmed iPS cell line, which only gives origin to germ cell lineage, we have performed a comparison between two different types of human cells, a human embryonic stem cell line (HS401) and the induced partially pluripotent germ cell line (ChiPSA) at whole transcriptome levels by RNA-Seq using SOLiD 3 System. The sequencing yielded millions of reads from the two cell lines and about 64% of the reads were mapped to the human genome in both lines. Out of these, 40% - 50% of the reads were uniquely mapped. Among 29409 evaluated genes/transcripts from 22 autosomal chromosomes and the X and Y chromosomes, we found that 20108 genes were expressed in both cell lines, while 6095 genes were not expressed in any of the two. Furthermore, 1825 genes were found uniquely expressed in ChiPSA, compared to 1381 genes solely expressed in HS401. Eighty-nine genes were highly differentially expressed. Our transcriptome data provide high-resolution information on the characteristics of the studied ES and iPS cell lines, adding important knowledge to research application of these cell lines.

1761/W

Role of the *ACACB* Gene in Human Metabolic Syndrome. *L. Ma, B.I. Freedman, M. Murea, A.K. Mondal, S.C. Elbein.* Internal Medicine, Wake Forest University Health Sciences, Winston Salem, NC.

The widely expressed acetyl CoA carboxylase β gene (*ACACB*) is a key negative regulator of fatty acid oxidation through carnitine palmitoyltransferase-1 (CPT-1). We hypothesized that *ACACB* expression would differ with insulin sensitivity (SI) in humans, and that expression levels would be controlled by DNA variation. We first evaluated *ACACB* expression in adipose and muscle taken from 22 African American (AA) and 40 Caucasian (CA) individuals who were divided equally between the extremes of SI. *ACACB* expression was increased by 1.7-fold in insulin sensitive CA subjects, but was not associated with SI in AA. These results were confirmed in a larger set of 107 CA, where *ACACB* levels remained correlated with SI (r=0.31; p=0.002, adjusted for age and gender), but not in 47 AA. Two local SNPs (rs2075259 & rs2075263) were associated with adipose *ACACB* expression in 62 non-diabetic subjects (40 CA; 22 AA; $p=3x10^{-8}$ and $5x10^{-7}$ respectively) in our prior microarray screening. These two SNPs, together with rs2075260 (V2141I), showed nominal associations with adipose *ACACB* expression by real time qPCR in 107 CA and 47 AA subjects (Stouffer p<0.001, recessive model). *ACACB* SNP rs2300455 was nominally associated with T2D (p= 0.00088) in publicly available data from the DIAGRAM consortium. We tested for association with body mass index (BMI), SI, acute insulin response to glucose (AIRG), and adipose *ACACB* expression for 8 *ACACB* coding SNPs. After adjusting for age and gender, subjects with allele T of synonymous SNP rs7135947 (C/T, Gly728Gly) had trend to lower adipose *ACACB* expression level (p = 0.03 in 47 AAs; p = 0.09 in 106 CAs; Stouffer p=0.01; additive model), and a lower BMI (p=0.0045, dominant model: TT+CT vs. CC; n=158) in AA, but not in CA. AA subjects with the T allele had higher AIRG (p=0.04) after adjusting for age, gender, protocol, BMI, and SI, and this association remained significant in all 497 subjects (AA and CA, combined p= 0.007, dominant model). Adipose *ACACB* expression was negatively correlated with BMI after adjusting for age and gender in CA (p=0.0002, r=-0.35, n=107), but not in AA (p=0.32, n=47). Allelic expression imbalance studies of 6 SNPs supported the existence of cis acting variants, as did total *ACACB* expression in transformed lymphocyte cell lines. Our data supports cis acting variants controlling *ACACB* expression in adipose and transformed lymphocytes, and may suggest ethnic-specific roles for *ACACB* in human glucose homeostasis.

1762/W

Pervasive Epigenomic Regulation in a Mouse Model of Maternal Obesity. *D. Serre[1], H. Miller[1], J. Hester[1], D. Buchner[2], J. Nadeau[2].* 1) Genomic Medicine, Cleveland Clinic, Cleveland, OH; 2) Dept of Genetics, Case Western Reserve University, Cleveland, OH.

Epidemiological studies and animal models have shown that maternal obesity during pregnancy increases the risk for the offspring to develop metabolic diseases in adulthood. However, the molecular bases underlying these life-long consequences remain poorly understood. We have developed a mouse model for studying the influence of maternal obesity and test how early developmental stresses are translated into lasting metabolic dysregulations. Briefly, we assigned C57BL/6J females to low-fat (LF, 10%kcal from coconut oil) diet or a high-fat (HF, 58%kcal from coconut oil) diet for 5 weeks before mating and maintained the pregnant dams on these diets throughout pregnancy and lactation. At weaning, we randomly assigned half of the offspring from LF and HF fed mice to a HF or LF postnatal diet to test if the nutritional conditions during early development could influence the response to an obesogenic environment. At nine weeks of age, we sacrificed the offspring and characterized in 80 animals the genome-wide gene expression profiles of six tissues (brain, liver, pancreas, muscle, adipose tissue and heart). Overall, we observed that postnatal diet influenced the expression of 30-45% of the genes in all tissues but liver where the effect is more pronounced (~60% of the genes were differentially expressed between offspring fed on HF and LF diet after weaning). In contrast, the maternal diet shows very heterogeneous effects across tissues: muscle and adipose tissues show no main effects of the maternal diet while in brain and liver roughly 35% of the genes are differentially expressed according to the maternal diet. In addition, the effect of the maternal diet varies dramatically depending on the postnatal nutritional conditions. To better understand the origin and consequences of these effects of maternal obesity on gene expression regulation, we have complemented the gene expression profiles with a comprehensive characterization of the genome-wide DNA methylation patterns in liver and an extensive quantitative assessment of plasma molecules.

1763/W

Profiling the transcriptome of human brain regions using high-throughput Capped Analysis of Gene Expression (CAGE) sequence analysis. *L. Pardo Cortes[1], P. Rizzu[1], M. Francescatto[1], M. Vitezic[2], H. Takahashi[2], N. Bertin[2], C. Daub[2], P. Carninci[2], P. Heutnik[1].* 1) Clinical Genetics, Section of Medical Genomics.VU medical Center, The Netherlands; 2) RIKEN OMICS SCIENCE CENTER, RIKEN YOKOHAMA INSTITUTE, Yokohama, Japan.

We are analyzing the transcriptome of post-mortem tissue from different aged human brain regions using CAGE to identify Transcription Start Sites (TSS) and their promoter regions. We prepared 25 CAGE libraries from total RNA isolated from 5 brain regions (caudate nucleus, frontal lobe, hippocampus, putamen and temporal lobe) from 5 subjects who died from non-neurological conditions. Mapping, expression normalization and clustering of the tags were carried out using automated pipelines (OSC, RIKEN). More than 14 million CAGE tags were mapped to unique positions in the human genome. We estimated differences in expression between regions using both global and pairwise tests based on uniquely mapped CAGE tags (TSS) present in at least 3 libraries. Over 76% and 47% of TSS originated from known RefSeq transcripts and from the promoter regions (-300 to +100bp)around RefSeq TSS, respectively. Many of the 'intergenic' TSS were represented by high tag counts, which suggests that these are not the result of background transcription. We divided the TSS into 3 groups according to their level of expression: highly expressed (HE; top 25%), moderately expressed (ME, middle 50%) and lowly expressed (LE; bottom 25%). Most (93%) of HE TSS were derived from known genes. Functional annotation analysis showed that HE genes were significantly overrepresented in categories such as metabolic processes, were located more often in mitochondria and were more often transcription factors. Unlike HE TSS, only 70% and 24% of ME and LE TSS, respectively were derived from known genes. We found that 21% of all TSS were differentially expressed (DE) across brain regions (1%FDR). The hippocampus accounted for most differentially expressed TSS and had the largest group of region specific TSS. Examples of these were TSS mapping to the RGL1, SOX5, and ITM2B genes. The most dissimilar regions were the hippocampus and caudate nucleus. DE genes between caudate and hippocampus included calcium channel genes (e.g. CACNA2D1, calmodulins) and transcription factors (e.g. TCF4 and CAMTA1). 19% of DE hippocampal TSS, mapped to intergenic regions. Our results shows that genes involved in metabolic processes are highly expressed throughout all brain regions. 19% of hippocampal TSS may represent novel promoters. ITM2B a gene involved in dementia was one of the most DE genes in hippocampus. This suggests that ITM2B is also an interesting target for expression studies of aging and cognitive decline.

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Gene expression signatures in blood distinguish autism spectrum disorder and brain expression profiles. *C.D. Collins[1, 7], S.W. Kong[2, 6, 7], I.A. Holm[1, 3, 7, 11], H. Liu[2], S.J. Brewster[1, 11], E. Hanson[1, 4, 5], H.K. Harris[1, 11], K.R. Lowe[1, 11], A. Saada[4], A. Mora[4], K. Madison[4], R. Hundley[4, 5], J. Egan[1], J. McCarthy[1], A. Eran[2], K. Vatalaro[1], M. Galdzicki[1], L. Rappaport[4, 7], L.M. Kunkel[1, 3, 7, 8, 9, 11], I.S. Kohane[2, 3, 7, 10, 11].* 1) Division of Genetics, Program in Genomics, Children's Hospital Boston, Boston, Massachusetts 02115, USA; 2) Informatics Program at the Harvard-Massachusetts Institute of Technology Division of Health Sciences and Technology, Children's Hospital Boston, Boston, Massachusetts 02115, USA; 3) Manton Center for Orphan Disease Research, Children's Hospital Boston, Boston, Massachusetts 02115, USA; 4) Division of Developmental Medicine, Children's Hospital Boston, Boston, Massachusetts 02115, USA; 5) Department of Psychiatry, Children's Hospital Boston, Boston, Massachusetts 02115, USA; 6) Department of Cardiology, Children's Hospital Boston, Boston, Massachusetts 02115, USA; 7) Department of Pediatrics, Harvard Medical School, Boston, Massachusetts 02115, USA; 8) Howard Hughes Medical Institute, Harvard Medical School, Boston, Massachusetts 02115, USA; 9) Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115, USA; 10) Center for Biomedical Informatics, Harvard Medical School, Boston, Massachusetts 02115, USA; 11) Autism Consortium, 10 Shattuck Street, Boston, Massachusetts 02115, USA.

BACKGROUND: Autism Spectrum Disorder (ASD) is a pervasive developmental disorder characterized by a triad of defects: impairments in social interaction and communication, and restricted and repetitive behaviour. The genetics underlying ASD is highly complex, with heritability estimates of greater than 90%. The genetic cause for a majority of cases has yet to be identified, but is likely to involve multiple genes. One approach to understanding the molecular changes that occur in ASD is through gene expression profiling. RESULTS: Here we report the results of a profiling study with peripheral blood gene expression data from 258 patients with ASDs and 158 controls enrolled in Boston area hospitals. We developed a 245-gene prediction model that achieves 80% cross-validation accuracy on a sample cohort of 90 male patients with ASDs and 55 male controls (AUC 0.83). More significantly, this model achieves 74% accuracy in an independent population of 168 patients with ASDs and 103 controls (AUC 0.78). Several immune and neuronal signalling pathways are significantly enriched in both populations. This signature also distinguishes post-mortem brain gene expression profiles of 11 patients with ASDs from 11 controls. RT-qPCR measures further validate these findings. CONCLUSIONS: Our findings suggest that changes in peripheral blood gene expression reflect those found in the ASD brain, as well as implicate the processes of neurodevelopment and immune signalling in disease.

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Significant *cis* regulatory effects are detectable for a large proportion of genes expressed in human lymphocytes. *E. Drigalenko[1], J.E. Curran[1], M.P. Johnson[1], M.A. Carless[1], J.W. Kent Jr[1], J. Peralta[2], T.D. Dyer[1], S.A. Cole[1], L. Almasy[1], A.G. Comuzzie[1], M.C. Mahaney[1], E.K. Moses[1], J. Blangero[1], H.H.H. Göring[1].* 1) Dept. of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX; 2) Universidad de Costa Rica, San Pedro, Costa Rica.

Much of the genetic variation underlying common human diseases are thought to be regulatory rather than structural in nature. Fine-scale statistical genetic analyses of genome-wide transcriptional profile data can be used to identify the genetic factors that are significantly associated with quantitative gene expression levels. This information in turn can be used to shed light on which genes and variants likely are the etiologically functional elements within complex disease candidate regions identified by genome-wide linkage and association analysis. By now it has been established that the proximal locus, *i.e.* the genomic region including and surrounding a particular gene, often contains functional genetic variants that determine the quantitative expression level. These variants are thought to act in *cis* and can have strong effects. The power to detect these *cis*-acting variants is much higher than for *trans*-acting variants located elsewhere in the genome because the former only requires examination of the proximal locus, and hence involves a much reduced multiple testing burden compared to genome-wide analysis. We have undertaken an examination of the frequency and strength of putative *cis* regulation in human lymphocytes in the San Antonio Family Heart Study. Using genome-wide transcriptional profiles data (Illumina; 16,681 significantly expressed and highly heritable transcripts) and SNP genotype data (Illumina; 542,944 SNPs after QC) from 1,189 Mexican Americans, and joint linkage and association analysis, we detect statistically significant evidence of *cis* regulation (at the false discovery rate of 0.05) for 43% of transcripts. In many cases the evidence is beyond all reasonable doubt (with p-values << $1.3x10^{-7}$, which corresponds to an empirically determined 0.05 genome-wide significance level). Detection of putative *cis* effects is maximized at a 20 kbp symmetrical window around genes, as compared to smaller or larger window sizes (we evaluated 0-1 Mb intervals), though some putative *cis* variants can be detected at a substantial distance. Overall, *cis* variants appear to be roughly equally likely to be positioned upstream or downstream of genes. ~4% of all SNPs genotyped in the genome show significant *cis* association results with at least one nearby transcript. We conclude that *cis* regulation is likely universal, impacting every gene, and that a database of *cis* variants should help in pinpointing which variants have the potential to be functional.

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Genome-wide search strategies for detecting *trans*-regulatory variants influencing human gene expression levels. *H.H.H. Goring[1], E. Drigalenko[1], J.E. Curran[1], M.P. Johnson[1], M.A. Carless[1], J.W. Kent Jr[1], J. Peralta[2], T.D. Dyer[1], S.A. Cole[1], L. Almasy[1], A.G. Comuzzie[1], M.C. Mahaney[1], E.K. Moses[1], J. Blangero[1].* 1) Southwest Foundation for Biomedical Research, San Antonio, TX; 2) Universidad de Costa Rica, San Pedro, Costa Rica.

While it is now well established that *cis*-regulation plays an important role in influencing quantitative gene transcription levels, much less is known about *trans*-acting factors. The search for such factors is complicated by the fact that it requires genome-wide searching, as there is no *a priori* candidate region for where these factors likely are located. Power is thus reduced due to the enormous multiple testing burden. We have performed a large-scale empirical comparison of three different genome-wide search strategies for detecting trans regulators, based on quantitative gene expression data and SNP genotype data for lymphocyte samples from 1,189 Mexican American participants in the San Antonio Family Heart Study. The three examined strategies are: (1) The customary genome-wide search, based on 542,944 SNPs. As we seek to identify *trans*-acting factors, we excluded the proximal locus (here taken to be 10 Mb around a gene). We used an empirically derived genome-wide 0.05 significance level (pointwise p-value of 1.3x10-7). (2) A genome-wide search using a subset of genotyped SNPs, namely putative *cis*-acting variants. We have identified ~20K SNPs that show significant association with the expression level of at least one gene located nearby. This strategy is based on the idea that such variants tag genomic regions harboring regulatory variants. The reason is that *cis*-acting factors may often have trans-acting effects, namely when the *cis*-regulated gene (such as a transcription factor) regulates the transcription of genes located elsewhere in the genome. (3) A search focused on specific candidate regions. We have generated a gene network based on the genetic correlations of transcript levels. Pairs of significantly genetically correlated genes that are located far from each other presumably include pairs where one gene acts as a trans regulator of the other gene. Hence, for such pairs of transcripts, we have an *a priori* candidate region, which we search using only the putative *cis*-acting SNPs located in the region. This approach also allows us to gain directional information about the relationship of these gene pairs. So far, we have identified significant putative trans associations for ~10% of transcripts, with about one half of those transcripts pointing to more than one trans locus. Our results suggest that smart search strategies can be useful in identifying subtle *trans*-regulatory variation influencing gene expression levels in humans.

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Transcription Altering *cis*-Regulatory SNPs (*cis*-rSNPs) In The Human Genome. *T. Pastinen[1], P. Lundmark[2], D.J. Verlaan[1], V. Adoue[1], B. Ge[1], A. Lundmark[2], U. Liljedahl[2], C. Enström[2], M. Ouimet[3], V. Gagné[3], V. Koka[1], K.L. Gunderson[4], R. Gwilliams[5], K. Rice[5], T. Kwan[1], D. Sinnett[3], P. Deloukas[5], F. Cambien[6], A.H. Goodall[7], W.H. Ouwehand[8], A.-C. Syvanen[2], . Cardiogenics Consortium[9].* 1) Department of Human Genetics, McGill University, Montréal, Canada; 2) Department of Medical Sciences, Uppsala University, Uppsala, Sweden; 3) Hôpital Ste-Justine, Université de Montréal, Montréal, Canada; 4) Illumina Inc., San Diego, California, USA; 5) Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK; 6) INSERM UMRS 937, Pierre and Marie Curie University and Medical School, Paris, France; 7) Department of Cardiovascular Science, University of Leicester, Leicester, UK; 8) Department of Haematology, University of Cambridge and NHS Blood and Transplant, Cambridge, UK; 9) The work of the Cardiogenics consortium is funded through the European Community's Sixth Framework Programme.

Cis-rSNPs can be directly interrogated and mapped in human cells using genome-wide allelic expression (AE) measurements on Illumina 1M BeadChips (NG 2009;41:1216). Expression is assessed for unspliced primary transcript, therefore providing a unique view of transcriptional variation at a population level. Previously, we have shown that in HapMap CEU lymphoblastoid cells (LCLs) up to 35% of transcripts harbor significant *cis*-variation that maps to common SNPs. A subset of these can be assigned at high confidence to full length primary transcripts, which are likely to represent effects on variable transcription rates. Fine-mapping of these variants in LCLs derived from CEU and YRI population using 1000 Genomes data reveals strong overlaps with functional regulatory sites independently described by the ENCODE project. Traditional approaches for validation of cis-rSNP function yields positive results. Extension of population-based AE-mapping to human cells of distinct lineages (purified monocytes and primary fibroblasts) indicates a pervasive influence of *cis*-rSNPs on transcription. Up to 60% of human genes that are expressed in all three cell types show strong evidence of *cis*-rSNPs that alter the expression of full-length primary transcripts in at least one cell type. Cell-type independent cis-rSNPs that alter transcription demonstrate strong predilection to immediate vicinity of transcription start sites (TSSs), whereas *cis*-rSNPs restricted to cell types map more distally from TSS. Furthermore, the effect size of *cis*-rSNPs is correlated with distance from TSS, suggesting that genetic variation in distal elements exert more subtle modulation of transcription. Large collection of *cis*-rSNPs in multiple cell types will enhance our ability to interpret genetic association studies: one fifth (365/1896) of GWAS hits published to date not only overlap significant (FDR 5%) cis-rSNP, but are also in strong LD (r2 above 0.5) with the top cis-rSNP that alter transcription. E.g. *cis*-rSNPs for LDLR and HMGCR that correlate with LDL GWAS hits as well as IL6R *cis*-rSNP for circulating CRP concentration are among these hundreds of functional SNPs. Overall, the mapped *cis*-rSNPs provide a large number of pathophysiological clues for common traits. We will discuss the implications of fine-mapping causal variants and the advantages of comprehensive catalogues of functional SNPs in the human genome across multiple cellular lineages.

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The impact of genetic polymorphism on inter-individual and inter-population differences in the response to glucocorticoids. *F. Luca, J. Maranville, A. Richards, X. Wen, D. Witonsky, M. Stephens, A. Di Rienzo.* Department of Human Genetics, University of Chicago, Chicago, IL.

Glucocorticoids (GCs) are steroid hormones produced by the human body in response to environmental stress. GCs modulate a large number of biological processes including cell survival and inflammation and are widely used as pharmacological agents in the treatment of common diseases such as cancer and asthma. GC action is largely mediated by the interaction with the GC receptor (GR), which activates a transcriptional cascade by regulating gene expression of direct targets. Because GCs modulate the response to environmental stressors, and human populations inhabit a wide variety of environments, we have hypothesized that the transcriptional response to GCs varies across human populations and that this variation is due to genetic variants that diverged in allele frequency possibly because of natural selection. To test this hypothesis, we analyzed the response to GC treatment (8 hours) in EBV-transformed lymphoblast cell lines (LCLs) from two HapMap populations, i.e. Yoruba from Nigeria and Tuscans from Italy (58 samples each). We performed eQTL mapping by applying a novel Bayesian approach. Specifically, we compared five different models under which genetic variation may affect gene expression in the presence/absence of GC treatment. This approach allows us to discriminate between genetic variants that affect gene expression equally (stable eQTLs) or differently (response eQTLs) in the presence and absence of GCs. We were able to identify 445 responsive genes (FDR<0.005) with stable eQTLs and 224 genes with response eQTLs (Bayes factor>100 for eQTLs in both cases). We also identified 307 genes (FDR<0.05) with significant differences in transcriptional response between populations. These differences are substantially reduced when we remove the effect of eQTLs (corresponding to genotype effect and allele frequency), suggesting a genetic basis for between population differences. Our study shows that genetic variation affects the transcriptional response to GCs and may account for some of the differences observed between populations in GC-related phenotypes. Additionally, our Bayesian approach to QTL mapping provides testable hypotheses of the molecular mechanisms underlying the identified QTLs. We are currently in the process of testing these hypotheses by performing genome-wide chromatin immunoprecipitation experiments for the GR and cooperating transcription factors.

1769/W

Transcriptional response signature of immune cells to IFN-β reveals distinct patterns in monocytes. *N. Avidan[1], N. Henig[1], T. Paperna[1], R.Y. Pinter[3], A. Miller[1,2].* 1) Pharmacogenetics and Translational Genetics Center Rappaport Faculty of Medicine & Research Institute, Technion-Israel Institute of Technology, Haifa, Israel; 2) Division of Neuroimmunology & Multiple Sclerosis Center, Carmel Medical Center. Haifa, Israel; 3) Department of Computer Science, Technion-Israel Institute of Technology, Haifa, Israel.

Monocytes, which have been acknowledged as important players in MS pathogenesis, are outnumbered by neutrophils and lymphocytes in peripheral blood mononuclear cells (PBMC). Interferon-b (IFN-β) is a widely used immuno-modulatory drug in MS and much effort has been invested to identify a gene expression signature associated with either good or poor response to the drug. We hypothesized that the monocytes unique expression profile might be obscured by T-cells, which are much more abundant in PBMC. Moreover, we suggest that this distinct response might underlie novel IFN-β functions and pathways. Using cell specific surface markers, CD14+ monocytes and CD3+ T lymphocytes were isolated from 3 healthy blood donors and exposed to IFN-β overnight. The expression profiles of the two cell types in response to IFN-β treatment were evaluated using Illumina HumanWG-6 microarray. Analysis was carried out using JMP Genomics and pathway enrichment was evaluated using GOrilla. Two-way analysis of variance revealed 280 and 47 genes with more than 2 fold change after FDR correction in monocytes and in T cells respectively, from which 38 were common to both cell types. Functional enrichment analysis revealed in T-cells the expected pathways of "immune system process"," response to virus", and "inflammatory response". However, although five times as many genes were differentially expressed in monocytes, less functional pathways were found to be enriched, suggesting that in monocytes IFN-β activates a signaling pathway that is in one way or another different from the established ones. The cell-specific IFN-β response was validated by RT-PCR for eight genes, and further validation on the protein level is in progress. We propose that in MS patients' monocytes expression profile may be better predictors for drug response than PBMC expression profile, and that these cell-specific immuno-modulatory pathways might have been overlooked. Future studies should evaluate the role of monocyte-specific genes, and possibly additional immune cell subsets, in the response profile of MS patients treated with IFN-β.

1770/W

Transcriptional profiling, brain ratio and the search of human genes involved in mental retardation. *P. Chiurazzi[1], F. Pirozzi[1], U. Moscato[2], P.J. van der Spek[3], G. Neri[1].* 1) Institute of Medical Genetics, Catholic University, Rome, Italy; 2) Institute of Hygiene, Catholic University, Rome, Italy; 3) Department of Bioinformatics, Erasmus University Medical Center, Rotterdam, The Netherlands.

Mental retardation (MR) is defined by a defect in intellectual functioning and adaptive behaviour. MR can be caused by genetic defects, environmental factors or a combination thereof. Among the monogenic causes of MR, the best characterized group is that of X-linked Mental Retardation (XLMR). Using available data downloaded from Gene Expression Omnibus (www.ncbi.nlm.nih.gov/geo), we reconstructed the expression profile of 20588 transcripts in 79 human tissues, including 30 samples derived from the central nervous tissue. We also calculated a "brain ratio" for all transcripts, estimating the relative level of expression in the nervous system compared to non-nervous tissues, and ranked them according to such ratio. Then we compared the brain ratio of all transcripts with that of the 90 known XLMR genes and of the approximately 1000 genes encoding for synaptic proteins listed in the Genes to Cognition or G2C catalogue (www.genes2cognition.org). Finally we compared the individual human chromosomes in order to identify which of them are richer in genes with high brain ratio. The analysis of the XLMR genes showed that a strong correlation exists between the brain ratio and the clinical presentation, namely a low brain ratio (indicating an ubiquitous expression in many tissues) predicts a syndromic presentation while a high brain ratio usually associates with a neurologic or "pure MR" clinical picture. G2C genes tend to have very high brain ratios as expected, given that they encode synaptic proteins and many of them have already been found mutated in human neurologic disorders that includes MR. Finally, when individual chromosomes are considered, only 3 chromosomes have more than 10% of genes expressed more than two-fold in brain compared to other tissues, namely chromosome 18 (12.9%), 13 (11.5%) and chromosome X (10.8%). In conclusion, although genes involved in the pathogenesis of MR may have a low brain ratio, it is likely that mutations in genes with a high brain ratio cause MR. Therefore, we suggest that knowledge of the brain ratio of individual genes contained in a given chromosomal region or linkage interval may be useful to prioritize candidates which should be analyzed first if the clinical condition is mainly characterized by MR.

1771/W

Batch effects in microarray experiments: performance evaluation of six algorithms. *C. Chen, D. Zhang, J. Badner, E. Gershon, C. Liu.* Psychiatry, University of Chicago, CHICAGO, IL.

The microarray expression study is currently a popular approach to explore a variety of biological questions. However, the technology per se remains unresolved "batch effects" in the thousands of microarray papers published every year: the systematic error introduced when samples are processed in multiple batches. Although thoughtful experimental design can reduce batch effect but not eliminate it except completing a study in one batch. Therefore, an additional step is helpful to adjust microarray data for batch effect before analysis. A number of programs using a variety of algorithms are now available. Here, we systematically compare six of these programs and demonstrate ComBat outperforms the others in multiple measures of precision and accuracy. We also show that it is essential to standardize replicate data at the probe level, due to the sizeable probe effect in microarray data which can inflate the correlation among replicates.

1772/W

Gene Expression Meta-Analysis in Schizophrenia and Bipolar Disorder. *D. Greco[1], K. Qian[2], A. Di Lieto[2], J. Corander[2], P. Auvinen[2].* 1) University of Helsinki Central Hospital, Helsinki, Finland; 2) University of Helsinki, Finland.

Background: The differential diagnosis of schizophrenia (SZ) and bipolar disorder (BD) is based solely on clinical features and upon a subset of overlapping symptoms. Within the last years, an increasing amount of clinical, epidemiological and genetic data do not support the model that bipolar disorder and schizophrenia would be independent syndromes, challenging the Kraepelinian dichotomy. Methods: We performed meta-analysis of 140 individuals' gene expression microarray data obtained from post mortem prefrontal cortex of bipolar disorder and schizophrenia patients and matched controls from independent experiments. Results: We found 535 and 213 genes specifically altered in bipolar disorder and schizophrenia respectively. Of these, 30 genes were shared between the syndromes. In addition, we identified genes of the transcriptional and post-transcriptional machineries altered in bipolar disorder and genes of the development changed in schizophrenia. Conclusions: Our results confirm that, on the gene expression level, bipolar disorder and schizophrenia can still be considered independent diagnoses according to the clinical model of the Kraepelinian dichotomy.

1773/W

Genome-wide transcriptome analysis of human placenta. *J. Kim[1], L. Lin[2], S. Shen[3], J.C. Murray[1,4], Y. Xing[2,3,5].* 1) Department of Anatomy and Cell Biology, Univ Iowa, Iowa City, IA; 2) Department of Internal Medicine, Univ Iowa, Iowa City, IA; 3) Department of Biostatistics, Univ Iowa, Iowa City, IA; 4) Department of Pediatrics, Univ Iowa, Iowa City, IA; 5) Department of Biomedical Engineering, Univ Iowa, Iowa City, IA.

The placenta is a multifunctional organ with transport, metabolic, endocrine, and immunologic activities that are vital to fetal development and pregnancy maintenance. Malfunction of the placenta can cause fetal growth impairment, prematurity, and other pregnancy complications, and identification of genes important for normal placental function may contribute to a better understanding of mechanisms underlying these adverse pregnancy outcomes. In this study, we characterized the placental transcriptome by conducting gene- and exon-level expression profiling in human placental amnion (N=23), using Affymetrix GeneChip Human Exon Junction Array. We first sought to identify genes distinctively expressed in the placenta by comparing with the gene expression patterns in other normal human tissues (cerebellum, epithelium, and blood). Our analysis revealed 236 differentially expressed genes (> 2-fold, FDR < 1%) that are enriched for nucleic acid and ion binding, transporter activity, and transcription regulation activity. Expression levels of selected genes including *TRIM29*, *CCL4*, and *SFRS14* were validated by quantitative real-time polymerase chain reaction (qRT-PCR). We next sought to identify genes that are involved in the process of normal labor, thus, when altered, could potentially contribute to undesirable obstetric complications. To this end, we subgrouped the placental amnionic samples into those obtained before and after the onset of labor. We found that a total of 42 genes were differentially expressed between the labor (N= 12) and non-labor (N=11) groups with 25 genes upregulated and 17 genes downregulated in the former group (≥ 1.5-fold, FDR < 1%). The differential expression of selected genes including *BMP2*, *GABRP*, and *PLIN2* was confirmed by qRT-PCR by analyzing identical samples used in the microarray experiment. Our study revealed gene-expression patterns unique to normal placenta and transcriptional changes associated with normal labor, which may lead to an improved understanding of placental biology as well as pathobiology. Examination of detailed isoform-level changes and expression patterns in other anatomical parts of the placenta may provide valuable clues to further understanding the pathophysiology of placenta-associated complications.

1774/W

Defects in protein metabolism pathways in a conditional muscle-specific transgenic mouse model over-expressing Pitx1. *S.N. Pandey[1], J. Cabotage[1], R. Shi[1], Y.W. Chen[1,2].* 1) Research Cenetr for Genetic Medicine, Children National Medical Center, Washington , DC 20010; 2) Department of Integrative Systems Biology and Pediatrics, George Washington University, Washington, DC 48109.

We previously showed that paired-like homeodomain transcription factor 1 (PITX1) was specifically up-regulated in patients with facioscapulohumeral muscular dystrophy (FSHD) comparing to 11 other neuromuscular disorders by expression profiling. We hypothesized that the up-regulation of PITX1 in skeletal muscles caused muscle atrophy in FSHD. To test the hypothesis we generated tet-repressible muscle-specific Pitx1 transgenic mice in which over-expression of Pitx1 is induced in skeletal muscles by doxycycline withdrawal. The Pitx1 transgenic mice showed muscle atrophy phenotype after over-expressing Pitx1 for 5 weeks. To study the molecular mechanisms of the muscle wasting, mRNA profiling of muscles collected from mice over-expressing Pitx1 for 3 weeks (n=5) was performed using mouse430_2 Affymatrix microarrays. Expression level of mRNA was calculated using two algorithms (MAS5.0 and PLIER). Using Welch T test in Genespring 7.3.1 with cut-off value of p< 0.01, 3280 genes were significantly differentially expressed between the Pitx1 transgenic mice and the control littermates. Ingenuity pathway analysis (IPA) was used to further categorize genes into functional groups and networks. The IAP results showed that genes involved in amino acid metabolisms and protein synthesis were significantly down-regulated. Interestingly, some ubiquitin-conjugating enzymes that are known up-regulated in muscle atrophy such as NEDD4l and atrogin-1/MAFbx showed changes in the opposite direction. The results suggest that muscle atrophy induced by Pitx1 may not share common mechanisms with other conditions commonly causing muscle atrophy.

1775/W

Enhanced ROC (EROC) for discovery of differentially expressed transcripts in microarray data. *D. Wroblewski, E. Wroblewska*. BioFormatix, Inc., San Diego, CA.

Purpose. To test a new methodology for identification of genomic biomarkers in microarray experiments with small number of replicates.

Methods. We use a robust, non-parametric estimate of noise distribution that is derived through resampling of analyzed data set. At least two replicates are required per condition. The differentially expressed genes (transcripts) are identified with non-parametric ROC (Receiver Operating Characteristic) uni-variate analysis. The approach viability is shown through comparison with three widely used 'benchmark' methods (RankProd, SAM, MAANOVA) and through knowledge-based analysis of results.

We used Mouse Gene Atlas (GNF, San Diego), which contains expression data for 78 normal mouse tissues obtained with Affymetrix mouse array (MOE430), with two experimental repeats per tissue. To produce comparisons for experiments with a larger number of repeats, we generated simulated data. Lists of differentially expressed probes were produced by each of the methods for pair-wise comparisons of tissues. Tissues with diverse levels of similarity were compared.

Summary of Results. EROC is effective in discovering differentially expressed probes in experiments with small number of experimental replicates. Overall, it performs better than the benchmark methods. Several metrics of performance were considered. (1) When the same detection criteria are used, EROC finds similar number of up/down regulated probes, independent of the number of experimental repeats. For other methods, the number of detected probes increases significantly with the number of repeats. (2) EROC probe lists show high consistency for experiments with different number of replicates. RankProd shows somewhat higher consistency that may be related to its ranking being highly correlated with fold change. (3) EROC shows reasonable and consistent correlation between fold change and ranking. For SAM and MAANOVA, the correlation coefficient increases with the number of experimental repeats. (4) EROC shows superior (but comparable to RankProd) ability to detect genes that are known to be expressed in certain tissues.

1776/W

MicroRNA expression profiling in the human cartilage growth plate. *Y. Xue[1], V. Funari[1,2].* 1) Genomics Core, GCRC Department, Cedars-Sinai Medical Center, Los Angeles, CA, 90048; 2) Medical Genetics Research Institute, Cedars-Sinai Medical Center, Los Angeles, CA, 90048.

INTRODUCTION The endochondral growth plate is a well-organized developmental structure with a spatial distribution of chondrocytes in different proliferation and differentiation stages. MicroRNAs (miRNAs), noncoding RNAs of 18-25 nucleotides, are generally believed to regulate gene expression at the post-transcriptional level by inducing degradation of target mRNAs that involve cell proliferation, differentiation and apoptosis. Identifying miRNAs expressed in growth plates and their prospective targets will enable further characterization of miRNAs role in endochondral ossification and skeletal dysplasias. METHODS AND RESULTS Total RNA isolated from four human cartilage samples were subdivided, then pooled into two subgroups. Commercially available total RNA from 16 tissues were subdivided into two pools of eight assorted tissues. RNAs from the two pooled cartilage samples and the two pooled non-cartilage samples were labeled and hybridized to Affymetrix GeneChip miRNA array. Cartilage miRNA data were compared to non-cartilage miRNA data to identify differentially expressed miRNAs. Forty-three miRNAs were found significantly altered in cartilage with 17 up-regulated and 25 down-regulated. MiRNA 140, specifically expressed in cartilage, was found up-regulated nine fold in cartilage tissue. A cartilage gene enriched list of 2500 genes derived from a previous microarray analysis was used to predict the mRNA targets. Target genes of three miRNAs (miRNAs 433, 140, 424) with the most significant changes and number of targets were chosen for Gene Ontology (GO) analysis and found significantly correlated with changes in intracellular organelle, transcription regulation and developmental processes, respectively. CONCLUSION The first miRNA profile for human cartilage tissue was identified. The finding of differentially expressed cartilage miRNAs and their targets will help enable the study of gene regulation in the development of growth plate.

1777/W

Using transcription modules to identify pathways perturbed in Williams-Beuren syndrome. *C.N. Hor[1], G. Csardi[2,3], M.-T. Zabot[4], C. Fusco[5], S. Bergmann[2,3], G. Merla[5], A. Reymond[1].* 1) Centre for Integrative Genomics, University of Lausanne, Lausanne, Switzerland; 2) Department of Medical Genetics, University of Lausanne, Lausanne, Switzerland; 3) Swiss Institute of Bioinformatics, Lausanne, Switzerland; 4) Centre de Biotechnologie Cellulaire, Hospices Civils de Lyon, groupement hospitalier Est, BRON, France; 5) Laboratory of Medical Genetics, IRCCS "Casa Sollievo della Sofferenza", San Giovanni Rotondo, Italy.

The global dysregulations caused by the Williams-Beuren Syndrome (WBS) deletion have been only partially characterised. We profiled the transcriptomes of skin fibroblast cell lines from WBS patients and compared them to matched controls. We identified 366 differentially expressed genes that were significantly enriched in extracellular matrix genes, major histocompatibility complex (MHC) genes, as well as genes the products of which localise to the actin cytoskeleton, microsome and vesicular fractions. We then used public expression datasets from human fibroblasts and the Iterative Signature Algorithm (ISA) to establish "transcription modules", i.e. subsets of genes that exhibit a coherent expression profile over a subset of microarray experiments. The majority of these modules are highly enriched in genes with common functional annotation, but they have the added advantage of including genes that have no or fragmented annotation. Hence, this modular approach increases the power to identify pathways dysregulated in WBS patients, thus providing additional candidates for genes and their interactions modulating the WBS phenotypes. Dysregulated modules are often interconnected and share multiple common genes, suggesting that intricate regulatory networks connected by a handful of central factors are disturbed in WBS. Our results emphasize the role of the extracellular space in the pathophysiology of WBS and provide novel clues about other affected processes. We identified, for example, variations in the expression of GABA receptor components that could have consequences at the cellular and synaptic level and common features with other syndromes, such as dysregulation of the Di George Syndrome critical region 2 gene (DGCR2).

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Conditional expression of TGFβ1 in skeletal muscles causes endomysial fibrosis and myofiber atrophy. *J.H. Narola[1], S.N. Pandey[1], R.L. Marathi[1], A. Glick[3], Y.W. Chen[1,2].* 1) Department of Genetic Medicine, Children's National Medical Center, Washington, DC; 2) Department of Integrative Systems Biology and Department of Pediatrics, George Washington University, Washington DC; 3) Department of Veterinary and Biomedical Sciences, Pennsylvania State University, PA.

To study the effects of TGF-β1 over-expression by skeletal muscles, we generated a tet-repressible conditional muscle-specific TGF-β1 transgenic mouse model (mCK-tTA/TRE-TGF-β1). The TGF-β1 transgene was induced when the mice were 4 weeks old. Among the 24 TGF-β1 mice examined, 50% of them showed muscle weakness and reduced body weight within 2 weeks after TGF-β1 gene induction, 30% showed the phenotype after 5-10 weeks and 20% showed no phenotype during our observation period (19 weeks). We defined the mice that showed phenotype within two weeks of TGF-β1 overexpression as early phenotype (EP) and the rest as the late phenotype (LP). H&E staining of skeletal muscle sections showed that the myofibers of TGF-β1 transgenic mice were significantly smaller in both the EP ($p<0.001$) and LP ($p<0.01$) groups comparing to the controls. In addition, the myofibers of the mice in the EP group were significantly smaller than those in the LP group ($p<0.001$). Pico sirius red staining showed that the endomysial collagen accumulation was significantly higher in the TGF-β1 mice in both EP (9.3±1.6%, $p<0.005$) and LP groups (3.2±0.15%, $p<0.001$) comparing to controls (1±0.3%). The TGF-β1 level in blood sera and in muscle was examined using enzyme-linked immunosorbant assay (ELISA). In the sera, active TGF-β1 and total TGF-β1 (active and latent) level was significantly higher in the EP group comparing to control, while there was no significant difference between the LP group and the controls. In the muscle, active TGF-β1 ($p<0.01$) and total TGF-β1 level ($p<0.005$) in the EP was higher than the control group but no significant difference between the LP group and the control. The studies showed that over-expression of TGF-β1 causes muscle wasting and endomysial fibrosis with individual variability, which suggests involvement of additional regulatory mechanisms that affect the responses to the TGF-β1 expression in the transgenic mice.

1779/W

DUX4, an FSHD candidate gene, induces pathways involved in DNA damage and hypoxia. *V. Sharma[1,2], R. Shi[1], J. Narola[1], N. Harafuji[1], Y. Chen[1,3].* 1) Center for Genetic Medicine, Children's National Medical Center, Washington, DC 20010; 2) Institute for Biomedical Sciences, George Washington University, Washington, DC 20037; 3) Department of Integrative Systems Biology and Department of Pediatrics, George Washington University, Washington, DC 48109.

Facioscapulohumeral muscular dystrophy (FSHD) is linked to the deletion of the D4Z4 arrays at chromosome 4q35 subtelomeric region. Each D4Z4 array contains a double homeobox 4 (DUX4) open reading frame. We previously showed that the DUX4 functioned as a transcription factor and regulated expression of paired-like homeodomain transcription factor 1 (PITX1) which was identified specifically upregulated in FSHD by expression profiling. In this study, we identified additional downstream targets and regulatory pathways of DUX4 by expression profiling C2C12 myoblasts transfected with expression vectors containing DUX4. Expression vectors containing homologues of DUX4, namely DUX4c and DUX1, and insertless vectors were used as controls. The expression profiling study was conducted using the Affymetrix 430_2 microarrays. The data was analyzed using Affymetrix MAS 5.0 followed by Weltch t test using Genespring GX 7.3.1. We identified 869 genes to be differentially expressed in the cells expressing the DUX4 gene compared to the insertless control ($p < 0.05$). Among them, 756 genes were specific to DUX4. The 5 top ranked canonical pathways identified using the Ingenuity Pathway Analysis were activation of IRF by cytosolic pattern recognition receptors, pyrimidine metabolism, ATM signaling, mitotic roles of polo-like kinase, and alanine, and aspartate metabolism. The 5 top ranked biological functions were cell cycle, RNA post-transcriptional modification, DNA replication, recombination and repair, cardiovascular system development and function, and infection mechanism. The data suggest that DUX4 is likely responsible for induction of DNA damage which in turn triggers a DNA repair response through multiple canonical pathways such as the IRF signaling pathway, ATM signaling pathway as well as the mitotic signaling pathway of polo-like kinase. The ATM signaling and polo-like kinase pathways are involved in cell-cycle checkpoints. Additionally, the DUX4 gene could also be involved in ischemic conditions as evidenced by upregulation of the alanine/aspartate metabolism signaling and IRF signaling pathway, which are frequently found to be misregulated in hypoxic conditions.

1780/W

Simultaneous gene expression profiling of protein-coding and long non-coding RNA. *A. Bergstrom Lucas[1], M. Guttman[2], P. Tsang[3], G. Lin[1], J. Donaghey[2], J. Rinn[2], L. Bruhn[3].* 1) Genomics R&D, Agilent Technologies, Santa Clara, CA; 2) Broad Institute of MIT and Harvard, Cambridge, MA; 3) Agilent Labs, Agilent Technologies, Santa Clara, CA.

Recently thousands of large intergenic non-coding RNA (lincRNA) transcripts were identified in human and mouse cells from genome-wide chromatin-state maps. Through the comparison of lincRNA expression profiles to known protein-coding gene pathways, lincRNAs have been implicated in diverse biological processes such as stem cell pluripotency, innate immunity, and cell cycle regulation and many lincRNAs have been shown to play a role in these pathways through regulation of gene expression. To enable systematic profiling of all lincRNAs and protein-coding genes, we have developed both human and mouse microarrays comprised of all known protein-coding mRNAs and lincRNAs thus allowing for simultaneous detection from a single sample. In validating these new array designs, RNA samples from a variety of mouse tissues were labeled and applied to the new mouse arrays to detect differences in coding and non-coding gene expression profiles. Using this approach, we are able to identify novel differentially expressed lincRNAs. Here we show examples of how profiling mRNA and lincRNA from the same sample can allow researchers to further define the role of lincRNAs in gene regulation. Together, these results highlight the power of simultaneously profiling lincRNAs and protein-coding genes on the same array.

1781/W

Gene regulatory network modeling using L1 regularized graphical models. *X. Zhang[1,2], J. Listgarten[1], C. Kadie[1], W. Wang[2], D. Heckerman[1].* 1) Microsoft Research; 2) Univeristy of North Carolina at Chapel Hill.

Understanding the mechanisms of gene transcriptional regulation through analysis of high-throughput gene expression data is a central problem in computational systems biology. Although extensive research has been done to estimate gene regulatory networks, two challenges need to be better addressed. The first challenge is the high-dimension, low-sample-size problem, wherein the number of dimensions (genes) is much larger than the number of samples. The second challenge is how to model our (partial) knowledge about transcription factors and other confounding factors. We present a sparse graphical model to address these two challenges. Our model incorporates known transcription factors and introduces hidden variables to represent unknown transcription and confounding factors. The expression level of a gene is thus modeled as a linear combination of the expression levels of known transcription factors and hidden factors. To address the high-dimension, low-sample-size problem, we add an L1 regularization penalty to the log likelihood function to impose sparseness on the learned model. Using large gene expression data measured with 39,296 oligonucleotide probes from 1109 human liver samples, we demonstrate that our model better predicts out of-sample-data than a model with no hidden variables. We also show that some of the gene sets associated with hidden variables are strongly correlated with Gene Ontology categories and could therefore correspond to newly identified regulators.

1782/W

The use of a water-soluble, inert chemical matrix to stabilize RNA in the liquid state at room temperature. *H. Martinez, D. Wong, C. Shi, B. Dalby, R. Nunez, M. Saghbini.* GenVault Corporation, Carlsbad, CA.

The purpose of this study was to examine the ability of an inert, water-soluble chemical matrix composed of antioxidents and RNase inhibitors to preserve the integrity of purified RNA stored in the liquid state at room temperature for up to one month. High quality RNA purified from rat liver, human white blood cells or HeLa cells was examined. RNA samples were applied to 1.5ml tubes containing a chemical matrix, known as GenTegra RNA, mixed to solubilize the chemicals with the RNA, and air-dried. Following dry state storage for up to two weeks, samples were rehydrated in molecular-grade water and stored at room temperature (25°C) or refrigerated (4°C) for two to four weeks. Controls stored frozen at -80°C, or in liquid state at 25°C and 4°C in the absence of the chemical matrix were also examined. RNA integrity was assessed using the RNA 6000 Nano Chip on the Agilent 2100 Bioanalyzer and by gel electrophoresis. Rat liver, human white blood cell or HeLa RNA stored in the dry state for up to two weeks in the presence of a chemical matrix, rehydrated, and stored at 25°C or 4°C for up to four weeks was indistinguishable from RNA stored frozen at -80°C. Conversely, RNA stored in the absence of the chemical matrix at 25°C or 4°C exhibited significant degradation during the same time period. RNA stored in the chemical matrix has been used successfully for gene expression analysis on the Affymetrix GeneChip Human Genome U133 Plus 2.0 Array and the Illumina Human HT-12 Expression BeadChips. In these studies, a greater than 96% correlation was observed between samples stored in the presence of the chemical matrix and controls stored at -80°C. Here, we have shown that a water-soluble, inert chemical matrix has the ability to stabilize purified RNA from various sources for up to one month when stored in the liquid state at room temperature or refrigerated. Storing RNA in the liquid state saves time and eliminates the potentially damaging effects of freeze-thaw cycles for RNA samples that will be used in multiple experiments. RNA stored in this manner can be successfully analyzed on the leading gene expression platforms.

1783/W

A Comparison of Next Generation Sequencing Technologies for Quantifying Transcriptional Response and Host-Agent Interaction. *A. Sivakumar[1, 2], J. Proescher[1], D. Drewry III[1], S. Kinahan[1], F. Ahmed[1], L. McNew[3], A. Chakravarti[2], E. Van Gieson[1].* 1) Johns Hopkins University Applied Physics Laboratory, Laurel, MD; 2) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 3) Edgewood Chemical and Biological Center, Aberdeen Proving Ground, MD.

Greater understanding of the host-agent interaction at the molecular level in the spatial and temporal context of exposure is needed to help identify better and earlier markers of infection, guide regulations for exposure limits, and identify targets for therapeutics. Next generation sequencing (NGS) technologies are poised to revolutionize transcriptional profiling, yet experimental workflow and data analysis processes are still evolving. In this study, a lymphoblastoid cell line from the fully sequenced HapMap sample, NA18507, were exposed to two different doses of bleomycin (10 μg/mL and 20 μg/mL) in order to quantify differential transcriptional response compared to the control treatment. The bleomycin/lymphoblastoid challenge represents a well-characterized system that offers an opportunity to compare sequencing-based expression analysis to other modalities of expression analysis previously performed with this challenge system. Our efforts compared the Roche/454 and Illumina/GAII NGS platforms for transcriptional profiling. Currently available computational tools and statistical packages were evaluated to identify capabilities and limitations of these processes to characterize mechanisms of agent interaction. The Roche/454 protocol resulted on the order of 1 x 10^5 reads per treatment with an average length of 200 bp. In contrast, the Illumina/GAII protocol resulted on the order of 1 x 10^7 reads per treatment with a length of 100 bp. The unique length and depth characteristics of each NGS platform were analyzed to investigate unmapped reads and provide insight into novel splice junction discovery. Both platforms agreed with validation performed using RT-PCR and Q-PCR for known bleomycin-associated genes (SIAH2, CDKN1A, DDB2, and FDXR). In addition, both platforms were shown to be in concordance with each other for known housekeeping genes. The primary metric to quantify gene expression was Reads per Kilobase of Exon Length per Million Mapped Reads (RPKM; Mortazavi et. al, 2008) and its advantages and disadvantages, as compared to alternative statistics are examined. Finally, the potential of NGS technologies to uncover novel transcripts expressed through such treatments is also discussed.

1784/W

Identification of DNA-associated Proteins by Sequence-Specific Capture and Mass Spectrometry. *H. Guillen Ahlers[1], S. Mirza[1], S. Zhang[1], M. Zickus[1], R. Cole[1], M. Zelembaba[1], M. Chesnik[1], C-H. Wu[2], S. Chen[2], Y. Yuan[2], G. Kreitinger[2], M. Scalf[2], M.R. Shortreed[2], L.A. Cirillo[1], L.M. Smith[2], M. Olivier[1].* 1) Wisconsin Center of Excellence in Genomics Science, Medical College of Wisconsin, Milwaukee, WI; 2) Wisconsin Center of Excellence in Genomics Science, University of Wisconsin, Madison, WI.

Recent advances in genomics and proteomics have brought us closer to reaching a detailed and comprehensive understanding of our genome and how it is regulated. Numerous proteins mediate DNA stability, control its activity, and regulate transcription of the genetic information. However, currently no technologies exist that allow the dissection of these protein-DNA interactions in a comprehensive global manner, and examine alterations in disease. To overcome this challenge, we report on the development of an entirely novel technology. Unlike ChIP-chip methodology, where DNA sequences that interact with individual known proteins are characterized, the Wisconsin Center for Excellence in Genomics Science (CEGS) utilizes an oligonucleotide capture technology to isolate targets of interest in a sequence-specific manner in order to analyze protein complexes attached to these regions. In an initial study, the mouse insulin-like growth factor-binding protein 1 (IGFBP1) promoter region was used as an in vitro model system. Specific capture oligonucleotides were designed and attached to gold surfaces using linker chemistry and amino-terminated oligonucleotides. Hybridization was optimized to sequester PCR-products containing an exposed single-stranded overhang. After on-chip protease digestion, FoxO1 binding to the DNA sequence was detected by tandem mass spectrometry using an LTQ XL mass spectrometer. Due to its high binding affinity, the reaction was carried out without a cross-linking step between FoxO1 and the DNA. Our analysis demonstrates efficient capture of FoxO1-DNA complexes in a sequence-specific manner. Capture technology and mass spectrometry allowed the detection of 1 pMol of captured FoxO1 protein using a PCR product with a FoxO1 binding site while an alternative PCR product with a mutated binding site did not lead to the detection of FoxO1. The on-chip digestion and sample preparation can be performed on small array surfaces (<5 mm2). Additional efforts are under way to allow the selective capture of restriction fragments directly from genomic DNA, rather than specific PCR products. This work was funded by the Wisconsin Center for Excellence in Genomics Science through NIH/NHGRI grant 1P50HG004952.

1785/W

Regulatory network construction from co-regulatory QTLs. *K.S. Kompass, J.S. Witte.* Epidemiology & Biostatistics, UCSF, San Francisco, CA.

The gene regulatory networks that control human cellular health and disease remain poorly understood. With inexpensive, high-throughput methods for profiling gene expression and genotype, transcriptional regulation in humans can be dissected by examining naturally occurring genetic variation. We previously developed a multistep method to identify trans loci governing gene co-regulation (Kompass and Witte, submitted) where significant associations between regulatory loci and gene clusters are called co-regulatory QTLs (crQTLs). We extend this work to model crQTLs in gene regulatory networks, building on recently developed Bayesian methods for eQTLs that incorporate genetic and other existing information to improve prediction (Zhu et al., Cytogenet Genome Res 2004). We apply this approach to an endometrial cancer dataset, where tumors were expression-profiled and samples genotyped with commercial arrays (Salvesen et al., PNAS 2009) using the R package 'deal' (Boettcher and Dethlefsen, JSS 2003). Regulatory priors were estimated from the data for each locus by comparing the distributions of crQTL association p-values to the overall background distribution. From this analysis, the gene PTPRD was predicted to be a key regulatory node interacting with GRIP1, HS3ST3A1, INPP4B, SLITRK3, PPARGC1A, PTBP2, and CCNG1. PTPRD is a tyrosine phosphatase with key roles as a tumor suppressor in multiple cancers (Veeriah et al., PNAS 2009), and these results suggest that PTPRD acts coordinately through various downstream genes to exert its effects in multiple pathways. Because several of these predicted interactors also have putative roles in cancer, future studies should examine the interactions between these genes as they shape the development of neoplasia. In summary, by extending crQTL results with a Bayesian network, we were able to distinguish putative regulatory networks for disease.

1786/T

Comparison of copy number variations using array-based SNP genotyping and massive parallel sequencing data. *A. Koike, K. Kiumura.* Cent Res Lab, Hitachi, Ltd, Kokubunji, Japan.

Array-based technologies and massive parallel sequencing technologies enabled us to detect copy number variations (CNVs) on a genome-wide scale. Especially, with the increased use of high density SNP arrays for genome wide association studies, CNV analysis based on these SNP arrays is becoming widely applied to explore disease-associated CNVs. However, due to the noise of SNP array data, the accurate CNV detections are quite thorny issues and several CNV detection methods have been developed. In this study, we calculated CNVs of one Yoruba individual and one trio family using publicly available affymetrix 6.0 SNP array data and massive parallel sequencing data and compared them to clarify their characteristics. In sequencing analysis, reads were first mapped on the reference genome with allowing edit distance 2 and considering multiple mapping sites. The depth of read coverage in each 200bp window was then calculated. Finally, continuous low coverage regions and high coverage regions longer than 1kbp were detected with correcting GC content bias and multiple mapping read bias. The influence of multiple mapping reads was considered by simulation data and their mapped data. For the array analysis, Circular Binary Segmentation, Gaussian process with adaptive criteria, and Hidden markov model were used. When compared with these results, about 80% of duplications and 93% of deletions of commonly detected CNVs by three array analysis methods were also detected by sequencing data. The Hidden Markov model showed the highest overlapping ratio of CNVs with sequencing results in all analysis methods and the ratio was about 50% in duplications and about 90% in deletions. The overlapping ratio was expected to be related to the re-producible CNV ratio in each array analysis (consistencies of CNV detection of different arrays using the same sample) and the consistency ratio of trio data (the ratio of CNVs in children which are observed in their mothers or fathers). Acknowledgement: This work was partly supported by the contract research fund "Integrated Database Project" from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

1787/T

Genotyping of Copy Number Variations Using Model-based Clustering. *N. Kumasaka[1], H. Fujisawa[2], N. Hosono[1], A. Takahashi[1], M. Kubo[1], N. Kamatani[1].* 1) Ctr Genomic Med, RIKEN, Tokyo, Japan; 2) The Institute of Statistical Mathematics, Tokyo, Japan.

Copy number variations (CNVs) have been investigated as biological markers to identify the genes relevant to common diseases and adverse effects. A typical statistical method to identify CNVs is the hidden Markov model, but it often fails via commercial genome-wide SNP genotyping platforms because of the lack of SNP marker density. We propose a model-based clustering method using a bivariate Gaussian mixture model with the Kullback-Leibler divergence penalty, which provides fast and reliable genotyping of CNVs. Using the fluorescence intensity data from the Illumina 610k platform, 3,378 CNVs with deletions were genotyped for a sample of 25,857 subjects. Around 300 subjects were randomly selected within the sample to validate CNV calls of 13 probes using Taqman Real-Time PCR Assay. The concordance of calls between the two independent assays, Illumina and Taqman, undertaken on these subjects was 99.71% (95% CI: 99.46 - 99.84%).

1788/T

Array CGH analysis of copy number variation in 200 Asian individuals. *D. Lee, Y. Ju, S. Lee, J. Kim, J. Seo.* GMI, Seoul national university, Seoul, Seoul, Korea.

Copy number variants (CNVs) accounts for the majority of human genomic diversity in terms of base coverage. But we still can't understand most part of it. In previous studies, we found over 3000 putative Asian specific CNVs. Further more, we developed CGH Array Reference-free Algorithm (CARA), to infer reference-free personal CNVs across aCGH platforms, and designed customized array CGH chip especially for calling Asian specific CNVs. Using these methods, we analyzed 200 individuals, 45 Chinese, 45 Japanese, 45 Mongolian, 45 Korean, 20 yoruban, and 20 European, to validate the previous results and find novel CNVs. We could see the difference of allele frequency among the populations. We expect the result of this study will help the following studies and especially for studying Asian, the database we made can be the foundation stone to start. More over, this study will help understand a lot of veiled aspect of CNVs.

1789/T

Mobile element insertion polymorphisms from the 1000 Genomes Project. *C. Stewart[1], D. Kural[1], M.P. Stromberg[1], W.P. Lee[1], J.A. Walker[2], M. Konkel[2], A.M. Stutz[3], J.O. Korbel[3], M. Batzer[2], G. Marth[1], 1000 Genomes Project.* 1) Dept Biol, Boston College, Chestnut Hill, MA; 2) Dept Biol, Sciences, Louisiana State University, Baton Rouge, LA; 3) Genome Biology Unit, EMBL, Heidelberg, Germany.

Previous studies suggest that thousands of mobile element insertion (MEI) polymorphisms exist between any two unrelated individuals. These can have significant phenotypic consequences via interrupting exons, altering promoters, or altering splicing. Only by cataloging the specific insertion sites and measuring allele frequencies within populations will we be able to understand the functional consequences of these polymorphisms and their importance for personal genomics. MEI's can be observed from next-generation sequencing data (NGS) either directly as insertions in a given sample, or indirectly as deletions in the sample (corresponding to insertions in the reference genome). The direct detection of insertions presents the greater computational challenge. To address this challenge, we developed two different computational approaches, one Read-Pair (RP) and the other a Split-Read (SR) sequence based detection method. We applied these methods to the 1000 Genomes Project pilot data in both the high coverage family trio samples and the 150 low coverage unrelated samples. In the entire dataset, we identified a total of 5364 insertions, of which 4496 contain Alu elements, 789 L1s, and 79 SVA elements. The false discovery rate in this set was lower than 5% for either detection method, with a combined detection sensitivity exceedinög 80% in the high coverage family trio samples, and for events with 10% allele frequency or higher in the low coverage dataset. The number of direct insertions is comparable to the 4251 MEI deletions detected in the same samples. This study represents the largest set of MEI loci found to-date. We estimated the total number of MEI polymorphisms between two unrelated high coverage individuals (one CEU and one YRI sample both from HapMap) to be 3300+/-300 based on both direct and indirect detection. We measured the Allele Frequency Spectra (AFS) on the full pilot data, and find a higher fraction of low frequency alleles in YRI samples than the CEU or CHB/JPT samples. We also carried out Principle Component Analysis (PCA) which revealed population specific patterns of insertion loci equivalent to PCA analyses in the same populations using SNP data. This approach is generally applicable to any whole genome NGS dataset, including those collected for association studies to assess the contribution of MEI events to explain disease phenotypes.

1790/T

Whole-Genome Sequencing Analysis of Structural Variation and Genomic Stability in Human Induced Pluripotent Stem Cells (iPSC). *A. Urban[1], Y. Zhang[2], A. Kocabas[3], B. Bi[3], A. Abyzov[4], J. Mu[4], A. Szekely[2], D. Palejev[3], M. Gerstein[4], S. Weissman[2], F. Vaccarino[3].* 1) Psychiatry, Stanford University, Stanford, CA; 2) Genetics, Yale University, New Haven, CT; 3) Child Study, Yale University, New Haven, CT; 4) MBB, Yale University, New Haven, CT.

There are frequent reports about genomic instability in embryonic stem cells (ESC) in culture. Even at low to medium resolution of analysis, ESC are found to be prone to accumulation of Copy-Number and other Structural Variation (CNV/SV) events in their genomic sequence. Here we investigate the genomic stability of another promising tool in the arsenal of stem cell research, that of iPSC. Fibroblast cells are obtained by biopsy from human donors and then de-programmed into a stem cell-like pluripotent state. At each stage of the process we extract genomic DNA and generate medium-coverage (i.e. at least 4x-8x genomic sequence coverage) paired-end whole-genome sequencing data. We are using the Illumina HiSeq 2000 instrument to sequence paired-end constructs with an insert-length of 700 bp, generating 2x100 nt of sequence per construct and a minimum of 60 million, but up to 120 million, mapped paired-end reads per stage. We use this data to carry out paired-end mapping [following approaches described in Korbel, Urban, Affourtit et al., Science 2007; Korbel et al., Genome Biol. 2009], split-read analysis and read-depth analysis and can thus determine in a comprehensive fashion the catalog of sequence variants from point mutations and small InDels to large and very large (i.e. thousands to hundreds of thousands or millions of bp) CNV and SV, including deletions, duplications and insertions as well as copy-number neutral events such as inversions and balanced translocations. We discuss our findings regarding the occurrence of such sequence variation events in the genomes of iPS cells relative to the genomes of the corresponding primary tissue from the human donor.

1791/T

Genomic differences in discordant couples of sisters with MECP2 mutation: classic Rett versus Zappella variant. *F. Ariani[1], R. Artuso[1], FT. Papa[1], MA. Mencarelli[1], A. Rosseto[1], M. Mucciolo[1], V. Disciglio[1], E. Grillo[1], I. Meloni[1], M. Pollazzon[1], F. Mari[1], M. Zappella[2], J. Hayek[2], DH. Yasui[3], JM. LaSalle[3], A. Renieri[1].* 1) Dept Molecular Biol, Univ Siena, Siena, Italy; 2) Child Neuropsychiatry, Univ Siena, Italy; 3) Medical Microbiology and Immunology, Rowe Program in Human Genetics, School of Medicine, Univ of California, Davis, CA.

Previous studies suggest that MECP2 mutation type and XCI are not sufficient to explain the wide phenotypic variability observed in Rett syndrome ranging from severely affected girls (classic RTT) to girls recovering motor and language function (Zappella variant RTT). We previously suggested a digenic model in which the phenotype is strongly dependent on the status of a second gene while the MECP2 mutation alone leads to X-linked recessive mental retardation (Renieri et al J Mol Med 2003). We present here array-CGH analysis (Agilent 105K) with 15kb resolution in two couples of Rett sisters with discordant phenotype (one classic RTT and one Zappella variant RTT), balanced XCI, and the same MECP2 mutation (http://www.biobank.unisi.it). We identified 7 CNVs and 9 CNVs in the two couples, respectively. One of them located on 8p11.23, extending 158 kb and containing two genes is shared by both couples and the variation is in the same direction: the classic RTT girls have a double copy. Expression and functional analysis of these genes is ongoing. It is also possible that the key player gene(s) is(are) located in a region not shared by the two couples and that the couple with normal copy number has functional modulation by SNP variation. For this reason we analyzed the gene content of the remaining regions for containing MeCP2 target genes (Yasui et al Proc Natl Acad Sci U S A. 2007) or for being previously reported to be involved in phenotype modulation. Interesting MeCP2 target genes were found to be located in 16p11.2, NFATC2IP and SPIN1 genes, in 1p36.3, CROCC, in 5p15.33, ZDHHC11, and in 15q11.2, HERC2P3. The variation of this last region was previously reported associated in patients with MECP2 mutation (Longo et al Eur J Hum Genet 2004) and the variation is in the same direction: the classic RTT girls have half a copy. Expression analysis on the above reported genes indicated that for only half of them the CNV at genomic level corresponds to mRNA variation, narrowing the groups of the key players.

1792/T

A Comparison of Genotyping Technologies to Clinically Assess SULT1A1 Copy Number Variation. *A.B. Freeman[1], M. Arefayene[1], J.A. Baker[1], Y. Guo-Avrutin[1], B. Staton[1], S. Hebbring[2], R. Weinshilboum[2], K. Hillgren[1].* 1) Eli Lilly and Company, Indianapolis, IN; 2) Mayo Clinic, Rochester, MN.

Human cytosolic sulfotransferase 1A1 (SULT1A1, Ch16) catalyzes the conjugation of several endogenous substrates and xenobiotics (Hildebrandt et al., 2004). Large variations in the levels and activity of human SULT1A1 have been attributed to inherited genetic variability, including copy number variations or CNVs (Price et al., 1989). SULT1A1 variability has potential clinical implications in individuals who conjugate therapeutic drugs and hormones via this enzyme.

Previous internally-generated data indicated that quantitative PCR (qPCR) was the most reliable technology to clinically assess CNVs. More recently, Hebbring et al. reported using fragment analysis to assess CNVs (2007). Therefore, we assessed the SULT1A1 CNVs in 145 liver DNAs using three genotyping methods; qPCR, fragment analysis, and quantitative SNP genotyping (qSNP) for three variants (rs9282861, rs750155, and rs3760091).

Overall, SULT1A1 CNV genotype calls from the three methodologies produced an 89.0% concordance rate with average frequencies of 5.2%, 61.8%, 27.6%, 6.2%, and 0.7% for 1, 2, 3, 4, and >4 SULT1A1 copies, respectively. Since >80% of the liver samples were obtained from Caucasians, the SULT1A1 CNV distribution observed in the cohort was consistent with previous reports in the Caucasian population (Hebbring et al., 2007). Liver samples containing 1, 2, 3, and 4 SULT1A1 copies contributed 2.1%, 54.1%, 34.2%, and 9.6% of the 333 total SNP alleles, respectively. In addition, the "C" alleles of rs9282861, rs750155, and rs3760091 had frequencies of 70.9%, 53.0%, and 39.9%, respectively.

Comparing the three technologies, 96.5% concordance of SULT1A1 genotype calls was observed between fragment analysis and qSNP genotyping in the liver samples. Quantitative PCR was the most discordant technology with only 89.0% and 92.4% concordance with fragment analysis and qSNP, respectively. Contrarily, the qSNP methodology was highly accurate, but was unable to genotype heterozygotes independently. Although additional studies are required, these results suggest that fragment analysis is the most accurate and independent methodology of the three we tested to assess SULT1A1 copy number.

1793/T

Evaluation of a novel, single molecule, amplification-free method for the detection of Copy Number Variation (CNV). *T. Guettouche[1], Y. Cardentey[1], M.A. Pericak-Vance[2], J.R. Gilbert[2], D.J. Hedges[2].* 1) Oncogenomics Core Facility, Sylvester Comprehensive Cancer Center, University of Miami Miller School of Medicine, Miami, FL; 2) John P. Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL.

Copy number Variation (CNV) is a major component of the genomic diversity present within the human population. Several methods for genome-wide discovery of structural variants now exist , including array-based genomic hybridization and High throughput SNP genotyping arrays (i.e. GWAS arrays). More recently, structural variation has also been inferred from Next Generation Sequencing data . As CNV calls made from each of these approaches can be subject to appreciable false positive rates, detected variants of interest are generally corroborated by an independent laboratory method. The method of choice for CNV confirmation has primarily been real-time quantitative PCR. Here we report the evalution of a novel copy number assay based on the Nanostring nCounter system. The nCounter system uses a hybridization-based single molecule capture approach that does not require amplification of input DNA. Individual tripartite complexes of the target molecule, a biotin labeled probe and a fluorescently labeled ("barcoded") probe are directly counted. We compared the performance of the nCounter system at 30 putative CNV loci in a set of 96 human samples, all of which have data at these same loci from ABI Taqman CNV assays. A subset of these samples also have data available from Agilent aCGH 244k custom arrays and Illumina 1M Infinium arrays. Preliminary results show that the Nanostring method exibits 86% agreement with the ABI Taqman CNV assays across all studied loci and individuals. When three problematic repetitive loci are excluded, concordance rises to 96%. We are presently continuing our evaluation process to include a total set of 300 individuals. Our initial results suggest that the nCounter system will provide a promising alternative for the validation of copy number variants.

1794/T

Genome-wide association study of CNVs in Korean right isomerism patients. *E. Seo[1,2], J. Lee[1], K. Kim[1], I. Park[1,3].* 1) Genome Research Center for Birth defects and Genetic disorders, Asan Med Center, Seoul, Korea; 2) Dept. of Laboratory Medicine, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea; 3) Dept. of Pediatrics, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea.

Right isomerism is characterizaed by asplenia, mirror-image symmetrical right lungs and complex cardiac anomalies with bilateral, morphologically identical right atrial appendages. Total incidence of heterotaxy syndrome including right isomerism is very low, but some reports suggested that right isomerism is more prevalent in oriental children. Although mutations have been identified in genes, NODAL, CFC1, ACVR2B, LEFTYA within the nodal signal transduction pathway of the left-right asymmetry, mutation prevalence of each genes is approximately 2 to 10%. Previous study has proposed a gene-dosage-sensitive genetic interactions between nodal signals in asymmetrical patterning. We performed copy number variations (CNVs)-association studies to identify dosage-sensitive genes susceptible to right isomerism. In order to explore the presence of CNVs, we designed a custom CGH 60K microarrays (Agilent) to cover 1562 genes involved in heart development or heart defects as well as other 5000 genes located in important chromosomal loci. Array CGH was performed on 39 Korean right isomerism patients and 100 Korean healthy individuals using NA 10851 DNA as a reference, and Agilent Genomic Workbench 6.0 was used to analyze microarray data and CNV-association. The CNVs detected in this study were validated through real-time quantitative PCR using TaqMan probes for specific regions. We found four statistically significant CNV loci in three genes, IGKC, COX6A2, and FOXC2. The 40-kb loss of proximal region of IGKC was detected in 26 patients, but not in 100 controls. Inversely, the 60-kb gain of distal region of IGKC was much frequently found in controls. The third was 357-bp gain on 16p12 detected in 16 patients and 18 controls. This region contains only one gene, COX6A2 involved in the heart/muscle isoform of cytochrome c oxidase subunit. The 1-Kb gain on 16q24 in 8 patients and 9 controls, FOXC2 (forkhead box C2, mesenchvme forkhead 1), as a key regulator of adipocyte metabolism.

1795/T

Genomewide high-resolution aCGH analysis of a patient with mild dysmorphism and behavioral problems. *R.R. Haraksingh[1, 2], A.E. Urban[3], A. Szekely[4], S. Weissman[4], M.P. Snyder[2].* 1) MCDB, Yale Univ, New Haven, CT; 2) Genetics, Stanford School of Medicine, Stanford, CA; 3) Psychiatry, Stanford School of Medicine, Stanford, CA; 4) Genetics, Yale School of Medicine, New Haven, CT.

Copy Number Variantion (CNV) represents a major source of human genetic diversity that may be inherited or occur de novo in individuals. These events occur by both recombination and replication based mechanisms and act as substrates for further genomic rearrangements and genome evolution. The CNVs reported in the human genome are catalogued in the Database of Genomic Variants (DGV, http://projects.tcag.ca/variation/) and comprise both benign and pathogenic variants. Several well-documented, pathogenic deletion/duplication events are routinely tested for in cytogenetic laboratories. However, novel, clinically relevant CNV haplotypes are not revealed in an unbiased way by these methods. Here we report the high resolution CNV profile of a family trio with unaffected parents and affected son. The patient is a middle aged adult male with mild but distinct dysmorphic features and a distinct constellation of behavioral problems including prominent anxiety and phobias along with more subtle autistic features. The trio was initially analyzed by Chromosome Microarray Comparative Genome Hybridization (CM-CGH) on an Agilent 180K array that tests for more than 140 microdeletion/duplication syndromes. The proband was found to possess a maternally inherited 93kb duplication on chromosome 15q21.2. This duplication has not been documented in the DGV and affects two genes; the entire TMOD3 gene and the last exon of the TMOD2 gene. Tropomodulins have been implicated in learning and memory function and in animal models of autism. Further high-resolution array CGH using a NimbleGen 2.1million whole genome tiling array (Roche-NimbleGen) revealed several other CNVs of unknown significance that have not been reported in the DGV. These include a 39kb deletion in the proband on 5q22.1 in a highly conserved intron of the TMEM232 gene. It is possible that a subset of the CNV complement of the proband including novel and private events working in concert may account for part of the phenotype by a multiple-hit mechanism.

1796/T

Identification and characterization of copy number changes: case study of familiar hemoglobinopathy. *L. Noskova[1], H. Hartmannova[1], V. Stranecky[1], L. Veprekova[2,3], S. Kmoch[1,4].* 1) Institute of Inherited Metabolic Disorders, First Faculty of Medicine, Charles University in Prague and General Teaching Hospital, Ke Karlovu 2, Prague 2, Czech Republic; 2) Pediatric Clinic of the General Teaching Hospital, Ke Karlovu 2, Prague 2, Czech Republic; 3) Trombotic centre CHL VFN, Karlovo náměstí 32, Prague 2, Czech Republic; 4) Center for Applied Genomics, Prague, Czech Republic.

Copy number variations (CNVs) are together with single nucleotide polymorphisms (SNPs) the main source of genetic variability in men. With regard to the extent of CNVs, they may posses significant phenotypic impact and are linked to group of pathological conditions and clinical syndromes. Using methods of DNA arrays, quantitative PCR, standard PCR and sequencing, we have identified and characterized large deletion in family with autosomal dominant familiar hemoglobinopathy. Hemoglobinopathies are very heterogenic group of disorders caused by the presence of abnormal structural variants of globin proteins or by underproduction of normal globin genes due to defects in α- or β- globin chain syntheses (thalassemias). We have studied family diagnosed by hemoglobinopathy with following clinical symptoms: preterm birth of severe anemic child with marked microcytosis and hepatopathy of various intensity, mild to severe anemia and marked microcytosis after several months of blood transfusions. We have performed mapping analysis using Affymetrix GeneChip Human Mapping 250K array and found heterozygous deletion 765 kb in length at chromosome 11: 5, 088, 500 - 5, 853, 400. This region contains β - globin cluster involving genes of β - globin chain (globin β, γ1, γ2, ε). To refine break in deleted region, we have designed real-time PCR probes using Roche Universal Probe Library. Narowing of the interval allowed sequencing of delimited region. Finally, fast and simple assay for detection of deletion in other family members is set up. This study was supported by the grant funded by the Ministry of Education of the Czech Republic (MSM0021620806).

1797/T

Unprecedented resolution for the detection of inherited and de novo structural variation by mate-pair sequencing of a family trio. *W. Kloosterman[1], V. Guryev[2], M. van Roosmalen[1], R. Hochstenbach[1], K. Duran[1], M. Poot[1], E. Cuppen[1, 2].* 1) Department of Medical Genetics, University Medical Center Utrecht, Universiteitsweg 100, 3584 CG, The Netherlands; 2) Hubrecht Institute and University Medical Center Utrecht, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands.

Structural genomic variation may contribute to phenotypic diversity and disease. While copy number changes have well been studied, the nature and prevalence of for instance copy-neutral genomic rearrangements and indels is poorly understood. Here we performed genome-wide sequencing of a long mate-pair library with 2.5 kb insert size of a child with severe psychomotor retardation and complete absence of expressive speech. The same methodology was used for sequencing of both parents to discriminate between inherited and acquired structural genomic variants. We obtained more than 10x average clone coverage throughout the genome. We detected hundreds of balanced and unbalanced structural rearrangements of which several occurred de novo in the child. Sanger sequencing identified breakpoints of all de novo structural variations at base-pair resolution. We fine-mapped the breakpoints of the de novo t(1;10;4)(p23.4;q21;q23) translocation in the child that was previously identified by karyotyping. The other de novo events were insertions, deletions and inversions in the vicinity of the breakpoints on chromosome 10 and 4. One of these de novo events disrupts the PCDH15 gene and may be involved in part of the phenotype(s) of the child. Most of the de novo events were accompanied by microdeletions, micro-homology or micro-insertions at the breakpoints. Our results provide an insight into the generation rate of de novo structural events in the human genome and emphasize the need for high-resolution methods to determine genetic aberrations in individuals with congenital anomalies.

1798/T

Increased Sensitivity of Copy Number Variation Detection Using a High-Density Array CGH Platform. *R. Selzer, C. Shaw, K. O'Moore, T. Richmond, J. Geoghegan, N. Jiang, R. James, J. Luckey.* R&D, Roche NimbleGen, Inc, Madison, WI.

Array CGH methods are widely used to investigate DNA copy number variation (CNV) associated with complex disorders. Disease-association studies have become increasingly focused on CNVs, and recent reports show links between CNVs and schizophrenia (Stefansson 2008; Stone 2008), autism (Sebat 2007; Marshall 2008; Glessner 2009), and cancer. More recently, the focus of CNV research has migrated to detection of rare variants, with an allelic frequency of less than 5% (Conrad 2009, Manolio 2009). In an effort to increase detection of rare variants as well as more common CNVs, we sought to develop the highest density oligo array available for CNV detection, as well as a more sensitive algorithm for CNV detection on NimbleGen arrays. The NimbleGen 2.1M and 3x720K CNV arrays contain empirically optimized probes, with the most comprehensive collection of targeted regions available, including Asian population-specific CNV regions. Utilization of the CNV arrays enabled detection of several hundred CNVs per individual. When compared to whole-genome tiling arrays, 2- to 3- fold more CNVs were detected with the CNV arrays. The NimbleGen CNV arrays detected 300% and 245% more CNVs, respectively, and the higher probe density allowed 2-fold higher detection of CNVs shorter than 1 kb when compared to competitor arrays. The increased sensitivity of CNV detection was aided by an improved segmentation algorithm (segMNT), included in NimbleScan v2.6 software. A comparison between the segMNT v1.1 and segMNT v1.2 algorithms shows increased sensitivity in detection of CNVs resulting in about 2-fold more CNVs detected per sample with segMNT v1.2.

1799/T

Intrachromosomal mitotic non-allelic homologous recombination is the major molecular mechanism underlying type-2 *NF1* deletions. *H. Kehrer-Sawatzki[1], A.C. Roehl[1], T. Mussotter[1], J. Vogt[1], A. Zickler[1], J. Högel[1], N.A. Chuzhanova[2], K. Wimmer[3], L. Kluwe[4], V.F. Mautner[4], D.N. Cooper[5].* 1) Insititue of Human Genetics, University of Ulm, Ulm, Germany; 2) School of Science and Technology, Nottingham Trent University, UK; 3) Division of Human Genetics, Medical University, Innsbruck, Austria; 4) Department of Maxillofacial Surgery, University Medical Centre, Hamburg-Eppendorf, Germany; 5) Institute of Medical Genetics, School of Medicine, Cardiff University, UK.

Non-allelic homologous recombination (NAHR) is responsible for the recurrent rearrangements that give rise to genomic disorders. Although meiotic NAHR has been investigated in multiple contexts, much less is known about mitotic NAHR despite its importance for tumorigenesis. Since type-2 *NF1* microdeletions frequently result from mitotic NAHR, they represent a good model in which to investigate the features of mitotic NAHR. We have used microsatellite analysis and SNP arrays to distinguish between the various alternative recombinational possibilities, thereby ascertaining that 17 of 18 type-2 *NF1* deletions, with breakpoints in the SUZ12 gene and its highly homologous pseudogene, originated via intrachromosomal recombination. This high rate of intrachromosomal NAHR causing somatic type-2 *NF1* deletions contrasts with the interchromosomal origin of germline type-1 *NF1* microdeletions, whose breakpoints are located within the NF1-REPs (low-copy repeats located adjacent to the SUZ12 sequences). Further, meiotic NAHR causing type-1 *NF1* deletions occurs within recombination hotspots characterized by high GC-content and DNA duplex stability, whereas the type-2 breakpoints associated with the mitotic NAHR events investigated here do not cluster within hotspots and are located within regions of significantly lower GC-content and DNA stability. Our findings therefore point to fundamental mechanistic differences between the determinants of mitotic and meiotic NAHR.

1800/T

Copy number variations unmask recessive mutations and identify novel candidates for Bardet-Biedl syndrome. *K.J. Meyer[1,2], D.Y. Nishimura[3,4], J.S. Beck[3,4], L.K. Davis[5], E.M. Stone[2,4,6], T.H. Wassink[1,2], V.C. Sheffield[2,3,4].* 1) Department of Psychiatry, University of Iowa, Iowa City, IA; 2) Interdisciplinary Genetics Program, University of Iowa, Iowa City, IA; 3) Department of Pediatrics, University of Iowa, Iowa City, IA; 4) Howard Hughes Medical Institute, University of Iowa, Iowa City, IA; 5) Department of Psychiatry, University of Illinois, Chicago, IL; 6) Ophthalmology and Visual Sciences, University of Iowa, Iowa City, IA.

Bardet-Biedl syndrome (BBS) is a heterogeneous autosomal recessive disorder characterized by obesity, retinopathy, renal malformations, hypogonadism, and mental retardation. Although a rare disorder, BBS serves as a model to study these and other phenotypes that are individually common in the general population. Mutations in 12 different genes, *BBS1-12*, account for approximately 70% of BBS; causative mutations have yet to be discovered in the remainder of cases. In recent years genome-wide studies of copy number variants (CNVs) have been successful in indentifying rare chromosomal anomalies that confer risk to develop genetically complex disease. In this study, we apply copy number detection technology to a simple Mendelian disorder in an effort to identify novel disease genes and to define a role for CNVs in BBS. Thirty-two BBS patients from non-consanguineous families with unknown mutation were hybridized to the Affymetrix Genome-Wide Human SNP Array 5.0 and analyzed for copy number variants. From the data set, 11 CNVs that were novel to the Database of Genomic Variants and absent from our control set of 500 individuals were classified as high interest candidate regions for BBS. We identified one patient hemizygous for *BBS10* and detected a frame shift mutation in the remaining copy of BBS10 by direct sequencing. In addition, we identified a deletion of one exon of *MARK3*, a promising candidate gene, which is predicted to cause a frame shift and premature stop of the protein. Thus our analysis of this dataset supports the interrogation of CNVs in individuals with Mendelian disorders who harbor an unknown mutation.

1801/T

Genic and Nongenic Human DNA is biased against Nullizygosity. *F. Alkuraya[1,2,3], H. Khalak[1], F. Imtiaz[1], S. Wakil[1], L. Abu Safieh[1], M. Aldahmesh[1], M. Al-Dosari[1], D. Monies[1], N. Kaya[1], M. Al-Hamed[1], F. Alzahrani[1], L. Al-Jbali[1], K. Ramzan[1], N. Al-Tassan[1], H. Shamseldin[1], R. Shaheen[1], M. Al-Rashed[1], B. Baz[1], S. Hagos[1], N. Abu Dhaim[1], M. Hashem[1], B. Meyer[1], A. Alazami[1].* 1) Dept Gen, KFSH & RC, Riyadh, Saudi Arabia; 2) Dept Ped, KKUH & KSU, Riyadh, Saudi Arabia; 3) Dept Anat & Cell Bio, Alfaisal University, Riyadh, Saudi Arabia.

Copy number variants (CNVs) represent an important source of human genome diversity. Whether “benign” hemizygous CNVs are also tolerated in the nullizygous state is largely unknown. This question is difficult to address systematically because the relatively low frequency of individual CNVs means that a prohibitive number of normal individuals have to be screened for detection of the CNV in question in a nullizygous state. We have reasoned that an alternative approach is to study “normal” individuals whose parents are first cousins since their genomes are likely to be enriched for nullizygous CNVs and thus lend themselves to a rapid cataloging of nullizygous CNVs i.e. dispensable DNA in humans. We have studied 451 such individuals and found a highly significant bias against the occurrence of nullizygous changes. This has led us to hypothesize that the low frequency of nullizygous CNVs in public databases cannot be solely explained on the basis of low population frequency of individual CNVs and that a biological factor is likely to exist. To confirm this, we analyzed an independent cohort of 18 multiplex consanguineous families for which the parental genotypes are also available. By directly examining the shared hemizygous CNVs among the first cousin parents in each family we again found a highly significant deviation from the expected 25:50:25 Mendelian distribution of the resulting genotypes with the nullizygous genotype being grossly underrepresented. Importantly, this bias is not restricted to genic CNVs and is not accounted for by a high rate of miscarriages. Therefore, our data suggests a greater importance for “junk” DNA than previously thought in very early human development.

1802/T

Familial idiopathic clubfoot is associated with recurrent microduplications of chromosome 17q23.1q23.2 containing TBX2/TBX4. *D. Alvarado[1], H. Aferol[1], K. McCall[1], J. Huang[3], M. Techy[3], J. Buchan[4], P. Gonzales[4], M. Dobbs[1,5], C. Gurnett[1,2,3].* 1) Orthopaedic Surgery, Washington Univ, St. Louis, MO; 2) Pediatrics, Washington Univ, St. Louis, MO; 3) Neurology, Washington Univ, St. Louis, MO; 4) Division of Biology and Biomedical Sciences, St. Louis, MO; 5) St. Louis Shriners Hospital for Children, St. Louis, MO.

Idiopathic clubfoot is a common musculoskeletal birth defect for which few causative genes have been identified. To identify the genes responsible for idiopathic clubfoot, we screened for genomic copy number variants (CNVs) with the Affymetrix Genome-wide Human SNP Array 6.0. A recurrent chromosome 17q23.1q23.2 microduplication was identified in 3 of 66 probands with familial isolated idiopathic clubfoot. The chromosome 17q23.1q23.2 microduplication segregated with autosomal dominant clubfoot with reduced penetrance in all three families. Short stature was common and one female had isolated developmental hip dysplasia. Skeletal abnormalities consisted of broad and shortened metatarsals and calcanei, small distal tibial epiphyses, and thickened ischia. Several skeletal features were opposite to those recently described in the reciprocal chromosome 17q23.1q23.2 microdeletion syndrome associated with developmental delay, cardiac and limb abnormalities. The 17q23.1q23.2 region contains the T-box transcription factor TBX4, a likely target of the bicoid-homeodomain transcription factor PITX1 previously implicated in clubfoot etiology. This chromosome 17q23.1q23.2 microduplication is the most common cause of idiopathic clubfoot identified to date, and provides strong evidence linking clubfoot etiology to abnormal early limb development.

1803/T

Germline and somatic CNVs in 4 Mexican American monozygotic twin pairs. *A. Blackburn[1], H.H.H. Göring[2], S. Kumar[2], M. Carless[2], J. Curran[2], R. Duggirala[2], J. Blangero[2], D. Lehman[3].* 1) Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, TX; 2) Southwest Foundation for Biomedical Research, Department of Genetics, San Antonio, TX; 3) Department of Medicine, Division of Clinical Epidemiology, University of Texas Health Science Center, San Antonio, TX.

Copy number variants (CNVs) occur commonly in the human genome, and account for more variation on a per nucleotide basis than single nucleotide polymorphisms (SNPs). There are conflicting reports regarding the contribution of CNVs to high levels of somatic mosaicism. SNP arrays are now commonly used to identify CNVs, however the results may vary by array and by statistical methodology. In this study we address three issues related to copy number variation by comparing 4 MZ twin pairs of Mexican American descent from the San Antonio Family Heart Study (SAFHS) and Family Diabetes/Gallbladder Study (SAFDGS). All four twin-pairs and four available parents were genotyped on the Illumina 1M Duo Beadchip. Individual twins were also genotyped on the 1M, 550, and 510 Beadchip platforms from Illumina. First we assessed the ability of the PennCNV package, and its supplementary tools, to identify germline CNVs by observing concordance between MZ twins on the same platform. Second, we assessed the concordance of germline CNV calls from PennCNV between platforms. Third, we assessed the extent of somatic mosaicism by calculating the absolute value of the difference between b-allele ratios for heterozygous SNPs on chromosome 6 between MZ twins and between duplicate samples. Chromosome 6 was chosen for its intermediate size to ease computation for initial investigation. We identified an average of 53 CNVs across the genome for samples genotyped on the 1M Duo using PennCNV. Genomic wave adjustment improved the concordance between MZ twins. ~50% of CNV calls had concordant breakpoints, suggesting that a minimum of 50% of CNV calls made by PennCNV on the 1MDuo platform have correct breakpoint identification. Overall, ~69% of CNVs inferred using PennCNV from the 1M Duo were observed in both MZ twins. We observed that the same CNVs can be detected across platforms, and that discordant breakpoints can be due to platform coverage differences. In our preliminary exploration of somatic mosaicism on chromosome 6, similar percentages of markers fell 1, 2, 3, and 4 standard deviations from the mean absolute difference between b-allele ratios for both MZ twins and duplicates. This suggests that the high level of somatic mosaicism previously implied may be within the normal variation for the assay. Further, out of 39 CNV calls on chromosome 6, all 7 discordant calls between MZ twins contained 0 heterozygous SNPs. We are extending the investigation to the entire genome.

1804/T

Detection of clinically relevant exonic copy-number changes by array CGH. *P.M. Boone[1], C.A. Bacino[1,2,3,4], C. Shaw[1,2], P.A. Eng[2], P.M. Hixson[2], A.N. Pursley[2], S-H.L. Kang[1,2], Y. Yang[2], J. Wiszniewska[2], B.A. Nowakowska[2], D. del Gaudio[2], Z. Xia[1], L.L. Immken[5], J.B. Gibson[5], A.C-H. Tsai[6], T.E. Reimschisel[7], L. Potocki[1,4], T. Gambin[1,8], M. Sykulski[1,9], M. Bartnik[1,10], K. Derwinska[1,10], B. Wisniowiecka-Kowalnik[1,10], S.R. Lalani[1,2,3,4], F. Probst[1,4], W. Bi[1,2], A.L. Beaudet[1,2,3], A. Patel[1,2], J.R. Lupski[1,2,3,4], S.W. Cheung[1,2], P. Stankiewicz[1,2,10].* 1) Dept. of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Medical Genetics Laboratories, Baylor College of Medicine, Houston, TX; 3) Dept. of Pediatrics, Baylor College of Medicine, Houston, TX; 4) Texas Children's Hospital, Houston, TX; 5) Clinical Genetics, 'Specially for Children, Austin, TX; 6) Dept. of Pediatrics, The Children's Hospital, University of Colorado School of Medicine, Aurora, CO; 7) Dept. of Pediatrics, Vanderbilt University School of Medicine, Nashville, TN; 8) Institute of Computer Science, Warsaw University of Technology, Warsaw, Poland; 9) Institute of Informatics, Warsaw University, Warsaw, Poland; 10) Dept. of Medical Genetics, Institute of Mother and Child, Warsaw, Poland.

Array comparative genomic hybridization (aCGH) is a powerful tool for the molecular elucidation and diagnosis of disorders resulting from genomic copy-number variation (CNV). However, intragenic deletions or duplications - those including genomic intervals of a size smaller than a gene - have remained beyond the detection limit of most clinical aCGH analyses. Increasing array probe number improves genomic resolution, although higher cost may limit implementation and enhanced detection of benign CNV can confound clinical interpretation. We designed an array with exonic coverage of selected disease and candidate genes and applied it clinically to identify losses or gains throughout the genome involving at least one exon and as small as several hundred base pairs in size. In some patients, the detected copy-number change occurs within a gene known to be causative of the observed clinical phenotype, demonstrating the ability of this array to detect clinically relevant CNVs with sub-kilobase resolution. Examples include: *MECP2* and Rett syndrome; *PTEN* and Bannayan-Riley-Ruvalcaba syndrome; *CREBBP* and *EP300* and Rubinstein-Taybi syndrome; *CDKL5* and X-linked infantile spasms; *ZDHHC9* and X-linked mental retardation; *FAM58A* and STAR syndrome; *HPRT1* and Lesch-Nyhan syndrome; *SCN2A*/*SCN3A* and infantile seizures; *STXBP1* and infantile seizures; *NRXN1* and developmental delay; *TCF4* and Pitt-Hopkins syndrome; *IL1RAPL1* and X-linked mental retardation; *EYA1* and branchiootic syndrome; and *JAG1* and Alagille syndrome. These conditions represent a variety of disease processes, including neurodevelopmental disorders, an enzyme deficiency syndrome, and other recognizable patterns of human malformation. Furthermore, many of these exonic CNVs have not been previously reported for these known disease genes. Perhaps of even greater interest, this approach may also enable elucidation of gene function for some of the large number of annotated genes in the human genome whose functions remain unknown. Thus, our approach allows new mutations to be described for known genetic conditions and also could potentially elucidate gene function. In summary, we demonstrate the utility of a custom-designed, exon-targeted oligonucleotide array to detect intragenic copy-number changes in patients with unexplained syndromic and non-syndromic mental retardation, congenital anomalies, and other clinically relevant phenotypes.

1805/T

Characterizing neutral drift in copy number variations of duplicating regions: model and test for non-neutral drift. *A. Caspi.* University of Pennsylvania. Philadelphia, PA.

With the recent survey of common copy number variants (CNVs) in several human populations [WTCC, 2010]*, it is now possible to characterize neutral drift in copy number variation across the human genome. We propose a model of neutral copy variation on duplicating genomic regions which assumes an underlying Markov process guides random drift in copy numbers. We learn the model parameters using a set of 1000 duplicating CNV regions selected from CNV's previously shown to be reliably genotyped by both Agilent CGH microarrays and Illumina Infinium genotyping platform. We removed any CNV's that could arise via deletions, to avoid confounding the model of selective pressure on deletions with those on duplications. This set was reported as impoverished in CNVs associated with diseases or complex traits, and the majority of genes overlapping these regions were annotated as functioning in the periphery of cellular networks. We use this set as a surrogate for randomly drifting CNVs. Using the learned model, we develop a method based on inter- and intra-population comparisons to test for duplicating CNV regions deviating from drift characteristics. Our test takes as input the allelic distribution of a CNV region for each of several subpopulations studied. In the present study, we observe frequencies for three HapMap populations: individuals of European Ancestry (CEU), in Yoruba (YRI), and in Tokyo and Beijing (CHP+JHT). We performed the test on the full set of over 5000 reliably genotyped CNV's assayed in 450 HapMap samples from the above populations. The test aims to identify regions for which observed copy number frequencies depart from those expected by the assumed background model of drift. We provide a ranking of CNV regions ordered in ascending likelihood that they exhibit non-neutral pressure. The analysis reveals strong candidate CNV regions, of which the top 16 contain the full set of three CNV's previously identified by [WTCC, 2010] as potentially disease associated regions. Given the reported paucity for disease-associated variants in this set, both the low number of candidate regions and the inclusion within this set of all regions identified in the original study are a testament to a successful application of the test. We propose this test as a solution for detecting CNV duplications under selective pressure in reliable genotyping data for forthcoming non-common copy number variant assays. *['common' is defined as minor allele frequency >5%].

1806/T

Evaluation of copy number variation in an African American type 2 diabetes and diabetic nephropathy Genome-Wide Association Study. *J.N. Cooke, N.D. Palmer, L. Lu, J.W. Chou, C.W. McDonough, B.I. Freedman, C.D. Langefeld, D.W. Bowden.* Wake Forest University, Winston-Salem, NC.

Type 2 diabetes (T2D) and T2D-associated end-stage renal disease (T2D-ESRD) disproportionately affect African Americans. We have assessed the contribution of copy number variation (CNV) to T2D or T2D-ESRD in African Americans. Using the Affymetrix 6.0 array, we performed a genome-wide association study (GWAS) on 965 African Americans with T2D-ESRD and 1,029 healthy African Americans recruited in the southeastern United States, interrogating 946K copy number probes across the genome. CNV data was analyzed using Genotyping Console 4.0 with version 30 library and annotation files. CNVs were categorized by deletion (CNV<2) and insertion (CNV>2) status and then used as predictors in a logistic regression model with age, gender, and Principal Component 1 adjustment for admixture to evaluate the odd ratios (OR) between cases and controls. A regional analysis of 1130 known CNVs identified association with an insertion on chromosome 1 in the pancreatic amylase precursor gene (AMY2B/2A) captured by CNP76 (p=6.20x10-5, OR=1.63 (CI 1.28-2.08)). This CNV has a deletion frequency of 43.2% in cases and 39.4% in controls. This is a previously identified common insertion; however, there have been no prior reports of association with disease. We also performed a secondary analysis by probe to detect de novo CNVs and novel, common CNVs in African Americans. This analysis identified a deletion on chromosome 14 in the gene encoding the alpha subunit of the T-cell antigen receptor (TCRA). The p-value for the nine probes spanning chr14:21959617-21967020 was 4.03x10-5, OR=0.07 (CI 0.02-0.24). The frequency of the deletion was approximately 0.3% in cases and 2.6% in controls, thus representing an uncommon variant not captured by common/known copy number polymorphism analysis. Adjacent probes located 186kb upstream and 32kb downstream of this region also support evidence of association (p=4.03x10-5-9.43x10-3). Other top hits from the by-probe analysis included a deletion on chromosome 1 in the gene encoding glutathione S-transferase μ2 (GSTM2; p=1.80x10-4-2.10x10-4, OR=2.04, CI=1.40-2.97 for three probes spanning chr1:110027431-110028023). The frequency of this deletion was 12.3% in cases and 7.8% in controls. We have observed both common and novel CNVs that appear to contribute to T2D and/or T2D-ESRD. These results suggest that CNVs contribute to susceptibility for T2D and/or T2D-ESRD in the African American population.

1807/T

Identification of gene copy number variations using quantitative real-time PCR for whole genome amplified DNA. *C.L. Dagnall[1,2], A. McCary[1,2], A.A. Hutchinson[1,2].* 1) Core Genotyping Facility, SAIC-Frederick, Inc., NCI-Frederick, Frederick, Maryland, USA; 2) Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, DHHS, Bethesda, Maryland, USA.

Copy number variations (CNVs) are structural variations defined as a DNA segment of at least 1 kb in size, for which copy number differences have been observed in the comparison of two or more genomes. CNVs have been shown to be present in many cancers and have also been used to predict metastatic capabilities of some cancers. They have also been associated with other immune diseases, neurological disorders, and drug metabolism. Our laboratory currently uses the Applied BiosystemsTM TaqMan® Copy Number assays to accurately detect CNVs using gDNA as template material. This is a duplex real-time qPCR assay which compares the amplification of the target CNV to the amplification of a reference gene, known to have two copies in all human subjects, to determine the copy number of the target CNV relative to that of the reference gene. The target CNVs of interest to be assessed in this project are the GSTT1 (glutathione S-trasnferase theta 1) gene and GSTM1 (glutathione S-transferase M1) gene, and the reference gene used is RNase P. Since gDNA samples can be quickly exhausted using various analytical platforms, there is a need for pre-analysis processing to increase the quantity of the samples. Whole genome amplification (WGA) is a method that synthesizes duplicate copies of the template human genome and is capable of generating many micrograms of high molecular weight DNA from just nanograms of input material. Previous PCR-based methods of WGA have been generally abandoned due to problems created during amplification, including limited fragment length, allelic dropout, and amplification bias due to preferential binding of primers to certain DNA regions. The current WGA protocol of GE Healthcare's illustraTM GenomiPhi V2 and GenomiPhi HY kits are based on multiple displacement amplification (MDA), a non-PCR-based process using the Phi29 DNA polymerase which boasts excellent processivity and high fidelity. Here we assess the performance of WGA products on validated copy number assays to determine if wgaDNA can provide accurate quantitation suitable for determination of relevant copy number changes in the GSTM1 and GSTT1 genes. Included is a comparison of results and raw data values of gDNA, wgaDNA, and pooled wgaDNA samples from the same individuals for the two genes of interest. The results of samples from variable biologic sources (blood, buccal, and fibroblast) are also examined. Funded by NCI Contract No. HHSN261200800001E.

1808/T

Nervous System Genes Are Enriched In Regions Affected By Dosage Changes in Hirschsprung Disease (HSCR). *B. Doan, S. Arnold, Intl. HSCR Genetics Consortium, A. Chakravarti.* Institute Genetic Medicine, Johns Hopkins Medicine, Baltimore, MD.

Patients with chromosomal mutations led to the identification of the HSCR susceptibility genes *RET*, *EDNRB*, and *ZFHX1B*. *RET* mutations are likely necessary to cause HSCR with mutations found in ~87% of patients. We conducted a GWAS on 220 trios using 500K SNPs (Affymetrix) to search for additional genes, and identified a second common risk locus, *SEMA3*. We also used these data, together with published gene expression and structural variation (SV) databases, to characterize the burden of dosage changes on HSCR risk using an integrative genome wide approach. Normalization and segmentation of log2 ratios (L2R) on raw allelic intensities were performed using ITALICS to generate gene dosage events (GDE); genotypes were called using CRLMM. GDE were filtered based on >2 probes and >0.25 absolute value L2R, and genotypes were cleaned using >0.95 confidence scores. Samples with >50 events were excluded since these arose from nosier than average array intensities. Inheritance (segregating/de novo) status, and Mendel errors (ME) were calculated for each event. In 218 offspring, 1333 events were identified: 102 >1Mb, 973 1kb-1Mb, and 259 <1kb: deletions were twice as frequent, 927 vs 406. For regions with >10 ME, 24 events >100kb were identified, mostly as segregating deletions (22 del/2 dup; 18 seg/6 de novo). A barcode analysis on human gene expression data identified all genes expressed in brain and gut to represent CNS and ENS specific genes. Additionally, 79 genes were identified from prior studies as HSCR susceptibility genes. For the 1333 GDE, 724 overlapped a RefSeq gene, and of these, 584 overlapped a SV/indel from databases. Of the 140 events that did not overlap any known SV (67 unique genes) none matched known HSCR genes: however, 24 (36%) were identified as CNS or ENS genes. Of the events overlapping a SV, 1085 unique genes were affected: 18 were known HSCR genes (23% of 79 HSCR genes), and 231 (21%) were of CNS/ENS origin. Additionally, a deletion involving the *SEMA3* locus was found. Thus, through an integrative approach, we have identified a series of dosage changes in affected offspring that both overlap HSCR susceptibility genes and have an enrichment of CNS/ENS expressing genes. These genes usually overlapped SV regions, suggesting that common dosage variation can be clinically relevant to disease. The most promising genes include: *TFF1*, *TFF3*, *SOD1*, *S100B*, *ERBB4*, *NAV2*, *PLEKHA1*. These results provide new pathways for studying HSCR genetic etiology.

1809/T

DGV and DGVa - Curating and archiving structural variation data. *L. Feuk[1], M. Manker[2], J. Hinton[3], M. Maguire[3], J. Foong[2], J.R. MacDonald[2], B. Thiruvahindrapduram[2], I. Lappalainen[3], P. Flicek[3], S.W. Scherer[2].* 1) Rudbeck Laboratory, Dept Genetics & Pathology, Uppsala University, Uppsala, Sweden; 2) The Centre for Applied Genomics, The Hospital for Sick Children, Toronto, Canada; 3) The European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, UK.

The Database of Genomic Variants (http://projects.tcag.ca/variation/) was developed in 2004 to provide information about structural variation (SV) in the human genome for biomedical research. The main user group of the database and its associated genome browser are clinical geneticists utilizing the data to facilitate the interpretation of structural variation data from patients. Currently, the database holds nearly 90,000 published variants representing approximately 15,000 independent chromosomal loci. The recent explosion of data from diverse SV studies now necessitates that a more formal public data archive be developed. Two such efforts have recently been launched, entitled DGVa (started by European Bioinformatics Institute) and dbVAR (started by the National Center for Biotechnology Information), respectively. These databases provide accessioning and archiving of structural variation data. The main focus of DGV going forward will be to curate, interpret and visualize SV data to facilitate biomedical research and genetic diagnostics. DGV will work with DGVa, and accessioned data from select studies will be transferred from the archive to DGV, where the data will be further curated and made available to the user community. A new DGV database structure will ensure compatibility with SV data in the archival databases, accommodate better search functions and facilitate clinical interpretation of patient data. We will describe the DGV-DGVa collaboration, the development of the databases and how these changes will benefit the user community.

1810/T

Recurrent and Non-recurrent Genomic Rearrangements in IKBKG locus, causing IP, are generated by different mechanisms and may involve the contiguous G6PD gene. *F. Fusco[1], M. Paciolla[1,2], F. Napolitano[1], A. Pescatore[1], E. Esposito[1], MB. Lioi[2], MG. Miano[1], MV. Ursini[1].* 1) Dept Human Molecular Genetics, IGB-ABT-CNR, Naples, Italy; 2) University of Basilicata, Potenza, Italy.

Incontinentia Pigmenti (IP) is an X-linked dominant disease caused by mutation in the IKBKG/NEMO located in a structurally complex region of Xq28. IKBKG gene maps in LCR1 while a second non-functional pseudogene copy, IKBKGP/deltaNEMO maps in LCR2, in opposite direction to IKBKG. Moreover, IKBKG is organized head-to-head with G6PD gene with which it shares a common bidirectional CpG promoter. The presence of clusters of repetitive sequences in the homologous LCRs (>99%) has a destabilizing effect, and might predispose the IP locus to generate benign or pathological rearrangements by different mechanisms. A recurrent genomic deletion of exon 4-10 of IKBKG (IKBKGdel) caused by nonallelic homologous recombination (NAHR) between two adjacent MER67B repeats is the most frequent cause of IP. Whereas, two recurrent benign copy number variations (CNVs), IKBKGPdeletion and MER67Bduplication, produced by (NAHR) due to misalignment between MER67B repeats located in the two LCRs may occur in the IKBKG locus. We identified also nonrecurrent rearrangements, which generate unique pathological CNVs in IKBKG locus. We investigated nine IP subjects by real time PCR and haplotype mapping and we identified six unique pathological CNVs, including four genomic deletions and two duplications. Each rearrangement includes IKBKG, or its conserved 5'-regulatory sequences. Breakpoint sequence analysis reveals that, differently from the predominant NAHR mechanism in recurrent rearrangement, various molecular mechanisms, including nonhomologous end joining, Alu-Alu-mediated recombination, and replication-based mechanisms (e.g., FoSTeS and/or MMBIR) can generate non-recurrent IKBKG pathologic rearrangements. In three IP subjects, the generation of pathological CNVs involve the pseudogene: in two cases the IKBKG mutation arose by gene conversion, in one case it occurred through intra chromosomal inversion between gene and pseudogene. Of note, one of the genomic deletions extends outside the coding sequence. In this case an Alu-Alu-mediated recombination removes the bidirectional IKBKG/G6PD promoter, spanning the exon 1B and 1C of IKBKG, in telomeric direction and the exon 1 and 2 (containing the ATG) of G6PD, in centromeric direction. This deletion abolishes G6PD transcription, while IKBKG is transcribed from a secondary distal promoter. The genetic and functional consequences of this nonrecurrent rearrangement on the expression of the two contiguous genes will be discussed.

1811/T

A Comprehensive Functional Study of CNVs as Trait-Associated Polymorphisms and as Expression Quantitative Trait Loci. *E. Gamazon[1], D. Nicolae[1,2], N. Cox[1].* 1) Section of Genetic Medicine Department of Medicine University of Chicago Chicago, IL; 2) Department of Statistics University of Chicago Chicago, IL.

A recent study (WTCCC, Nature 2010) of CNVs and 8 complex disorders involving 16,000 cases and 3,000 controls concluded that common CNVs that can be typed on existing platforms are likely to have been interrogated by SNP-based genome wide association studies (GWAS) and unlikely to have a major role in the genetic basis of complex diseases. The same study also reported a paucity of enrichment of association signals among CNVs involving exonic deletions. We conducted a comprehensive functional study of CNVs well-tagged by SNPs (r2 > 0.8) by analyzing their effect on gene expression and their association with disease susceptibility and other traits. We tested whether these CNVs were disproportionately more likely to be functional than frequency-matched SNPs as trait-associated loci or as expression quantitative trait loci (eQTLs) influencing phenotype by altering gene regulation. Our study found that well-tagged CNVs are significantly enriched for eQTLs as well as for cis eQTLs; furthermore, these CNVs show an overrepresentation of reproducible trait associations relative to frequency-matched SNPs. Strikingly, we observed that these CNVs are more likely to simultaneously affect multiple expression traits than frequency-matched SNPs. The enrichment in reproducible trait associations holds robustly when we restrict the analysis to autoimmune diseases and to metabolic traits. Gene Ontology analysis shows that previously reported enrichment in immune response and response to external biotic stimuli for genes in CNV regions generalizes to the target genes of CNVs as eQTLs. Given these strong findings on the functional relevance of CNVs, we created an online resource of expression associated CNVs using the most comprehensive population-based map of CNVs (Conrad et al, Nature 2010) to supplement our earlier studies on eQTL SNPs and to inform future studies of complex traits (Nicolae et al, PLoS Genetics 2010). Our genome-wide gene expression analysis of HapMap CNVs shows that an even greater proportion predicts transcript levels than observed among the well-tagged CNVs in the WTCCC study. Collectively, our various findings show that caution must be exercised in assessments of the contribution of CNVs to complex human traits and in dismissing the possibility that taggable CNVs may indeed underlie certain complex phenotypes.

1812/T

Multiplexed, single-assay validation of whole genome microarray copy number data. *G. Geiss, N. Elliott, B. Birditt, P. Hengen, W. Buckingham.* Research and Development, NanoString Technologies, Seattle, WA.

We have developed a method for multiplexed measurements of copy number variants in genomic regions that can be performed in a single assay without the need for technical replicates. The assay utilizes color-coded barcodes to directly measure copy number variants with as little as100ng of genomic DNA. Since no amplification or technical replicates are required, the assay is extremely easy to use requiring less than 1 hour of hands-on time for 12 samples. Using a strategy of designing 3 probes per region of interest, we simultaneously measured 20 variant regions across 100 DNA samples from the International HapMap Consortium and compared the data to publically available microarray-based copy number results. Here, we show that this method accurately confirmed the published copy number over 94% of the time. Results from technical performance experiments indicate the assay is 99% reproducible across multiple users, days, and cartridges. Currently, using 3 probes per region, the assay can be customized for detection of up to 183 regions of genomic DNA and has been shown to be compatible with DNA isolated from saliva, blood and FFPE tissues. In summary, the nCounter copy number variation assay is an ideal choice for validating copy number variants obtained via whole genome sequencing or microarray analyses or screening large numbers of samples for defined sets of CNVs.

1813/T

Efficient Algorithm for Tandem Copy Number Variation Reconstruction in Repeat-rich Regions. *D. He, N. Furlotte, E. Eskin.* University of California Los Angeles, Los Angeles, CA 90095, USA.

Copy Number Variations (CNV) are one type of structural variations that have attracted much recent attention due to their effect on gene expression, disease status and other observable phenotypes. The recent development of high-throughput sequencing techniques has enabled the generation of reads with high genome coverage, which has lead to the development of new methods for identifying and even reconstructing CNVs. Such methods map reads to a reference genome and interrogate mapping inconsistencies, which might indicate the presence of a CNV. These methods work well when CNVs lie within unique genomic regions. However, the problem of CNV identification and reconstruction becomes much more challenging when CNVs are in repeat-rich regions, due to the multiple mapping positions of the reads. In this study, we propose an efficient algorithm to handle these multi-mapping reads such that the CNVs can be reconstructed with high accuracy even for repeat-rich regions. To our knowledge, this is the first attempt to both identify and reconstruct CNVs in repeat-rich regions. Our experiments show that our method is not only computationally efficient but also accurate.

1814/T

Exploring copy number variation in somatic mosaicism within human brain regions in neurodegeneration. *S. Iraola-Guzman[1], R. Rabionet[1], H. Mattlin[2], A. Ferrer[2], I. Ferrer[4], E. Martí[1], X. Estivill[1,3].* 1) Genes and Disease Program CRG-UPF and CIBERESP, Barcelona, Spain; 2) Microarray Core Facility, CRG, Barcelona, Spain; 3) Experimental and Helath Sciences Department, Pompeu Fabra University, Barcelona, Spain; 4) Anatomo-pathological service, IDIBELL-Hospital of Bellvitge, Barcelona, Spain.

The existence of genetic mosaicism in humans is well documented. For instance, about 1-2% of Down Syndrome cases are caused by mosaicism between normal and trisomic cells. Although traditionally mosaicism has been mostly used for cytogenetic variants, it refers to the presence of two populations of cells with a different genotype; however, it is assumed that somatic cells are genetically identical within a single individual. Nevertheless, a previous study has shown differences in copy number among various tissues in three individuals, suggesting that humans are commonly affected by somatic mosaicism for “de novo” copy number variants (CNVs). In order to analyze the presence of somatic mosaicism of CNVs within different brain regions, we have performed high-density array-CGH of six areas (amygdala, frontal cortex, hippocampus, hypothalamus, substantia nigra and pons) against the corresponding cerebellar vermis (CV) in six different individuals (2 Parkinson’s disease, 2 Alzheimer disease and 2 Controls), including a self to self-hybridization. We have detected several regions showing differential hybridization between areas, including ten regions common to at least two individuals, and 22 regions shared between at least two different areas in a single individual. Regions shared among more than two areas have been selected for further validation either by MLPA, real time qPCR or by allele-specific PCR, depending on the type of CNV. Confirmation of somatic mosaicism for structural variants could have an important impact in neurodegenerative disease. Most genetic studies are performed on blood DNA, with the assumption that any variant related to disease, even if it is tissue specific, it can be detected in blood. If there is an important level of mosaicism within the brain, it could account for part of the missing genetic component in complex neurological disorders.

1815/T

De Novo Rates and Selection of Large Copy-Number Variation. *A. Itsara[1], H. Wu[2], J.D. Smith[1], D.A. Nickerson[1], I. Romieu[3], S.J. London[2], E.E. Eichler[1,4].* 1) Genome Sciences, University of Washington, Seattle, WA; 2) National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, NC; 3) National Institute of Public Health, Cuernavaca, Mexico (current affiliation: International Agency for Research on Cancer, Lyon, France); 4) Howard Hughes Medical Institute, Seattle, WA.

While copy-number variation is an active area of research, *de novo* mutation rates within human populations are not well characterized. Here, focusing on large (>100 kbp) events, we estimate the rate of *de novo* formation of copy-number variants (CNVs) in humans by analyzing 4394 transmissions from human pedigrees with and without neurocognitive disease. Analyzing Phase I Hapmap trios, we attempted to validate four candidate *de novo* CNVs in CEU samples using a combination of array-CGH and marker analysis in the extended CEPH pedigrees and found that all events were likely somatic or cell line artifacts. This demonstrates that a significant limitation in directly measuring genomewide CNV mutation is accessing DNA derived from primary tissues as opposed to cell lines. We conservatively estimated the genomewide CNV mutation rate using SNP microarrays to analyze whole blood derived DNA from asthmatic trios, a collection in which we observed no elevation in the prevalence of large CNVs. At a resolution of ~30 kb, nine *de novo* CNVs were observed from 772 transmissions, corresponding to a genomewide CNV mutation rate of $\mu = 1.2 \times 10^{-2}$ CNVs per genome per transmission ($\mu = 6.5 \times 10^{-3}$ for CNVs >500 kb). Combined with previous estimates of the CNV population prevalence and assuming a model of mutation-selection balance, we estimate significant purifying selection for large (>500 kb) events at the genomewide level to be $s = 0.16$. Supporting this, we identify de novo CNVs in an analysis of 717 multiplex autism pedigrees from the AGRE collection and observe a significant ($p = 1.4 \times 10^{-3}$) four-fold enrichment for *de novo* CNVs segregating to cases of multiplex autism versus unaffected siblings suggesting that many *de novo* CNV mutations may be contributing a subtle, but significant risk for autism.

1816/T

Evaluation of the performance of gDNA from saliva collected with Oragene®•DNA for the purpose of CNV analysis on the Agilent Human Genome CGH Array 244A. *R.M. Iwasiow[1], M.A. Tayeb[1], P. Hu[2], R.F. Wintle[2], S. Scherer[2].* 1) DNA Genotek, Ottawa, ON, Canada; 2) The Centre for Applied Genomics, The Hospital for Sick Children, Toronto, ON, Canada.

CGH microarrays are efficient, high-resolution tools for genome-wide profiling of copy number variation (CNV). The Agilent Human Genome CGH Array 244A, which contains over 236,000 distinct probes representing both coding and non-coding sequences, is useful for CNV analysis. The current study evaluates the performance of gDNA from saliva on the Agilent Human Genome CGH Array 244A. Oragene•DNA simplifies sample collection by providing an alternative to blood, eliminating phlebotomy cost and complexity while facilitating collection from subjects dispersed throughout the general population. Oragene•DNA is a non-invasive, self-collection device intended for collection of large quantities of high-molecular weight genomic DNA (gDNA) from saliva. DNA is stabilized at room temperature for extended periods enabling sample collection and transport via regular mail and flexibility to process in batches. Two saliva samples were collected from each of four donors using the Oragene•DNA kit and the gDNA extracted from each of these collections was analyzed. Performance of the gDNA from saliva was assessed by evaluating CNV calls from each sample and the reproducibility of the data was determined by comparing the results from two separate collections from the same donor. The Aberration Detection Method 2 (ADM-2) algorithm (with fuzzy zero correction) provided in the Agilent Genomic Workbench 5.0 software was used to analyze the data. Less than 29 CNVs per genome were detected in individual saliva samples and there was a high degree of concordance between replicate samples from the same donor. Additionally, >80% of the CNVs found in saliva were present in corresponding blood samples. The derivative log ratio spread (DLRS) for all saliva and blood samples was <0.2. All other metric parameters were within acceptable limits and no significant difference between blood and saliva samples was observed. The results indicate that gDNA isolated from saliva collected with Oragene•DNA is a suitable substitute for gDNA from blood for use on Agilent Human Genome CGH Arrays.

1817/T

Genome-wide detection of CNVs in Lithuanian patients with mental retardation. *V. Kucinskas[1,2], V. Dirse[1], A. Utkus[1,2], E. Preiksaitiene[1], J. Kasnauskiene[1,2], K. Männik[3], A. Kurg[3].* 1) Department of Human and Medical Genetics, Faculty of Medicine, Vilnius University, Vilnius, Lithuania; 2) Centre for Medical Genetics of Vilnius University Hospital Santariskiu Klinikos, Vilnius, Lithuania; 3) Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia.

Background: Mental retardation (MR) which occurs in about 1-3 % of population is etiologically very heterogeneous and remains unexplained in almost half of all cases. Whole-genome scanning technologies such as array CGH and SNP-CGH have enabled the detection of clinically relevant copy number variations in individuals with MR of unknown etiology. **Methods:** Twenty-three Lithuanian patients with MR/developmental delay (DD) and congenital malformations were enrolled in this study. We have used Infinium HD whole-genome genotyping HumanCytoSNP-12 (300K SNPs) and HumanOmniExpress (700K SNPs) BeadChips to detect significant copy number variations. Obtained data was analyzed with Illumina GenomeStudio, KaryoStudio and QuantiSNP software. **Results:** Two copy number variations with potential clinical relevance were detected. The first patient was a 12-year-old boy with moderate MR and speech delay. He was the second born child of healthy parents. Birth weight was 2550g, length 51 cm. At age 12 his height was 141 cm (3 centile), weight 27 kg (<3 centile), OFC 49 cm (<3 centile). He had microcephaly, sloping forehead, relatively large ears. Detected 1.8 Mb microdeletion at 17q21.33 contains among other genes *CACNA1G* and *CA10* which are important in neurological processes. The *CACNA1G* gene encodes voltage-dependent calcium channel which are thought to be involved in neuronal oscillations and resonance, pacemaking activity in central neurons, neurotransmission. The *CA10* gene encodes the protein which is acatalytic member of the alfa-carbonic anhydrase subgroup and it is thought to play significant role in the central nervous system, especially in brain development. *CACNA1G* and *CA10* might be good candidate genes for further studies to investigate whether these are involved in the development of phenotypical features of this patient. The second patient was referred because of MR/DD, particularly speech and language delay. Clinical phenotype was remarkable for several small areas of skin hypopigmentation and cafe-au-lait spots, mild joint hypermobility and medial deviation of the feet. 1.7 Mb subtelomeric microduplication at 4q35.2 was found which partially overlaps with similar 2.5 Mb microduplication reported in DECIPHER database. The research leading to these results is funded by the European Community's Seventh Framework Program [FP7/2007-2013] under grant agreement n° 223692, CHERISH project.

1818/T

Exploring De Novo Copy Number Variants in Three-Generation Families of Mongolia. *S. Lee[1,4], H. Park[1,5], YS. Ju[1,6], HJ. Kim[1,4], JI. Kim[1,2,3], JS. Seo[1,2,4,6].* 1) ILCHUN Genomic Medicine Institute, Medical Research Center, Seoul National University, Seoul, Korea; 2) Department of Biochemistry and Molecular Biology, Seoul National University College of Medicine, Seoul, Korea; 3) Psoma Therapeutics Inc., Seoul, Korea; 4) Department of Biomedical Sciences, Seoul National University Graduate School, Seoul, Korea; 5) Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA; 6) Macrogen Inc., Seoul, Korea.

After the discovery of copy number variation (CNV) a few years ago, we are starting to figure out that CNV is actually the huge portion of human genomic variation, and could affect a lot to many kinds of phenotypes we can imagine. Moreover, dislike SNPs, CNVs are known to be newly generated rather easily during cell division, so De Novo CNVs, which refers CNVs newly generated, could be discovered in the way of comparing CNVs of family members in different generations. In this study, we focused on this type of De Novo CNVs in three-generation families, and tried to approximate the true number of De Novo CNVs generated between parents and children. We used the DNA samples of three Mongolian families, each of which consists of 6 family members: grandparents, parents, and two children. We chose array comparative genomic hybridization (arrayCGH) as a CNV detection method, and used 1M custom design array. (Agilent Technology) This array has been designed to detect most of previously reported CNVs and simultaneously discover new kinds of CNVs by tiling the rest of probes throughout human genome. We used HapMap sample NA10851 as a reference DNA, and result files coming out of the arrayCGH experiments are transformed by a program, called 'CARA : CGH Array Reference-free Algorithm', which provides the correction of the effect of reference DNA. To remove false positive CNVs, we focused on one parent among family members, whose parents are the grandparents of our samples. Firstly, we collected De Novo CNV candidates which are detected in the target parent but not in grandparents, and then we selected CNVs which are transmitted to his or her children. As a result, we found 106, 35, and 102 De Novo CNVs in each family, and these CNV regions are now on validation using real-time PCR. Furthermore, we are also trying to propose candidate CNV regions which may result from somatic changes, and we could suggest more kinds of De Novo CNV candidates with children samples' CNV calls. With all these results together and further analysis on the sequence level, we are expecting to provide some clues about the mechanism of CNV generation.

1819/T

Copy Number Variants Associated with Refractive Error. *Y.J. Li[1, 2], D. Abbott[2], S. MacGregor[3], P.G. Hysi[4], A.W. Hewitt[5], D. Mackey[5], C.J. Hammond[4], T.L. Young[2].* 1) Department of Biostatistics & Bioinformatics, Duke Univ Med Ctr, Durham, NC; 2) Center for Human Genetics, Duke Univ Med Ctr, Durham, NC; 3) Genetics and Population Health, Queensland Institute of Medical Research, Brisbane, Australia; 4) Department of Twin Research and Genetic Epidemiology, King's College London School of Medicine, St. Thomas' Hospital, London, United Kingdom; 5) Centre for Eye Research Australia, University of Melbourne, Royal Victorian Eye and Ear Hospital, Melbourne, Australia.

Purpose: Copy-number variations (CNVs) are deletions, insertions, or duplications of chromosomal segments that may influence disease susceptibility. We conducted a genome-wide association of CNVs with refractive errors using twin samples. **Methods:** 3024 genomic DNA samples from a twin cohort were genotyped with the Illumina human610-quad beadchip. Both PennCNV and QuantiSNP programs, Hidden Markov modeling based methods, were used to detect CNV regions for each sample. The primary phenotype is spherical equivalent (SE=sphere+1/2(cylinder)). We tested whether a CNV event is associated with the average SE of both eyes (AvgSE). A linear regression was performed in the R package with AvgSE as an outcome variables and the CNV event (presence or absence) at a CNV SNP as a predictor variable. To fit model assumptions of normality, AvgSE was transformed using the ENQT method. Due to the insufficient sample size for CNV SNPs with low frequencies, CNV SNPs with a frequency less than 0.4% (12 samples) were excluded. CNV regions were prioritized based on a combination of significance thresholds of 0.0005 and 0.01 in either detection method. **Results:** Of 600,470 SNPs in the analysis, PennCNV detected 295,507 (49.2%) CNV SNPs while QuantiSNP detected 443,385 (73.8%) CNV SNPs. We excluded 85,337 (14.2%) CNV SNPs due to low sample counts. Five CNV regions met our criteria: 1) chr9: 106390295-106413750 ($p=0.01$, $4.58x10^{-5}$ for PennCNV and QuantiSNP, respectively); 2) chr10: 122759266-122777752 ($p=9.73x10^{-5}$, $9.73x10^{-5}$); 3) chr12: 20908303-20930416 ($p=3.39x10^{-4}$, $p=1.09x10^{-5}$); 4) chr17: 41730794-41780482 ($p=0.01$, $4x10^{-4}$); and 5) chr20: 51709859-51737515 ($p=0.007$, $1.4x10^{-4}$). Among these regions, chr10 region showed the best results with high consistency between PennCNV and QuantiSNP, but no genes mapped to this region. The most interesting regions were chr12, chr17, and chr20 - all with potential interval candidate genes. Notably, although PennCNV did not show an impressive p-value for the chr17, this region was detected by both methods when high myopia (SE < -5.00 diopter (D) for at least one eye) was compared to controls (-0.50 D < SE < +1.00D)($p=0.005$, 0.004). **Conclusions:** The present study identified potential loci with CNVs that may modify refractive error. Further investigation of these CNV intervals may lead to the identification of susceptibility genes for myopia.

1820/T

A region-based CNV calling algorithm. *T. Liu, A. Thalamuthu.* Genome Inst Singapore, Singapore, Singapore.

A crucial step in studies of CNVs in human disease is to correctly identify those CNV events at the first place. Once CNVs are successfully identified, all types of downstream association tests can be performed to test the relatedness of a disease and the copy-number-variation of a particular region. Below we describe a region-based CNV calling algorithm, which can be applied to the intensity data generated by various platforms. Our algorithm works with predefined regions of known copy-number variation, especially suited for regions of common variations. Our algorithm is very useful in two circumstances, 1) we can use it to verify the CNV calls in those potentially interesting regions defined by using standard HMM-algorithms 2) given supplemental information from other published data with reported copy-number variations, we can incorporate these prior information and improve the CNV calls in known reported regions. Based on LRR values of the samples, the general idea of our algorithm is to combine the multi-dimensional Gaussian Mixture Model (GMM) and model-based hierarchical clustering to cluster samples into discrete copy number classes (0,1,2,3 and 4). The three advantage of our algorithm are 1) we are able to incorporate relevant reported knowledge with the data under study, which may potentially boost the accuracy of the estimation of copy number; 2) our algorithm takes the entire sample into account during the calling process, hence our algorithm is expected to be more sensitive than the HMM algorithm; 3) our algorithm is region based, which enable us to make adjustments for the batch-effects more effective and straightforward. We have tested our algorithm with the 90 Hapmap samples from Chinese Han population. The intensities were obtained using the Affimetrix 500k chip, and converted to the LRR values. We compared our results with those given by the Birdsuite algorithm (Broad Institute, 2008). The average consistency of our algorithm and the Birdsuite algorithm over these regions is 92.4%. Yet, in 173 regions, the consistency of the two algorithms is lower than 90%. We show that in these regions, the copy-number calling by our algorithm is more reliable, comparing to the current the Birdsuite algorithm (which by the way relies heavily on the prior information), when the prior information was possibly misleading.

1821/T

Investigate the roles of DNA copy number variants in primary open-angle glaucoma. *Y. Liu[1], J. Gibson[1], C. Haynes[1], K. Hutchins[1], S. Schmidt[1], R.R. Allingham[2], M.A. Hauser[1,2].* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) Duke Eye Center, Duke University, Durham, NC.

Copy number variants (CNVs) have been shown to be involved in many human diseases, including autism, schizophrenia, and systemic lupus erythematosus. However, the role of CNVs in primary open-angle glaucoma (POAG) still remains unknown. POAG, as the most common form of glaucoma, affects more than 3 million people in the US. We used genome-wide Illumina microarrays containing over 610,000 genetic markers to genotype 100 primary open-angle glaucoma cases. At the same time, the genotype and raw intensity files were requested for over 900 Parkinson's disease controls from dbGAP in NCBI. PennCNV software was used to identify and analyze the CNV changes in POAG cases and controls. The CNV frequencies were compared between cases and controls using Fisher's exact test. We identified a heterozygous DNA copy number loss on the GALC gene (galactosylceramidase) in two POAG cases comparing to none in dbGAP controls (p=0.012). The association of this specific CNV loss was confirmed in our Caucasian case-control (470 cases and 468 controls) POAG dataset (p=0.03) using TaqMan-based realtime PCR CNV assays. The identified CNVs were further confirmed using chromosome 14 specific NimbleGen aCGH. The end points for CNV loss were defined so that CNV-specific PCRs were designed to further confirm the CNV loss. We have identified different end points for the CNV loss on GALC, suggesting the complexity of CNV changes at this locus. Very interestingly, the homozygous CNV loss of GALC accounts for 40-45% of Krabbe disease patients in the Caucasian population. Majority of the Krabbe disease patients show optic neuropathy or vision loss, which suggests a potential biological role of GALC in the pathogenesis of POAG. Our study will be the first to incorporate DNA copy number variants into the genetic association of glaucomatous optic neuropathy seen in POAG. To our knowledge, this is the first report describing the CNVs in the pathogenesis of POAG. This study provides important and novel information that, if corroborated, can be used to develop strategies for future genetic testing, diagnosis, and potential new therapies to treat POAG, a common blinding disease.

1822/T

Autoantibody profile associates with *C4* gene copy number in Systemic Lupus Erythematosus. *E. Lundstrom[1], I. Gunnarsson[1], J. Gustavsson[1], YL. Wu[2], K. Elvin[3], CY. Yu[2], LO. Hansson[4], A. Larsson[4], L. Klareskog[1], L. Padyukov[1], E. Svenungsson[1].* 1) Dept Medicine, Karolinska Inst & Hosp, Stockholm, Sweden; 2) Dept of Molecular Virology, Immunology and Medical Genetics, the Ohio State University, Columbus, Ohio, USA; 3) Dept of Clinical Immunology and Transfusion Medicine, Unit of Clinical Immunology, Karolinska Institutet, Sweden; 4) Dept of Clinical Chemistry and Pharmacology, Akademiska Hospital, Uppsala, Sweden.

Objectives: Systemic lupus erythematosus (SLE) is a heterogeneous and complex systemic autoimmune disease characterized by autoantibody profile. Copy number variation (CNV) has been shown to be one of many possible sources of phenotypic heterogeneity. The region with strongest linkage to SLE (6p21.3) harbors the genes *C4A* and *C4B* which are known to exhibit CNV. In this study, we aimed to analyze the associations between *C4* CNV and SLE. In addition, we analyzed the association of *C4* CNV with plasma levels of complement and autoantibodies among SLE patients. **Methods**: 283 SLE patients and 180 controls matched for age, sex and region of living participated. *C4* CNV was determined with RT-PCR. Plasma levels of C4, C3, and C3d were measured in patients by rate nephelometry and autoantibodies with enzyme linked immuosorbent assay. The Lupus anticoagulant (LAC) test was performed with a Dilute Russel Viper Venom method. Associations were determined with Chi-square and Mann-Whitney tests. **Results**: SLE patients displayed more often low gene copy number of *C4A* (<2) (OR: 2.3, 95% CI: 1.4-3.7) or <4 copies of 'C4 total' (C4A+C4B) (OR: 2.3, 95% CI: 1.4-3.6) compared to controls. No association between *C4B* CNV and SLE was observed. Presence of SSA/SSB was significantly associated with SLE patients carrying <2 copies of *C4A* or <4 copies of C4tot (C4A+C4B) (OR C4A: 1.8, 95% CI: 1.1-3.1) (OR C4tot: 4.1, 95% CI: 2.4-7.00). SLE patients carrying <2 copies of *C4A* or <4 copies of C4tot, were less likely to have β2GP1 IgG (OR C4tot: 0.4 95% CI: 0.2-0.7), anticardiolipin (aCL) IgM (OR *C4A*: 0.5, 95% CI 0.2-0.9) or a positive LAC test (OR C4tot: 0.3, 95% CI: 0.1-0.5). In addition, our data reveal association between low copy numbers (<4 copies) and low serum levels of C4 (p = 0.02) among SLE patients. No association between *C4* CNV and plasma levels of C3 and C3d was observed. **Conclusion**: Our study demonstrates a significant association between *C4* CNV and autoantibody profile in SLE. Low gene copy numbers of *C4* were strongly associated with the presence of SSA and SSB antibodies while SLE patients with high numbers of C4 gene copies were more likely to have aPL antibodies. Our data support previous findings that low gene copy number of *C4* is associated with SLE and with low levels of C4 protein in plasma. Further studies related to the complement system are warranted to understand how genetics is related to subgroups of SLE patients with different autoantibody profile.

1823/T

An oligonucleotide array for detecting length polymorphisms of long tandem repeats in the human genome. *R. Margolis[1], L. Yu[1], G. Benson[2], P. Warburton[3], L. Delisi[4].* 1) Dept Psychiatry, Johns Hopkins Univ Sch Med, Baltimore, MD; 2) Departments of Biology and Computer Science, Program in Bioinformatics, Boston University, Boston MA; 3) Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York NY; 4) Department of Psychiatry, Harvard Medical School, Boston VA Brockton Health Services System, Brockton, Massachusetts.

There are more than 1 million tandem repeats in the human genome. Repeats with units of 2-12 bp in length have emerged both as powerful markers for linkage studies and as mutations causing a number of human diseases. We hypothesize that polymorphisms of longer tandem repeats (unit length of 50 bp to >150,000 bp), relatively unexplored features of the human genome, may also contribute to normal human variation and to disease, including schizophrenia. To systematically address this issue, we developed an oligonucleotide array specifically designed to detect length variations of those repeats. we selected repeats that were at least 250 bp in length with units a minimum of 50 bp in length and of at least 95% identity. A total of 6,763 non-redundant repeats were selected from the human genome reference sequence for potential inclusion. Using the Roche Nimblegen singleplex Comparative Genomic Hybridization (CGH) 12X135K oligonucleotide platform, a 136,363-probe set was generated for detecting 4,064 tandem repeats (~60% of the potential repeats) with a coverage of approximately 4 million base pairs. Twelve patients with schizophrenia (labelled with Cy3) and one healthy control (labelled with Cy5) were used as test and reference samples, respectively. The log2 ratio of test to reference signal intensities were determined for each probe, and then a log2 ratio for each repeat was determined by calculating the mean log2 ratios of all probes hybridizing to a given repeat. In total, we detected 495 repeats with substantial length polymorphism (log2 ratio >0.5 or <-0.5). For each individual tested, we detected 100-200 repeats with lengths substantially different from the reference sample. The presence or absence of polymorphism was confirmed for selected repeats using PCR. We conclude that this oligonucleotide array will be a novel tool for detecting repeat length variations associated with diseases.

1824/T

Calling copy number variants in DNA of 8,000 African Americans: the CARe project. *YA. Meng[1], J. Korn[2], J. Nemesh[3], D. Altshuler[4], EJ. Benjamin[5], E. Boerwinkle[6], D. Bowden[7], C. Chiang[8], M. Fornage[9], J. Glessner[10], A. Kutlar[11], G. Lettre[12], M. Li[13], S. Musani[14], G. Papanicolaou[15], S. Redline[16], A. Reiner[17], S. Rich[18], D. Siscovick[19], X. Zhu[20], H. Hakonarson[21], JN. Hirschhorn[1,4,8], JG. Wilson[14], B. Keating[22], SA. McCarroll[4,8].* 1) Metabolic Disease Initiative, Broad Inst, Cambridge, MA; 2) Genetic Analysis Platform, Broad Inst, Cambridge, MA; 3) Computational Biology and Bioinformatics Program, Broad Inst, Cambridge, MA; 4) Medical and Population Genetics Program, Broad Inst, Cambridge, MA; 5) School of Medicine, Boston University, Boston, MA; 6) Human Genetics Center, University of Texas Health Science Center at Houston, Houston, TX; 7) Wake Forest University School of Medicine, Winston-Salem, NC; 8) Department of Genetics, Harvard Medical School, Boston, MA; 9) Institute of Molecular Medicine, University of Texas, Houston, TX; 10) Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, PA; 11) Department of Medicine, Medical College of Georgia, Augusta, GA; 12) Department of Medicine, Université de Montréal, Montreal Heart Institute, Quebec, Canada; 13) Department of Biostatistics and Epidemiology, University of Pennsylvania, Philadelphia, PA; 14) University of Mississippi Medical Center, Jackson MS; 15) Division of Prevention and Population Sciences, National Heart, Lung, and Blood Institute, Bethesda, MD; 16) Department of Medicine and Center for Clinical Investigation, Case Western Reserve University, Cleveland, OH; 17) Department of Epidemiology, University of Washington Seattle, WA; 18) Department of Public Health Sciences, University of Virginia, Charlottesville, VA; 19) Division of Cardiology, Department of Medicine, University of Washington, Seattle, WA; 20) Department of Biostatistics and Epidemiology, Case Western Reserve University, Cleveland, OH; 21) Department of Pediatrics, University of Pennsylvania, Philadelphia, PA; 22) Department of Cardiology, University of Pennsylvania, Philadelphia, PA.

We analyzed data from > 8,000 African Americans who were genotyped on the Affymetrix 6.0 Array to optimize detection of copy number variants (CNVs). Samples were from five cohorts in the NHLBI Candidate Gene Association Resource (CARe) project, including the Atherosclerosis Risk in Communities (ARIC) study, Cleveland Family Study, Coronary Artery Risk Development in Young Adults (CARDIA) study, Jackson Heart Study, and the Multi-Ethnic Study of Atherosclerosis (MESA). We used algorithms described in Korn et al. (Nat Genet 2008;40:1253-60) to call common (Canary) and rare (Birdseye) CNVs. Reference panels included data from both HapMap2 and HapMap3, and incorporated the more accurate and complete catalogs of copy number variation from the Genome Structural Variation Consortium (Nat Genet 2010;42:385-91). To construct prior probabilities for use in Canary, we applied a weighting of 4:1 YRI:CEU, reflecting typical ancestry of African Americans. We tested the impact on call rate of altering a variety of parameters and filters, as well as certain QC measures, such as apparent Mendelian inheritance errors in the Cleveland Family Study. Using CANARY, we analyzed over 1,000 diallelic CNVs that passed our QC filters, of which ~500 had minor allele frequency >1%. Parameters critical to CNV calling in African Americans included use of appropriate prior probabilities, careful attention to plate-specific artifacts, and judicious use of Hardy-Weinberg filtering in this admixed population. In ongoing work, we will assess the accuracy of these CNV calls by direct genotyping and will produce a set of CNVs for association testing with phenotypes that have been measured in the CARe cohorts.

1825/T

Population Analysis of Copy Number Variation Regions with SNP Genotyping Array. *S. Moon, Y. Kim, C. Hong, Y. Shin, D. Kim, M. Park, J. Lee, H. Kim, B. Han, B. Kim.* Division of Structural and Functional Genomics, Center for Genome Science, Korea National Intsitute of Health,KCDC, Seoul 122-701, Korea.

To date, thousands of genomic variants associating with susceptibility to human complex disease have been reported through GWA studies. In spite of successful findings of GWAS, it is still not sufficient to find the missing heritability of complex diseases. To make up for finding the missing heritability, copy number variation analysis has been considered as an alternative and a complement way. Copy number analysis is defined as a loss or gain of long segments of DNA and it is relatively common in the human genome. Although tens of thousands of CNVs and CNV regions have been discovered from various CNV studies, only minorities of these studies were focused on the Asian. Thus, for considering more precise CNV analysis, it is essential to add Asian-specific CNV on the CNV map. In this study, we undertook a genome-wide analysis of copy number variation in community-based Korean population. We used the Affymetrix genome-wide Human SNP array 6.0 platform (including more than 906,600 SNP and more than 946,000 CN probes) to carry out genotyping 3,703 healthy controls from the KoGES (Korean Genome Epidemiology Study). After quality control test check, two kinds of CNV detection tools such as GADA and DNAcopy were used to analyze CNV locus. In conclusion, we identified a number of both common CNV loci and rare CNV loci through the quality control process and we also merged clusters of overlapping CNVs at the same level into CNV event (CNVE) according to the WTCCC protocol. These findings will be confirmed by follow-up study and validation study using qPCR.

1826/T

Genome-wide high-resolution array-CGH analysis of copy number variation in human somatic tissues. *M. O'Huallachain[1, 2], A.E. Urban[3], M.P. Snyder[2].* 1) MCDB, Yale Univerisy, New Haven, CT; 2) Genetics, Stanford University School of Medicine, Stanford, CA; 3) Psychiatry, Stanford University, Stanford, CA.

Human genomic variation is a key factor in understanding human diversity. Genomic changes range from single nucleotide polymorphisms to large chromosomal aberrations. Structural variation including copy number variation (CNV) affects chromosomal regions on the kilobase to megabase scale. Many studies in the past several years have demonstrated the significance of copy number variation to human genomic variation. CNVs have been implicated in multiple human genetic disorders. These studies generally used easily accessible DNA from blood or blood-derived cell lines. It was widely assumed that all healthy cells, with very few specific exceptions in the immune system and germ line, which arise from the same zygote, possess the same genomic content. However, recent evidence indicates that copy number variation exists between differentiated tissues within individuals. Our study aims to uncover the scope of somatic copy number variation using technology not previously available. Here we report findings from array-CGH experiments using Nimblegen 2.1M probe arrays. This array has the potential to detect copy-number changes as small as a few kilobases using unbiased tiling across the whole genome. At this time, we have tested a total of 13 tissues collected post mortem from two individuals. Our analysis of these experiments suggests that there are approximately 30 locations of potential copy number differences between tissues within an individual ranging in size from ~2kb to ~1.5Mb. Several of the CNVs encompass genes while others occur in intergenic regions. A number of them have been previously indentified based on the Database of Genomic Variants while some appear to be novel. Our findings suggest that somatic copy number variation is more frequent than has previously been reported.

1827/T

Towards a comprehensive structural variation map of an individual human genome. *A.W. Pang[1,2], J.R. MacDonald[2], D. Pinto[2], J. Wei[2], M.A. Rafiq[2], D.F. Conrad[3], H. Park[4], M.E. Hurles[3], C. Lee[4], J.C. Venter[5], E.F. Kirkness[5], S. Levy[5], L. Feuk[6], S.W. Scherer[1,2].* 1) Department of Molecular Genetics, University of Toronto,Toronto, Ontario, Canada; 2) The Centre for Applied Genomics, The Hospital for Sick Children, Toronto, Ontario, Canada; 3) Wellcome Trust Sanger Institute, The Wellcome Trust Genome Campus, Hinxton, Cambridge, United Kingdom; 4) Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts, USA; 5) J. Craig Venter Institute, Rockville, Maryland, USA; 6) Department of Genetics and Pathology, Rudbeck Laboratory, Uppsala University, Uppsala, Sweden.

Several genomes have now been sequenced, with millions of genetic variants annotated. While significant progress has been made in mapping single nucleotide polymorphisms (SNPs) and small (<10bp) insertion/deletions (indels), the annotation of larger structural variants (SVs) has been less comprehensive. It is still unclear to what extent a typical genome differs from the reference assembly, and the analysis of the genomes sequenced to date have shown varying results for copy number variation (CNVs) and inversions. We have combined computational re-analysis of existing whole genome sequence data with novel microarray-based analysis, and detect 12,178 structural variants covering 40.6Mb that were not reported in the initial sequencing of the first published personal genome. We estimate a total non-SNP variation content of 48.8Mb in a single genome. Our results indicate that this genome differs from the consensus reference sequence by ~1.2% when considering indels/CNVs, 0.1% by SNPs and ~0.3% by inversions. The structural variants impact 4,867 genes, and >24% of structural variants would not be imputed by SNP-association. Our results indicate that a large number of structural variants have been unreported in the individual genomes published to date. This significant extent and complexity of structural variation, as well as the growing recognition of its medical relevance, necessitate it be actively studied in health-related analyses of personal genomes. The new catalogue of structural variation generated for this genome provides a crucial resource for future comparison studies. Finally, to continue the annotation of this human structural variation map, we are currently utilizing a number of next generation sequencing (NGS) technologies to discover novel variants, to refine variation breakpoints, and to elucidate the putative molecular mechanism in the formation of these variants.

1828/T

CONDR - COpy Number Detection with RNA-seq: A novel algorithm to infer copy number changes from RNA-seq data. *A. Ramachandran[1], M. Micsinai[2], I. Pe'er[1].* 1) Computer Science, Columbia University, New York City, NY; 2) NYU Cancer Institute, New York University School of Medicine, New York, NY.

Background: The recently emerged next generation sequencing technology applied to RNA (RNA-seq) is a powerful, high-resolution approach to quantitatively survey the transcriptome, revealing information about expression level as well as single nucleotide variants (SNVs). Current state-of-the-art algorithms easily detect SNVs from RNA-seq data, but copy number changes are more complicated to distinguish from transcription levels. To address this shortcoming, we developed a new computational method, CONDR, to identify abnormal copy number from RNA-seq data with application to germline copy number variants (CNVs), as well as somatic aberrations.

Methods: The CONDR algorithm uses an HMM to incorporate two sources of information: relative transcript abundance measured in Fragments Per Kilobase of transcript per Million mapped fragments (FPKM), and sequence information of mapped short reads that provides SNVs. These quantities are obtained from standard tools for RNA-seq mapping. In our HMM implementation we define six states that are biologically relevant and computationally feasible. The algorithm calls a copy number state, normal or any of the aberrant states for each exon along the genome. Aberrant states include homozygous deletion, heterozygous deletion, copy neutral loss-of-heterozygosity, chromatin change and copy number increase.

Computationally, the inference is based on each of the copy number states being characterized by particular fold changes in FPKM and rates of single nucleotide variants per kilobase. The modeled transitions are between the normal state and an aberrant state and their respective probabilities quantify the chances of initiation or termination of a copy number change in a particular gap between exons.

The emission probabilities are estimated from the expectations of FPKM and SNV rates for the normal state. Given the data, we use the forward-backward algorithm to compute the most likely state for each exon along the genome.

Results: We demonstrate CONDR performance on simulated and experimental data, where copy number change loci have been independently validated by qPCR.

1829/T

The impact of common copy number variation on gene expression phenotypes in asthmatics. *A.J. Rogers[1], J.H. Chu[1], K. Darvishi[2], J. Howrylak[1], B. Klanderman[1], R. Mills[2], I. Ionita-Laza[3], V. Carey[1], C. Lee[2], B.A. Raby[1].* 1) Channing Laboratory, Brigham & Women's Hospital, Boston, MA; 2) Department of Pathology, Brigham and Women's Hospital, Boston MA; 3) Columbia University, New York, New York.

Rationale: Common copy number variants (CNVs) constitute a substantial proportion of total genetic variation. However, the extent of their contribution to human phenotypic variation, as compared to single nucleotide variation, remains undetermined. Previously, an analysis using low-resolution CNV genotyping methods in HapMap lymphoblastoid cell lines estimated that ~17% of gene expression may be explained by CNVs (Stranger et al., Science 2007). The motivation of the current study is to evaluate the impact of CNVs on gene expression in a larger dataset using a higher resolution array, designed specifically for the purpose of CNV genotyping. **Methods**: Gene expression profiles from peripheral blood CD4+ lymphocytes from 153 Caucasian asthmatics were generated using Illumina HumanRef8 v2 BeadChip arrays. CNV genotyping was performed using a custom 180k Agilent CGH CNV genotyping array that interrogates ~20,000 known CNV regions identified in several recent high-resolution CNV discovery and sequencing projects, including the Structural Genomic Consortium, 1000 Genomes Project, and CNV genotyping in Asians (Park et al, Nat. Gen. 2010). Each CNV region was tagged with 6 to 9 probes and each sample was tested against a standard reference (HapMap NA10851). After filtering of minimally expressed genes, we measured the correlation of (log-transformed) transcription abundance with copy number (aCGH log2 ratio) for all CNV regions. **Results**: We observed strong correlation between CNV log2ratio and expression phenotype. Using p-value thresholds of $1x\ 10^{-4}$, $5x\ 10^{-5}$, and $1x\ 10^{-5}$, we found respectively 2487 (13%), 1619 (9%), and 589 (3%) of CNV associated with expression of at least one syntenic gene. The variance in gene expression explained by these CNVs was high (median 34%, range from 32% to 88%), and was considerably greater than that observed by SNP (median <22% for all eQTL studies reported to date). While most CNVs were associated with expression of only a single gene, more than 100 were associated with expression of at least 5 genes. **Conclusions**: The impact of CNV on gene expression is large. Though only a fraction of CNVs influence transcription, their phenotypic consequences on their target genes is greater than that observed for all but a limited number of SNP. Moreover, an important subset of expression-associated CNVs manifests "master regulator"-like effects. **Funding**: R01 HL093076, R01 HL086601, P01 HL083069, P41 HG004221, and K12 HL089990.

1830/T

***NRXN1* exonic deletions in psychiatric disorders - a mega-analysis of published data.** *D. Sato, D. Pinto, A.C. Lionel, A. Prasad, C.R. Marshall, S.W. Scherer.* The Centre for Applied Genomics and Program in Genetics and Genomic Biology, The Hospital for Sick Children, Toronto, Ontario, M5G 1L7, Canada.

Autism spectrum disorders (ASDs) are a genetically complex group of neurodevelopmental disorders with incidence of ~1% in the general population. Despite a strong genetic basis underlying ASDs and some genes now identified, the majority of the genetic causes still remain largely unknown. Over the past years, the International Consortium Autism Genome Project (AGP) has shown that multiple rare de novo and inherited copy number variations (CNVs) contribute to >10% of ASDs. In 2007, the AGP reported two de novo deletions disrupting the *NRXN1* gene in two female siblings with ASD (Szatmari et al. 2007). The *NRXN1* gene encodes a synaptic neuronal adhesion molecule and represents one of the best candidate genes for ASDs based on its well-known role in synaptogenesis and its interaction with neuroligins. After that first report of *NRXN1* deletions associated with ASD, subsequent studies have identified *NRXN1* deletions in other ASD cohorts as well as in schizophrenia (SCZ) patients. Such CNVs may result in the expression of different neurodevelopmental phenotypes. Thus, we compiled all available published and our own unpublished data on CNVs affecting the *NRXN1* gene from various neuropsychiatric disorders. We found that *NRXN1* CNVs could be observed in ASDs, SCZ, intellectual disability, attention deficit hyperactivity disorder and bipolar disorder. We observed a significant excess of exonic CNVs overlapping *NRXN1* in neuropsychiatric disorder cases compared to controls. In our latest AGP study (Pinto et al. 2010), we identified three exonic deletions and one duplication in *NRXN1* in four out of 996 ASD cases versus none in 4,964 screened controls ($P=4.7x10^{-3}$). All four CNVs were de novo, further supporting a role in ASD pathogenesis. Moreover, in a mega-analysis of all cases, we found exonic deletions in 15 of 3,632 ASD cases and none in 7,719 controls ($P= 3.7\times10^{-8}$), and a total of 42 exonic deletions in 16,618 psychiatric disorder cases and 9 in 52,690 controls corresponding to an odds ratio of 14.8 (95%CI 7.2-30.5, $P=2.4\times10^{-18}$). Although we cannot exclude an overestimation of these frequencies due to some individuals being reported in multiple papers, these findings warrant further follow-up in larger patient cohorts, and existing cross-disorder sets where raw genotyping data is available. In addition, we are generating iPS (induced pluripotent stem) lines from ASD cases with *NRXN1* exonic deletions for functional studies.

1831/T

Recurrent rearrangements of 16p13.11: further evidence for a pathogenic role of deletions in Autism Spectrum Disorders (ASD). *A.F. Sequeira[1,2,3], C. Correia[1,2,3], J. Almeida[4], S. Mouga[4,5], C. Cafe[4], F. Duque[4], D. Pinto[6], P. Szatmari[7], S.W. Scherer[6], G. Oliveira[4,5], A.M. Vicente[1,2,3], The Autism Genome Project Consortium.* 1) Instituto Gulbenkian de Ciência, Oeiras, Portugal; 2) Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa, Portugal; 3) Center of Biodiversity, Functional & Integrative Genomics (BioFig), Lisboa, Portugal; 4) Instituto Biomédico de Investigação de Luz e Imagem, Faculdade de Medicina da Universidade de Coimbra, Coimbra, Portugal; 5) Centro de Desenvolvimento da Criança, Hospital Pediátrico de Coimbra, Coimbra, Portugal; 6) The Center for Applied Genomics and Program in Genetics and Genomic Biology, The Hospital for Sick Children, Toronto, Ontario M5G 1L7, Canada; 7) Department of Psychiatry and Behavioural Neurosciences, McMaster University, Hamilton, Ontario L8N 3Z5, Canada.

Recent studies have established copy number variants (CNV) as an important class of rare variation that may cause or increase risk for autism. Deletions and reciprocal duplications of the chromosome 16p13.1 region have been reported in cases of intellectual disability (ID), autism and schizophrenia. Previous findings suggested that deletions are likely to be causal for the patient's phenotype, whereas the role of duplications remains uncertain. To clarify the relevance of 16p13.11 deletions and duplications in autism, we correlated CNVs in this region, identified through a whole genome CNV analysis carried out by the Autism Genome Project Consortium on 1275 patients, with detailed phenotypic data. We have identified 3 patients with rearrangements of the reported 16p13.11 microdeletion/microduplication region (~800kb). One patient carried a deletion, which was observed once in a total of 4964 control subjects of European ancestry. The deletion carrier is a male diagnosed with autism using the ADIR-R and ADOS, has mild ID (IQ=52), and presented severe behavioral problems (aggressiveness and coprolalia) from 2 years of age, but no language delay. Epilepsy and microcephaly, common features reported in some cases, were not present. The family history of this patient was positive for neuropsychiatric problems, with a sister with learning problems, an uncle with schizophrenia and a mother who had two depression episodes. The deletion was validated by qPCR and is larger than the typical deletion described for 16p13.11 (~1.5Mb), including ~15 Refseq genes. Candidate genes in the region include *NDE1*, *NTAN1*, and *RRN3*; mouse orthologues of these genes, when disrupted, result in abnormal neuronal phenotype and/or abnormal behavior. The other two patients with 16p13.11 rearrangements carried a duplication (~800Kb), inherited from their unaffected fathers. Both had normal IQ, language delay, no epilepsy and no dysmorphisms. The unaffected paternal half sister of one of the patients did not carry the rearrangement. Four duplications in this region were identified in the same control population. Together with previous observations, these findings suggest that 16p13.11 duplications may be rare benign or modulatory variants, while deletions are more likely to have a pathogenic role with a variable clinical presentation. Additional large cohorts including multiple psychiatric conditions are needed to fully understand the role of these variants in neuropsychiatric disease.

1832/T

Detection of chromosomal imbalances in children with neurologic disorder by array CGH. *E. Shin[1], K. Lee[1], J. Byun[2], B. Eun[2].* 1) Genome Res Ctr, NeoDin Med Inst, Seoul, Korea; 2) Dept of Pediatrics, Korea Univ Guro Hopital, Seoul, Korea.

Microarray-based CGH has enabled the identification of previously unrecognized submicroscopic chromosome alterations and elucidated the etiologies of known syndromes. We performed a genome-wide assay for pediatrics neurologic disorder using Roche NimbleGen 135K (Roche NimbleGen Inc., USA) arrays on 3 children with epilepsy and seizure. Case 1 was a 12 year old boy with epilepsy, severe seizure, eyesight and hearing impairment, and has lived as a vegetable state since 5 year old. A deletion spanning 2 OMIM genes with an estimated size of 110 Kb was identified, located on chromosome band 1p36.33. In addition, a duplication spanning 2 OMIM genes with an estimated size of 115Kb was identified, located on chromosome band 21q22.13. The deletion is probably the cause of the boy's phenotype, since his clinical features overlap greatly with 1p36 deletion syndrome mentioned in previous reports despite his MELAS mutation. Another genomic imbalance on chromosome 21, involving 2 genes such as TTC3, DSCR2, should be interpreted depending on clinical phenotype as this region is related to Down syndrome. Therefore, the array result of this case was arr 1p36.33(825,513-935,943)x1, 21q22.13(37,432,798-37,548,446)x3 Abnormal Male. Case 2 was a 6 year old girl with rett syndrome, epilepsy, seizure and a behavioral disturbance with rubbing her hands. In case 2, no genomic deletion or duplication was not detected using 135,000 probes, but the patient's phenotype with rett syndrome should be determined with PCR-direct sequencing method for MECP2 on chromosome Xq28. Case 3 was a 4 year old girl with lissencephaly type 1 on brain MRI, seizure and normal karyotype. A deletion spanning 6 OMIM genes those are not related to her phenotype, with an estimated size of 1.81 Mb was identified, located on chromosome band 16p13.11. The deletion of chromosome 16p13.11 is probably the cause of this case, since her clinical features overlap greatly with 16p13.1 deletion syndrome and the array result was arr 16p13.11p12.3(14,957,300-16,768,273)x1 Abnormal Female. Oligonucleotide array CGH offers increased resolution compared to BAC array CGH, FISH, and karyotype analysis. It has enabled the discovery of novel microdeletion and microduplication syndromes and the detection of genomic imbalances of clinical significance will increase knowledge of the human genome by performing genotype-phenotype correlation.

1833/T

A definitive haplotype map derived from homozygous genomes as a basis for disease gene study. *T. Tahira[1], Y. Kukita[2], K. Yahara[3], M. Sonoda[1], K. Yamamoto[1], N. Wake[4], K. Hayashi[5].* 1) Div Genome Analysis, Kyushu Univ/Med Inst Bioreg, Fukuoka, Japan; 2) Research Institute, Osaka Medical Center for Cancer and Cardiovascular Diseases; 3) Division of Life Science System, Fujitsu Kyushu Systems Inc; 4) Department of Gynecology and Obstetrics, Graduate School of Medical Sciences, Kyushu University; 5) Faculty of Agriculture, Kyushu University.

Unexpected complexity in the haplotypes of human genomes, including SNPs and copy number variations (CNVs), has been realized as a cause of variation in disease susceptibility. CNVs are abundant, possibly variable among populations, and can confer various phenotypic variations such as risks to complex diseases. Even with the aid of recently developed genome-wide microarrays, it is still difficult to define CNV status in the target region. Taggability of CNVs with adjacent SNPs is confirmed to some extent, but not fully investigated, because delineating haplotype structure including SNPs and CNVs is difficult in the analysis of diploid cells. We have been proposing determining definitive haplotype, by analyzing a collection of complete hydatidiform moles (CHMs) of Japanese, using high-density DNA arrays. CHMs are tissues carrying duplicated haploid genomes that originated from single sperms. All of the genetic variants in a CHM genome are homozygous. This makes CHMs more advantageous as materials over conventional diploid cells in detecting CNVs, because greater signal to noise ratios in hybridization signals are expected, and data interpretation is not bothered by possible heterozygosities of overlapping CNV segments. We previously analyzed CHM genomes using Affymetrix SNP 6.0 array and detected many new CNVs in addition to reported ones. We also showed that boundary of CNV is variable even in the same haplotype background, indicating that haplotype-preferential occurrence of CNV event (Kukita et al, Am. J. Hum. Genet., 2010). Here we extended SNP and CNV analyses by genotyping the same CHM samples with Illumina beadarrays. By merging genotyping results of Affymetrix SNP 6.0 and Illumina 1M-duo, we obtained a definitive haplotype map that included 1.7 million SNPs. For the markers common to these two platforms, genotyping results were more than 99.9% concordant. The merging of CNV genotype data of the two systems is underway. Our previous finding of haplotype-preferential recurrences of CNV events were confirmed.

1834/T

High-resolution breakpoint mapping of 38 regulatory and *FOXL2* encompassing deletions in BPES using targeted microarrays. *H. Verdin[1], B. D'haene[1], P. Lapunzina[2], J. Nevado[2], B. Menten[1], E. De Baere[1].* 1) Center for Medical Genetics, Ghent University, Ghent, Belgium; 2) Instituto de Genética Médica Y Molecular (INGEMM), Hospital Universitario La Paz, Universidad Autónoma de Madrid, Madrid, CIBERER, U783-ISCIII, Spain.

Blepharophimosis syndrome (BPES) is an autosomal dominant developmental disorder characterized by a complex eyelid malformation associated or not with ovarian dysfunction. Deletions encompassing *FOXL2* and located outside its transcription unit represent 12% and 5% of molecular defects respectively. In total we identified 28 *FOXL2* encompassing and 10 regulatory deletions, of which the exact breakpoints remained unknown for 36 of them. In order to gain more insight into the mechanisms underlying these rearrangements we aimed to determine the precise location of breakpoints. A second purpose was to further refine genotype-phenotype correlations for extra-ocular features. We used high-resolution tiling arrays with 52,800 probes spaced at a density of ~200 bp to map the breakpoints to an interval that allows validation by junction PCR and sequencing. For 25 deletions, both breakpoint regions could be delineated to less than 7500 bp. The breakpoint regions of seven deletions ranged from 500 bp to 35.5 kb. Four remaining deletions could not be delineated so far. The fine-mapping results were used to design breakpoint PCRs. With regard to genotype-phenotype correlations, an *ATR* encompassing deletion was found in five of eight BPES patients with reported microcephaly. In the three patients with a normal *ATR* copy number, a *cis*-ruption mechanism deregulating *ATR* transcription cannot be excluded however. Interestingly, in two patients with joint abnormalities the *SOX14* gene was located within the deletion, potentially implicating this gene in the pathogenesis of this extra-ocular feature. In conclusion, in the largest series of *FOXL2* encompassing and regulatory deletions reported in BPES, high-resolution breakpoint mapping provided insights in the mechanisms underlying the deletions, and refined genotype-phenotype correlations for extra-ocular features.

1835/T

Common and rare copy number variants in Southeast Asian populations. *H. Xu[1], X. Sim[1], R.TH. Ong[1,2], C. Suo[1], W.T. Tay[3], E.S. Tai[2,4], T.Y. Wong[3,4,5], K.S. Chia[1,2], Y.Y. Teo[2,6,7].* 1) Centre for Molecular Epidemiology, National University of Singapore, Singapore, Singapore; 2) Department of Epidemiology, National University of Singapore, Singapore; 3) Singapore Eye Research Institute, Singapore National Eye Centre, Singapore; 4) Department of Medicine, National University of Singapore, Singapore; 5) Centre for Eye Research Australia, University of Melbourne, Australia; 6) Department of Statistics and Applied Probability, National Unversity of Singapore, Singapore; 7) Genome Institute of Singapore, Agency for Science, Technology and Research, Sinagpore.

Copy number variants (CNVs) extend our understanding of the genetic diversity in humans. It has been reported that Caucasians and Asians share only a small fraction of their CNVs, and the frequencies of common CNVs vary significantly across populations. Since most CNV studies to date were conducted in Caucasians or small Asian samples, the distribution and characteristics of CNVs in Asian populations remain largely unexplored, especially for rare CNVs which have emerged as important genetic factors for complex traits. In the present study, we perform an in-depth investigation of common and rare CNVs in Southeast Asian populations. Illumina Human 1MDuo and 610KQuad arrays were used to genotype 3 population-based cohorts of Southeast Asian origin: Chinese (CHS, n=1945), Malays (MAS, n=2399) and Indians (INS, n=2217) in Singapore. CNVs were identified with pennCNV using stringent quality control criteria (e.g., SD of LRR <0.3, # SNPs/CNV >=10), and will be validated using quantiSNP. After QC we found ~16 CNVs/individual and the ratio of loss to gain events is ~2:1 in each population. The median size of deletions is shorter than that of duplications (CHS: 34.5kb vs. 63.5kb, MAS: 64.2kb vs. 83.4kb, INS: 60.8kb vs. 93.7kb). In each population, ~10% of the CNVs are considered novel as they do not overlap with any CNV in the DGV (Database of Genomic Variants). The frequencies of 90% of novel CNVs are lower than 0.5%, but >1/2 of them are shared by at least two populations and thus considered reliable calls. 60% of CNVs in each population overlap with refGene gene regions. CNVRs were constructed by merging overlapping CNVs from different individuals into discrete loci. In each population, about 4,000 unique CNVRs were defined, covering ~20% of the genome. The Malay and Indian populations share 75% of their CNVRs, while the Chinese population only shares 60% with either group. The population differentiation of these CNVRs will be further explored. To study rare CNVs, we have extracted CNVs with 0.5-5% frequency in each population, which constitute 15-20% of total CNVs. The median sizes are slightly smaller than the overall groups but the loss/gain proportions remain similar. 60% of rare CNVs overlap with refGene gene regions, and >60% are shared by at least two populations. Our samples have been quantified for various clinical traits, such as lipid levels and blood pressure, and we are investigating the effect of rare/novel CNVs on these phenotypes.

1836/T

The effect of diet in the evolution of human metabolic gene copy number variation among Korean and Mongolian population. *M. Yavartanoo[1, 2], J. Kim[1, 2, 4], Y. Ju[2], S. Lee[2, 3], J. Shin[2], H. Kim[2, 3], D. Lee[2, 3], J.S. Seo[1, 2, 3, 4].* 1) Department of Biochemistry and Molecular Biology, Seoul National University, college of Medicine, Seoul Korea; 2) Genomic Medicine Insitute, Medical Research Center, Seoul National University, Seoul 110-799 Korea; 3) Department of Biomedical Science, Seoul National University, Graduate School, Seoul, 110-799; 4) Psoma Therapeutic Inc., Seoul, 110-799.

The difference in the diet between agricultural societies and hunter gatherers may result to change in the copy number of genes that are related to digestion. According to previous studies, copy number of salivary amylase gene (AMY1) correlated positively with salivary amylase protein level such that individuals with high starch diet have, on the average, more copies of the AMY1 gene compared to those with low starch diet. The objective of this study is to determine the copy number variation of these different genes involved in milk, protein and fat digestion, namely, LCT, MCM6, AMY1, PNL1P, LIF, TRYX3 and BACE among Mongolians and Koreans who were known to have differences in diet. Method. Real time PCR was used to analyze the DNA samples. Results. Mongolian subjects were observed to have higher lactase gene copy number compared to Korean subjects. Conclusion. We have demonstrated that the pattern of variation in copy number of the human lactase gene is affected by the amount of intake of dairy products in their diet.

1837/T

Systematic detection of chromosomal abnormalities from multiple genome-wide association scans. *M. Yeager[1,2], K.B. Jacobs[1,2], L. Burdett[1,2], Z. Wang[1,2], L. Perez-Jurado[3,4], M.G. Cullen[1,2], S.J. Chanock[2].* 1) Core Genotyping Facility, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD 21702; 2) Division of Cancer Epidemiology and Genetics, NCI, NCI, NIH, Bethesda, MD 20892; 3) Department de Ciències Experimentals i de la Salut, Universitat Pompeu Fabra, E-08003 Barcelona, Spain; 4) CIBER de Enfermedades Raras, CIBERER, E-08003 Barcelona, Spain.

Over sixty thousand human DNA samples have been genotyped at the CGF using Illumina BeadArray assays, the vast majority derived from leukocytes found in blood samples and oral buccal cells (stratified squamous epithelium) and a small proportion from lymphoblastoid cell lines. While these samples were scanned to perform genome-wide association studies (GWAS) to detect heritable genetic risk factors of numerous cancer sites, the probe intensity data have been analyzed to detect large chromosomal abnormalities, including aneuploidy, tissue mosaicism, and large copy-number variants. Analyses were performed in two stages: 1) rapid detection of outlier missing genotype rates and heterozygosity using a sliding window algorithm along each chromosome to nominate samples for more in-depth study; 2) application of a more computationally intensive copy number segmentation algorithm on chromosomes nominated in stage 1 to estimate chromosomal breakpoints using normalized SNP probe intensity values adjusted for local GC and CpG sequence context. Probe intensity and allelic distribution plots were generated and annotated with the estimate CNV breakpoints for manual classification. This method is able to detect large chromosomal deletions and duplications, but not specific rearrangements or translocations. Although this effort is ongoing, preliminary results show significantly higher rates of chromosomal abnormalities than expected from published population rates for DNA extracted from both blood and buccal samples. Funded by NCI Contract No. HHSN261200800001E.

1838/T

Copy Number Variations in Three Singapore Populations. *R.Y.Y. Yong, L.S.H. Gan, S.H. Ng, E.P.H. Yap.* Defence Medical & Environmental Research Institute, DSO National Laboratories, Sinagpore.

Copy Number Variations (CNV) is now known to be an important class of global genetic variation in the human genome, and contributes to human genetic and phenotypic diversity. Therefore, cataloguing the pattern and frequency of CNV in the general populations is fundamental in understanding its role in human phenotypic diversity, and differentiating between benign verses pathogenic CNVs in future disease studies. We report here a preliminary database of autosomal CNVs larger than 10 Kb, detected in 304 healthy unrelated individuals (103 Chinese, 100 Malay and 101 Indian) representing 3 ethnic groups of Singapore. The samples were typed on high-density oligonucleotide microarray, Affymetrix SNP Array 6.0, and CNV called by GTC v3.0.2. In order to reduce false positive, we focused on CNV greater than 10Kb covered by at least 5 probes. Assessing only the autosomes, a total of 10,770 CNV events were recorded, which mapped to 1,884 non-redundant genomic regions (CNVR). About 38% of these CNVR are not reported in DGV (Database of Genomic Variants). Approximately 21.7% of these CNVR has a polymorphism frequency of >1%, while 6.3% has frequency of >5%. About 11.3% of the CNVR can be found at >1% in all 3 populations. The ratio of deletion verses duplication is about 2:1. The median size of deletion is 31 Kb while duplication is 67 Kb. The average CNV size and ratio of deletion verses duplication are similar across the 3 populations. There are higher number of population-specific CNV in the Indian, and more sharing of CNV between the Chinese and Malay. This work will provide a useful resource to assist future assessment of CNV in the context of human variation and disease susceptibility for Singapore populations. The work is on-going. We plan to do further validation through CNV-calling with other software and via wet-lab approach using real-time PCR for copy number quantification.

1839/T

Germline copy number variations in *BRCA1* mutation carrier. *K. Yoshihara[1], S. Adachi[1], A. Tajima[2], M. Sekine[3], T. Yahata[1], I. Inoue[2], K. Tanaka[1].* 1) Obstetrics and Gynecology, Niigata University, Niigata , Japan; 2) Molecular Life Science, Tokai University, Isehara, Japan; 3) Obstetrics and Gynecology, Nagaoka Red Cross Hospital, Nagaoka, Japan.

The lifetime risks of ovarian cancer for *BRCA1* mutation carriers show a wide range, from 24 to 44% by age 70 years. Therefore, the prediction of individual's lifetime risk for developing ovarian cancer in women with *BRCA1* mutation is very important in clinical management of *BRCA1* carriers. In this study, we aimed to investigate characteristics of germline copy number variations (CNVs) in *BRCA1* mutation carriers and to clarify the relationship between germline CNVs and susceptibility to ovarian cancer. Germline CNVs in 68 *BRCA1* carriers, 33 sporadic ovarian cancer patients and 47 healthy Japanese women were analyzed by both signal intensity and genotyping data using the Affymetrix Genome-Wide Human SNP Array 6.0. Total number of CNVs per genome was more frequent in sporadic group (mean, 24.0±5.3) than in *BRCA1* group (mean, 20.9±5.8; posthoc $p < 0.05$) or in normal group (mean, 20.5±5.8; posthoc $p < 0.05$). Amplifications per genome in sporadic group were more than in other two groups. On the other hand, deletions per genome in *BRCA1* group were more than in the others. Especially, microdeletions defined as segment size less than 50kb were significantly increased in *BRCA1* group (mean, 9.4±3.5) compared to sporadic group (mean, 6.5±3.3; posthoc $p<0.001$) or normal group (mean, 7.9±3.8; posthoc $p<0.05$). Moreover, several CNVs specific to *BRCA1* mutation carriers were detected in chromosome X. Although there was no difference in number of genomic regions between *BRCA1* mutation carriers unaffected with ovarian cancer and *BRCA1* mutation carriers affected with ovarian cancer, we identified some CNVs detected only in B*RCA1*-mutated daughter affected with ovarian cancer compared to *BRCA1*-mutated mother unaffected with ovarian cancer. We reported on the genome-wide profile of germline CNVs in *BRCA1* mutation carriers. Further study will be necessary to uncover the role of gernline CNVs in *BRCA1* mutation carriers.

1840/T

High resolution analysis of CNV breakpoints reveals potentially predisposing sequence motifs and variable mechanisms of genomic rearrangement. *H. Yu[1], E. Geiger[1], H. Xie[3], J. Perin[3], X. Gai[3], C. Haldeman-Englert[4], T. Shaikh[1,2].* 1) Department of Pediatrics, University of Colorado Denver, Aurora, CO; 2) IDDRC, University of Colorado Denver, Aurora, CO; 3) Bioinformatics Core, The Children's Hospital of Philadelphia, Philadelphia, PA; 4) Department of Pediatrics, Wake Forest University, School of Medicine, Winston-Salem, NC.

Copy number variations (CNVs) are an increasingly recognized cause of human disease and variation. Unstable genome architecture promotes the rearrangements which lead to CNVs associated with recurrent microdeletions and microduplications. A majority of these pathogenic CNVs appear to be mediated by non-allelic homologous recombination (NAHR) between segmental duplications (SDs). In this study, we have identified and analyzed 100 pathogenic CNVs, 70 deletions and 30 duplications. The sizes of CNVs ranged from 10Kb to more than 56Mb. The rearrangement breakpoints of 17/100 CNVs localized to highly homologous SDs, a significant enrichment, considering that SDs only make up 5% of the genome. Almost all of these 17 CNVs were found to be recurrent in multiple individuals and the frequency was directly proportional to the size and sequence identity shared between the paralogous SDs. The remaining 83 CNVs were mostly non-recurrent, singletons with a few instances of overlapping CNVs but with different breakpoints. We used custom-designed, tiling microarrays to refine the rearrangement breakpoints in a subset of these allowing the rapid cloning and sequencing of 42 breakpoints. Twenty-five breakpoints localized to repetitive DNA elements like Alus and LINEs, but none of these CNVs appeared to result from NAHR between repeats. The observation of microhomology and small nucleotide insertions at the majority of breakpoint junctions suggested alternative mechanisms for rearrangement for non-recurrent CNVs. These include, non-homologous end joining mechanisms (NHEJ) and/or replication-based mechanisms such as fork stalling and template switching (FoSTeS) or microhomology-mediated break-induced replication (MMBIR). We further analyzed each of the breakpoint regions for sequence motifs and palindromic sequences that have previously been identified at or near rearrangement breakpoints. Although many such "hotspot" motifs were identified, none were significantly enriched when compared to control sequences. Analysis using GLAM2 motif search program identified a Poly(-dA:dT) tract in the vicinity (average distance from BP is 85 bp) of 41/42 breakpoints. Poly(dA:dT) tracts have been shown to be important in nucleosome organization suggesting a correlation between breakpoints and chromatin structure. It will be important to determine if these variable rearrangement mechanisms are observed as more CNV breakpoints, both pathogenic and normal variants, are analyzed.

1841/T

Investigation of the break-point integrity of copy number variants suggests dynamic transmission. *F.R. Zahir[1], C.F. Boerkoel[1], J. Michaud[2], J.M. Friedman[1].* 1) Department of Medical Genetics and Child & Family Research Institute, Univ British Columbia, Vancouver, BC, Canada; 2) CHU Sainte-Justine Research Centre, Montreal, Quebec, Canada.

Copy number variants (CNVs) account for the most genomic variation between individuals. Recently described mechanisms for CNV formation highlight a replication based model (e.g., FoSTeS, MMBIR). We hypothesized that CNVs which have occurred via replication based models are less likely to preserve their structural integrity through cell divisions. We investigated the status of inherited CNVs in trios comprised of a child with idiopathic intellectual disability and both normal parents. We assessed CNV data obtained from 15 trios who were tested on Affymetrix 6.0 SNP chips®. 12 CNVs >100kb and involving >30 markers were identified that showed a 'shift' in one or more break-points in the child compared to that of the transmitting parent. Using quantitative PCR we confirmed and mapped break-point shifts in 3 of 5 CNVs tested in the child and both parents.

Two cases had inherited gains of chromosome 3q26.1. One child exhibited a shift of ~73 kb on one end of a paternally inherited CNV (i.e., the child's CNV was 73 kb longer than the corresponding CNV found in the father). The other child had a complex pattern of gained material interspersed with normal material on a maternally inherited CNV, whereas the parent had a single gain. No SNP probes were present in the affected region precluding parent-of-origin analysis for these cases. The third child had a paternally inherited duplication of chromosome 10q11.22. The child's CNV was 823 kb, while the father's was 802 kb. The region duplicated in the child but not the father involves the SYT15 gene. We found 8 informative SNP probes within the common CNV segment excluding sequence involved in segmental duplication. The mean ratio of the paternal allele:maternal allele for these SNPs was 1.8 (95% confidence intervals ±0.91), compared to a mean ratio of 0.98 (95% confidence intervals ±0.0055) for allele A: allele B for the entire chromosome 3 in the child, indicating that the duplication is indeed on the paternally transmitted chromosome.

In summary we have demonstrated that CNVs can be prone to breakpoint shifting during transmission. This work has important implications for our understanding of genomic stability. It re-addresses judging pathogenicity of inherited CNVs when there is a possibility for changes in CNV architecture during inheritance.

1842/T

Using family data as a verification standard to evaluate CNV calling strategies. *X. Zheng[1], J.R. Shaffer[1], C. McHugh[2], C. Laurie[2], M. Melbye[3], J.C. Murray[4], M.L. Marazita[1], E. Feingold[1].* 1) Biostatistics/Grad Sch PubHlth, Univ Pittsburgh, Pittsburgh, PA; 2) University of Washington, Seattle, WA; 3) Statens Serum Institut, Copenhagen, Denmark; 4) University of Iowa, Iowa City, IA.

A major statistical concern for all CNV detection algorithms is their reliability and repeatability. However, it is difficult to evaluate the validity of CNV calling strategies due to the lack of gold standard data that would tell us which CNVs are real. We propose that if called CNVs are reproducibly called in duplicate samples, or inherited from parent to child, then these can be considered validated CNVs. We used two large family-based Genome-Wide Association Study (GWAS) datasets from the GENEVA consortium (dental caries and premature birth) to look at concordance rates of CNV calls between duplicate samples, parent-child pairs, and unrelated pairs. Our goal was to make recommendations for appropriate ways to filter and use CNV calls in genome-wide association studies. We used PennCNV as our primary CNV-calling algorithm, and tested CNV calls using different datasets and different marker sets, with and without GC model adjustment, and with various filters on CNVs and samples. We found that using the Illumina core HumanHap550 SNP set, which was available for both studies, we saw duplicate concordance rates of approximately 55% and parent-child inheritance rates of approximately 28% in both datasets. This suggests an overall "reliability" rate of approximately 55% for these CNV calls. GC model adjustment, sample quality filtering, stratification on DNA sample type, and stratifying on the number of CNVs called for an individual all had very little effect on these reliability measures. Stratification on CNV size did have some effect, with duplicate concordance rates ranging from 31% for the smallest CNVs to 64% for the largest. Adding common CNV markers from the Illumina Human660W-Quad increased the number of CNVs called by a factor of six, but these common CNV calls had only slightly higher concordance and inheritance rates than the rare CNV calls. Overall, our results show that it is probably not possible to find a CNV calling strategy (including filtering and algorithm) that will give us a set of "reliable" CNV calls, at least using currently available chips. CNV calls will always need to be understood as having high error rates. But if we understand the features of that error process, we can still use them appropriately in genetic association studies. NIH grants DE018903, HG004423, T32 MH015169 and NIH contract HHSN268200782096C.

1843/T

Genomic methylation deserts are hotspots of structural mutability in the human genome. *J. Li[1,2], R.A. Harris[1], P. Stankiewicz[1], S. Cheung[1], A. Patel[1], S. Kang[1], C. Shaw[1], A.C. Chinault[1], L.D. White[1], T. Gambin[3], A. Gambin[4], J.R. Lupski[1,5,6], A. Milosavljevic[1,2].* 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, 77030; 2) Program in Structural and Computational Biology and Molecular Biophysics, Baylor College of Medicine, Houston, TX, 77030; 3) Institute of Computer Science, Warsaw University of Technology, Warsaw, Poland; 4) Institute of Informatics, Warsaw University, Banacha 2, 02-097, Poland; 5) Department of Pediatrics, Baylor College of Medicine, Houston, TX, 77030; 6) Texas Children's Hospital, Houston, TX, 77030.

Approximately 10% of the human genome may be structurally polymorphic at submicroscopic scale, a much larger fraction than covered by SNPs. Early analyses of disease-associated hypermutable loci revealed the role of Non-Allelic Homologous Recombination (NAHR) in mediating deletions, duplications and inversions between homologous Low Copy Repeats (LCRs) and led to the concept of genomic disorders. Despite these early advances, the mechanistic basis for the highly nonuniform distribution of structural instability and structural polymorphisms observed across the human genome remains to be discovered. Prompted by the recently discovered association of hypomethylation and structural instability in white-cheeked gibbons, we examined the relative magnitudes of genome-wide submicroscopic structural mutability in humans attributable to both LCR-mediated NAHR and hypomethylation. We combined evidence from human genome evolution, structural polymorphisms in the human population, and disease studies. Our results show that Loci comprising 1.5% of the human genome that are extremely hypomethylated in the germline, which we refer to as methylation deserts, harbor 15% of 522 structural rearrangements that occurred in the human genome since the branching of human and chimpanzee. Permutation testing indicates that 22% of human-specific structural rearrangements are attributable to hypomethylation, a significantly larger fraction than attributable to NAHR mediated by LCRs. We validate the result by designing a custom whole-genome oligo-aCGH chip probing more densely within NAHR-promoting LCR regions and by applying it to 400 human samples, identifying more than 2,000 CNVs. Analysis of these data combined with the publicly available structural variation data collected on 270 HapMap samples, 450 HapMap samples, 19,000 samples from eight disease studies, and 6,672 samples from a recent study by the International Schizophrenia Consortium confirms stronger association of structural mutability with germline hypomethylation than with LCR-mediated NAHR. The individuals carrying larger numbers of CNVs and schizophrenia patients tend to have a larger fraction of CNVs within hypomethylated regions, pointing to a new connection between the epigenome, mutability, evolution and human disease.

1844/T

The single-nucleotide structure of copy number variants (CNVs) and functional impact on gene expression. *Y. Ju[1,3], J. Kim[1,4], D. Hong[1], S. Lee[2], S. Kim[3], H. Park[5], C. Lee[5], J. Seo[1,3,4].* 1) Genomic Medicine Institute, Seoul National University, Seoul, Korea; 2) Department of Biomedical Science, Seoul National University College of Medicine, Seoul, Korea; 3) MacroGen, Seoul, Korea; 4) Psoma Therapeutics, Seoul, Korea; 5) Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

The fine-scale structure of genomic variants and its functional influence on gene expression Since 2004, CNVs have been discovered to determine what segments of human genome are frequently affected by deletion or amplification. However, its single-nucleotide level structure and the influence on gene expression are still under-ascertained. To characterize the structural and functional nature of human CNVs as a whole, we analyzed whole genome and transcriptome of 10 Asian individuals using massively parallel DNA and RNA sequencing (Illumina genome analyzer) as well as ultra-high resolution whole-genome tiling CGH arrays (Custom-designed Agilent platform), comprising 24 million probes. We identified ~ 700 accurate personal by combining read-depth data of sequencing coverage (average read-depth is 25x each individual) and the array CGH data. We characterized the precise breakpoint sequences of ~ 60% of the CNVs by analyzing the short-reads aligned on near the CNV breakpoint locations expected from the CGH microarrays. In addition we attempted to identify the location of copy number gain segments using short-read data. From the characteristics of breakpoint sequences, the mechanisms for CNV genesis are suggested. Then we figured out the influence of CNV on gene expression. Generally, CNV affected genes showed weak but positive relationship between copy number and expression level. However, expression of several specific genes was strongly controlled by CNVs, which suggests the potential influence CNVs on phenotypic variations and human complex diseases.

1845/T

Chromosome-wide mapping of long-range interactions involved in Smith-Magenis and Potocki-Lupski syndromes. *N. Gheldof[1], B. Lajoie[2], J. Molina[3], G. Ricard[1], J. Chrast[1], J.R. Lupski[4], J. Dekker[2], K. Walz[3], A. Reymond[1].* 1) Center of Integrative Genomics, University of Lausanne, Lausanne, Switzerland; 2) University of Massachusetts Medical School, Worcester, MA, USA; 3) University of Miami, Miami, FL, USA; 4) Baylor College of Medicine, Houston, TX, USA.

Copy number variations (CNVs) affect expression levels of the genes that map within the affected region, but also of genes located in the flanking regions. To understand the mechanisms at play, we studied gene expression and chromatin conformation in mice models of Smith-Magenis (SMS) and Potocki-Lupski (PTLS) syndromes, containing a microdeletion and its reciprocal microduplication, respectively, on mouse chromosome 11 (MMU11). We profiled the transcriptome of embryonic fibroblasts of mice with one, two, three and uniallelic two copies of the SMS/PTLS region in an otherwise identical genetic background. As expected, the most differentially expressed transcripts are mapping to the SMS/PTLS interval, but a significant proportion of most differentially expressed genes also map to the rest of MMU11. We hypothesized that these chromosome-wide effects might be caused by changes in long-range interactions along the entire chromosome. We therefore analyzed the chromatin structure of MMU11 of these four mouse strains by using the chromosome conformation capture carbon copy (5C) technology. We designed 1,748 5C primers that combined detect 708,651 long- and short-range interactions. Deep sequencing of the interacting fragments of the wild-type 5C library yielded 15 million reads. Further analysis of the data will allow comparing the chromatin structure and the presence of physical contacts between functionally interacting genomic elements in genotypes differing only by the number of copies of a CNV, and correlating these interaction maps with the observed differential gene expression. Detailed investigations of the different mechanisms by which CNVs alter the architecture of chromosomes are warranted to shed light on how CNVs influence gene expression. In addition, this study will provide a first comprehensive chromatin interaction map of an entire mouse chromosome.

1846/T

Mobile interspersed repeats are major structural variants in the human genome. *C. Huang[1,2], A. Schneider[3], Y. Lu[1,2], T. Niranjan[2], P. Shen[3], M. Robinson[1], J. Steranka[1,2], D. Valle[1], C. Civin[4,6], T. Wang[1], S. Wheelan[4,5], H. Ji[5], J. Boeke[2,4], K. Burns[3,4].* 1) Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore MD; 2) High Throughput Biology Center, Johns Hopkins University School of Medicine, Baltimore MD; 3) Department of Pathology, Johns Hopkins University School of Medicine, Baltimore MD; 4) Department of Oncology, Johns Hopkins University School of Medicine, Baltimore MD; 5) Department of Biostatistics, Bloomberg School of Public Health, Johns Hopkins University, 615 North Wolfe Street, Baltimore MD; 6) Center for Stem Cell Biology & Regenerative Medicine; Department of Pediatrics, University of Maryland School of Medicine, Baltimore MD.

Despite the importance of characterizing structural variants in the human genome, no high-throughput genome-wide approach exists for detecting interspersed repeats. Thus, degrees to which mobile DNAs contribute to genetic diversity, heritable disease, and oncogenesis are sources of speculation. We exploited transposon insertion profiling by microarray (TIP-chip) to map human L1(Ta) retrotransposons (LINE-1s) genome-wide. This identified numerous novel human L1(Ta) insertional polymorphisms with highly variant allelic frequencies. We also explored TIP-chip's usefulness in candidate phenotype-associated allele discovery in clinical cohorts. Our data suggest occurrence of new insertions is more than double previous estimates, and that these repeats are under-recognized as sources of human genomic and phenotypic diversity. We have just begun to probe the complexity of human L1(Ta) polymorphisms, and as TIP-chip is applied to Alu SINEs, it will expand the catalog of genomic variants further still.

1847/T

A statistical model for loss of heterozygosity among tightly-linked SNP genotypes. *P. Scheet[1,2,3], S. Vattathil[1,2], R. Xia[1,3], Y. Guan[4].* 1) Dept. of Epidemiology, U. of Texas M. D. Anderson Cancer Ctr, Houston, TX; 2) U. of Texas Graduate School of Biomedical Sciences, Houston, TX; 3) Div. of Biostatistics, U. of Texas School of Public Health, Houston, TX; 4) Dept. of Statistics, Dept. of Human Genetics, Univ. of Chicago, Chicago, IL.

In SNP microarray data from unpaired tumor samples or constitutional DNA only, inferring loss of heterozygosity (LOH) is typically conducted based on patterns of SNP genotypes, namely the existence of unusually long runs of homozygosity. However, run length alone does not take into account all available information, since local variation in allele and haplotype frequencies also affects the size of homozygous regions. Here we present a model that accounts for these population-specific features of the data to enable more precise inference of LOH due to somatic events such as mitotic recombination, chromosome deletion, or gene conversion.

The statistical model is based on a multiple-layer hidden Markov model (HMM). In traditional "single layer" HMMs for genotype data, the hidden states represent template or consensus haplotypes. In this model, the hidden state space is expanded to include an LOH process that, when active, renders alleles from a single haplotype to be presented as homozygous genotypes. We assume the separate processes that capture linkage disequilibrium and somatic events leading to LOH to be independent a priori, but dependent conditional on the data. Our model can be used to estimate boundaries and obtain posterior probabilities of LOH events. We also develop a likelihood ratio test to quantify the evidence of somatic LOH in specific regions.

We apply our method to simulated data and compare results to those from more restrictive implementations to test the utility gained from relaxing individual assumptions, such as allowing for varying allele frequencies, accounting for the dependence among alleles at nearby markers (linkage disequilibrium), and modeling uncertainty in observed genotypes. Our method accommodates genotyping error and phase uncertainty, and is tractable for thousands of samples from whole-genome data sources, such as SNP arrays and next generation DNA sequencing data.

1848/T

Molecular diagnosis of unknown alpha-thalassemia deletions. *M. Fallah[1], Z. Sharifi[1], P. Fouladi[1], F. Rahimi-nezhad[1], R. Vahidi[1], M. Feizpour[1], S. Kianfar[1], M. Sadeghi[1], H. Bagherian[1], S. Zinali[1, 2].* 1) Kawsar Human Genetics Research Center, Tehran, Tehran, Iran; 2) Dep't of Mol. Med., Biotech Research Center, Pasteur Institute of Iran, Tehran, Iran.

Iran is located in world thalassemia belt and alpha-thalassemia is very prevalent in the country. Most of alpha thalassemia mutations include large deletion of one or both alpha globin genes. It play an important role to make a correct decision for performing prenatal diagnosis (PND) and differentiating unknown alpha-thalassemia from normal HbA2 beta thalassemia. Multiplex ligation-dependent probe amplification (MLPA), a simple technique appropriate for investigation of large deletions with unknown border, was used to determine new deletion in cases suspected of having alpha-thalassemia without any known mutation in alpha-globin gene cluster. Couples referred to Kawsar Genomics Center for PND, investigated for common deletional alpha-globin mutations and point mutation using multiplex Gap-PCR and direct sequencing respectively. Those remained unknown, further investigated for other deletions by MLPA methods. After denaturation, hybridization and ligation, PCR-amplification was performed with the specific SALSA primers. Electrophoresis of PCR products performed using ABI-3130 genetic-analyzer. Data analysis performed using GeneMarker V1.6 software. Conventional molecular study of alpha globin gene mutation for common deletions and point mutations, revealed a mutation in more than 75% of cases. From those remained unknown, 50 suspected cases were investigated further using gene dosage study. MLPA study of globin gene cluster determined a variety of diverse deletion patterns in probe set in 36 (72%) cases. Deletion patterns expanding from 3.5 to 9.3 kb upstream HBZ up to exon 3 HBQ1 (3.7 kb downstream HBA1). Span of deletion lengths varied from at least 9.1 kb up to 67.2 kb. Our study, showed using MLPA can increase accuracy of prenatal diagnosis for alpha thalassemia especially when we face with cases suspected to have large deletion.

1849/T

RNA-seq uncovers the influence of copy number variants on transcriptome diversity. *E. Ait Yahya Graison, A. Reymond.* CIG, Univ Lausanne, Lausanne, Switzerland.

Copy number variation (CNV) of DNA segments has been identified as a major source of genetic diversity, but a comprehensive understanding of the phenotypic effect of these structural variations is only beginning to emerge. Our group and others established extensive maps of CNVs in wild mice and inbred strains. These variable regions cover ~11% of their autosomal genome. CNVs are suggested to shape tissue transcriptomes on a global scale and thus represent a substantial source for within-species phenotypic variation. The recently emerged RNA-seq method has brought transcriptome analysis to a new level, because it addresses both gene expression and alternative splicing events simultaneously. We took advantages of this new technology to unravel the effects of CNVs on expression at the nucleotide rather than locus resolution level. We generated by ultra high-throughput sequencing >230 millions 75-bp RNA-seq reads from brain of three mouse inbred strains (129S2, DBA/2J (D2) and C57BL/6J (BL6)) to monitor expression changes of transcripts that map within and outside genomic regions that vary in copy numbers. We used TopHat v1.0.13 and Cufflinks v0.8.2 to map, assemble and estimate the abundance of assembled isoforms, respectively. When compared to the BL6 reference sample, 13,656 isoforms (representing 9,445 genes) and 11,754 splicing variants (9,034 genes) are differentially expressed in 129S2 and D2 brain, respectively. Among these, we observed a significant enrichment for CNV genes (p-value=2.55e-06 for 129S2, p-value=9.405e-09 for D2) meaning that alternative transcripts that derive from genes varying in copy numbers show significantly more differential expression between strains. This confirms previous observations made on expression arrays but at exon-level, a resolution never achieved before. We also tested difference in splicing events between strains and showed that 371 and 320 genes exhibit significant differential splicing between isoforms in 129S2 and D2 brain samples, respectively, compared to the BL6 reference. We are currently analyzing liver transcriptome sequencing and are, thus, producing the first transcriptome comparison at the nucleotide level of normal individuals of a population. This study provides a unique opportunity to extensively gauge the influence of CNVs on the transcriptome complexity and regulation.

1850/T

Exploration of European Type 2 Diabetes loci in an African American sample. *S.S. An, N.D. Palmer, J.N. Cooke, L. Lu, J. Divers, B.I. Freedman, M.C.Y. Ng, C.D. Langefeld, D.W. Bowden.* Wake Forest University School of Medicine, Winston-Salem, NC.

Type 2 diabetes (T2D) has a high prevalence in the African American (AA) population. Studies to date have focused on identification of T2D susceptibility genes in European-derived populations. We have performed a genome-wide association study of 965 AA T2D with end stage renal disease (T2D-ESRD) cases and 1029 controls using the Affymetrix 6.0 array to directly genotype 832,357 and impute 2.9 million SNPs. From this analysis, we evaluated previously identified European-derived T2D loci in our AA population. Given our sample size, we have >80% power to detect an OR of 1.23 given a MAF of 20% at 5% significance level, which is consistent with published effect sizes. Our analysis of genotyped and imputed SNPs included 19 known index variants defined in studies of European-derived samples. Of these variants, only the imputed SNP rs7903146 in TCF7L2 was convincingly associated with T2D in AA (p=3.2x10-6). Additionally, genotyped SNP rs486745 in JAZF1 showed a trend (p=0.09). These variants were have risk and protective effects against T2D with OR=1.41 and 0.88, respectively. Locus-wide analysis was performed by expanding to a ±10kb region around the gene or neighboring genes for intergenic variants. In the locus-wide analysis, 197 genotyped and 611 imputed SNPs were nominally associated with T2D with p£0.05. These variants are candidates for replication genotyping in independent sample sets. With the exception of rs7903146 in TCF7L2, no other imputed SNPs were found to have a stronger association with T2D compared to the genotyped SNPs among the 19 loci. Based on this analysis, the SNP with the strongest association with T2D in the directly genotyped variants, rs10906180, is in CAMK1D (p=8.5x10-5 OR=1.32). The European T2D index SNP, rs12779790, is located in an intergenic region between genes CDC123 and CAMK1D. However, this analysis points to CAMK1D as the T2D-susceptibility locus and warrants further evaluation. Within these genes, European LD blocks range between 2-164kb, with an average block size of 43kb, whereas Yoruba LD blocks range between 0-46kb, with an average block size of 13kb or 30% of the average Caucasian LD block. The 30kb difference in LD block size between European and Yoruba populations may facilitate the search for causal variants in the AA population. These results suggest that the genetic architecture of T2D in the AA population is unique and invaluable in the efforts to identify causal T2D variants.

1851/T

Changing with the times: the human reference genome. *V.A. Schneider[1], P. Flicek[2], T. Graves[3], T. Hubbard[4], D. Church[1].* 1) NCBI, Bethesda, MD 28092; 2) EBI, Hinxton, Cambridge, CB10 1SD, U.K; 3) The Genome Center at Washington University, St. Louis, MO 63108; 4) The Wellcome Trust Sanger Institute, Hinxton, Cambridge, CB10 1SA, U.K.

Major efforts to sequence and assemble the human genome culminated in 2004, resulting in a reference assembly of very high coverage and quality. Since that time, the human reference genome has played critical and diverse roles in many different aspects of biological research. Among the many important findings facilitated by the reference genome was the discovery of an unanticipated degree of genetic variation between individuals, coupled with the realization that the extant assembly is insufficient in its representation of these often divergent and complex genomic regions. Providing alternative assemblies of such regions is a major focus of the Genome Reference Consortium (GRC), the group responsible for the reference genomes of human and mouse (http://genomereference.org). In 2009, the GRC released the current version of the human reference genome, GRCh37, which for the first time places alternate locus representations into a chromosome context. We will review these alternative assemblies and demonstrate their importance to personal genomics projects (especially those utilizing short read technologies) and improving phenotype to genotype correlations. The GRC also strives to correct sequencing errors and close the remaining gaps in the reference genome. While such assembly updates are necessary to provide the most accurate reference sequence and are crucial to researchers working in poorly-represented regions, frequent assembly releases disrupt the coordinate system relied upon by other researchers working at the whole-genome level. To balance the need for timely updates with the need for a relatively stable coordinate system, the GRC has now developed a system of assembly patches. The patches consist of new or updated sequence contigs defined outside the coordinate system of the current assembly that can be released between full assembly updates. Patches will be incorporated into the reference assembly at the time of the next full update. We will present the first set of assembly patches for GRCh37 and review their relationship to the current and future reference genomes, as well as their biological importance. The work presented is a collaborative effort of the GRC member institutions: NCBI, EBI, The Genome Center at Washington University and The Wellcome Trust Sanger Institute.

1852/T

Jumping Genomes and Hanging Breakpoints: Novel sequencing methods for characterizing balanced rearrangements in developmental disorders. *M. Talkowski[1,2,5], C. Ernst[1], S. Liu[1,4], C. Hanscom[1], B. Mudduk-rishna[4], A. Heilbut[1], A. Kirby[1,5], T. Ohsumi[4], M. Borowsky[4], M.J. Daly[1,2,5], C.C. Morton[3,5], J.F. Gusella[1,2,5].* 1) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA; 2) Departments of Genetics and Neurology, Harvard Medical School, Boston, MA; 3) Departments of Obstetrics and Gynecology, Reproductive Biology, Pathology, Harvard Medical School, Boston, MA; 4) Department of Molecular Biology, Massachusetts General Hospital, Boston, MA; 5) Program in Medical and Population Genetics, Broad Institute of M.I.T. and Harvard.

The recent technical revolution in genomics has greatly enhanced our ability to survey common variation and copy number changes associated with complex disorders, yet the contribution of balanced rearrangements remains unclear. Current array based methods are blind to their presence and traditional molecular approaches are inadequate to define precise breakpoints rapidly. In an effort to address this void in autism and related developmental disorders, we designed a series of sequencing experiments to precisely characterize balanced genomic rearrangements to base pair resolution. As a baseline analysis of the simplest approach to this problem we performed whole genome sequencing to map rearrangement breakpoints from small and large insert paired end libraries using published Illumina methods. Next, we developed two large-insert “jumping” library methods that use custom bar coded internal adapters to improve genomic coverage and diversity. Occasionally, patient samples are mapped to megabase resolution or less by cytogenetic methods and thus do not require whole-genome sequencing. To enable rapid survey of these rearrangements in a high throughput manner, we designed a novel capture sequencing strategy. We used individual-specific array-based probes of known genomic sequence to capture regions of interest in eight subjects with different chromosomal translocations. The pool of captured DNA fragments were sequenced on a single lane of a flow cell without indexing, thus delivering substantial improvements in time and cost over traditional approaches. We further created an algorithm to exhaustively identify split reads spanning structural variation breakpoints that would otherwise fail to align. Our experiments successfully detected translocation and inversion breakpoints in children with autism and other developmental disorders. The results suggest several causal loci in previously defined microdeletion syndromes, including 2q23.1 and 2q33.1, and novel targets for autism spectrum disorders. We also find significant complexity at the sequence level in many chromosomal rearrangements, and analyses of genomic signatures mediating such events are ongoing. Our methods have implications ranging from de novo assembly to clinical genetic mapping. The results suggest that highly optimized next-generation sequencing can be leveraged to characterize the contribution of these under-investigated sources of genomic variation to complex disease.

1853/T

NIH Roadmap Epigenomics project data at NCBI. *I. Fingerman, T. Barrett, R. Cohen, T. Hassan, Z. Jiang, P. Ledoux, R. Muertter, L. McDaniel, W. Ratzat, A. Soboleva, X. Zhang, G. Schuler.* National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, 45 Center Drive, Bethesda, MD 20892, USA.

Epigenetics refers to a gene activity state that may be stable over long periods of time, persist through many cell divisions, or inherited through several generations, all without any change to the primary DNA sequence. Epigenetic mechanisms include DNA methylation, post-translational modification of histone proteins and non-coding RNAs. These mechanisms of epigenetic regulation contribute to the epigenome. The distribution of methylated DNA, histone modifications, and non-coding RNA expression can be specific to a particular organism, a particular tissue, or a particular cell. The epigenome is dynamic, influenced by environmental factors and extracellular stimuli, and can change in response to these factors. Misregulation of epigenetic events has been observed in various cancers and human diseases. To better understand this, the NIH Roadmap Epigenomics Mapping Consortium was launched with the goal of producing a public resource of human epigenomic data to catalyze basic biology and disease-oriented research. The Consortium leverages experimental pipelines built around next-generation sequencing technologies to map DNA methylation, histone modifications, chromatin accessibility and small RNA transcripts in stem cells and primary *ex vivo* tissues selected to represent the normal counterparts of tissues and organ systems frequently involved in human disease. Data from the NIH Roadmap Epigenomics project are hosted at NCBI’s Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/roadmap/epigenomics/). This data listings page allows users to browse and query project metadata, as well as download original sequence, alignment and track data files. Furthermore, Roadmap Epigenomics project data have been uploaded to NCBI’s new dedicated Epigenomics database at http://www.ncbi.nlm.nih.gov/epigenomics/. This resource enables users to explore and visualize Roadmap Epigenomics data alongside hundreds of other richly-annotated epigenomics datasets. It provides a unique interface to search and navigate epigenomic data in the context of biological sample information, as well as tools to select and view multiple sets of epigenomic data as tracks on genome browsers.

1854/T

Genome-wide search for a candidate gene in Idiopathic CD4+ T-cell lymphocytopenia by combination of SNP-based homozygosity mapping and next-generation sequencing. *A. KITAMURA, K. YASUTOMO.* Univ Tokushima Graduate Sch, Tokushima, Japan.

Idiopathic CD4+ T-cell lymphocytopenia (ICL) is defined by persistent CD4+ T-cell lymphopenia (< 300 cels/ul or < 20% of total T cells) in the absence of any infections including HIV-1 or drug therapies associated with reduction of CD4+ T lymphocyte. The clinical phenotype of ICL ranges from no symptom to life-threatening opportunistic infections and ICL patients are also susceptible to autoimmune disorders and malignancies. The causative gene of ICL has not been identified because of a very rare disease. We identified one consanguineous Japanese family with 2 affected and 1 unaffected sibling whose parents were first cousins. As the affected siblings presented frequent opportunistic infections and malignancy from an early childhood, bone marrow transplantations were performed on affected siblings, which restored CD4+ T lymphocyte counts. To identify an underlying gene, we performed a genome-wide homozygosity mapping using the high density SNP microarray (Human610-Quad, Illumina) in three siblings and revealed 6 homozygous regions that spanned from 4 Mb to 20 Mb. These loci spanned total 73.6 Mb and harbored 1233 genes. Next, we resequenced whole exons in two affected siblings by combination of target enrichment system and next-generation sequencing approach, because of the high number of candidate genes and low throughput of traditional Sanger sequencing. We used SureSelect Target Enrichment System (Agilent Technologies) to capture whole exons in the human genome and sequenced the captured fractions on the Illumina Genome Analyzer 2. Mean sequencing coverage was approximately 20, and more than 99% of the exons were captured and sequenced to sufficient depth. We detected tens of thousands genetic variants in each affected siblings and found approximately 4000 novel variants in each affected siblings by comparing the variants to dbSNP database. We found 269 identical homozygous variants in two affected siblings including missense and nonsense changes, and selected 64 novel variants located in the SNP homozygous regions. Further validation and analysis of these variants are currently underway and will be reported. We conclude that it is likely to identify a candidate gene of an autosomal recessive disorder even with single consanguineous family by combination of SNP-based homozygosity mapping and next-generation sequencing.

1855/T

Method for testing relationship between SNP probability and adjacent complex multilocus effect. *K. SHIBATA, A. FUJIYAMA.* National Institute of Informatics, Tokyo, Japan.

Next-generation sequencing technology has been useful for identifying variations between individuals. Thus far, base quality values are used to discern true allelic variations from sequencing errors. In practice, however, while sequence generated by next-generation technology has been aligned to some reference, these mapped to reference assemblies vary greatly in there coverage and complex multilocus effect and then are blind to most novel sequences including true individual variants. Here, we have developed a computational tool that tests the relationship between single nucleotide polymorphism (SNP) probability and adjacent complex multilocus effect. The applicability and accuracy of the methods are evaluated using real data from Illumina GAIIx Analyzer.

1856/T

Validation of a mouse model for Usher Syndrome type 1F (USH1F), a hearing loss disorder in humans. *A.A. Torres[1], S.M.G Massironi[2], J.L. Guenet[3], A.L.B. Godard[1].* 1) General Biology Dept, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil; 2) Department of Immunology, Universidade de São Paulo, São Paulo, Brazil; 3) Unit of Mammals genetics, Institute Pasteur, Paris, France.

The mutation *rodador* is an autosomal recessive disorder characterized by hearing loss, circling behavior and balance dysfunction. Examination of cochlear histology revealed abnormal stereocilia and linkage analysis of recombinant animals using microsatellite markers mapped the mutation on chromosome 10, between 29.0 and 49.0 cM. The characterization of the region allowed the selection of Protocadherin 15 (Pcdh15) as a strong candidate gene as it is involved with hearing function, and many mouse models have already been described and show phenotype similar to *rodador*. Pcdh15 is a member of the cadherin superfamily of calcium-dependent adhesion molecules, and is a component of the extracellular filaments that control morphogenesis and function of stereocilia on mechanosensory cells of the inner ear. We aimed to identify the mutation by sequencing Pcdh15 exons. Forty-one exons, including intronic flanking sequences, were sequenced and an AT-to-GC transition was found in intron 25. Three rodador mice were sequenced: all of them showed the same alteration compared to the control animal from the parental BALB/c strain. C57BL/6, DBA and NZB mice were sequenced to exclude the possibility of this alteration being a polymorphism among strains, and they all presented an Adenine at the referred position. This alteration led to a switch of a dinucleotide ApA for ApG at a position close to the acceptor splice-site. Our hypothesis is that it may have created a stronger intronic cryptic splice-site that could lead to the incorporation of 8 bases from the intronic region into the mRNA, altering the ORF and resulting in a truncate protein. Evidence supporting this hypothesis includes: 1) approximately 80% of known mutations caused by ENU are AT-to-TA or AT-to-GC; and 2) 26% of ENU-induced mutations cause abnormal splicing. We intend to validate this mutation by performing Real-Time PCR tests to evaluate the expression levels of Pcdh15 for control and mutated mice, and sequencing the cDNA portion that contains the junction of exons 25 and 26, to verify whether intronic bases were incorporated. Specific primers have been designed, RNA samples collected from the inner ear and cDNA molecules produced. In humans, mutations of PCDH15 cause hearing loss and Usher Syndrome Type 1F (USH1F). The *rodador* mouse might be a good model for studying hereditary hearing loss and may aid in the comprehension of the mechanisms involved with mechanotransduction and hearing function.

1857/T

Draft sequencing of 1,000 genomes to study the genetics of quantitative traits: data production. *F. Busonero[1], B.J. Tarrier[2], E.A. Ketterer[2], G.R. Abecasis[1], C.A. Brennan[2].* 1) Biostatistics, University of Michigan, Ann Arbor, MI; 2) DNA Sequencing Core, University of Michigan, Ann Arbor, MI.

Genome-wide association studies, which systematically assess the association between common single nucleotide polymorphisms and a trait of interest, have led to rapid advances in our understanding of complex traits. Although they have been particularly successful, a large proportion of the genetic component of most of the analyzed traits and diseases remains unexplained. This can be attributed to different reasons, including the fact that the assessed variants were restricted to the design of the commercially available chips and to the catalog of HapMap variations. To overcome this issue, we planned a large scale low-pass whole genome sequencing (1,000 individuals at a minimum depth of 2X) from the SardiNIA project. Sequence data production is ongoing at the University of Michigan DNA Sequencing Core (UMDSC), taking advantage of existing laboratory infrastructure. Upon receipt, each sample is genotyped on the Sequenom platform with a set of 40 SNP markers that are known to have minor allele frequencies close to 50% in a variety of populations. These markers are present in the Illumina Metabochip and Affymetrix 6.0 arrays, and these genotypes will allow samples to be tracked through the lab and to be compared to pre-existing array genotype data, where available. Paired-end Illumina libraries are generated for each sample, as described by Quail M.A. et al. 2008, with several improvements, including automation of purification steps. Actual sequencing capacity, 3 Illumina Genome Analyzers IIx, allows for about 400 GB of DNA sequence data to be generated each month. With each run we are able to sequence 8 samples with an average of 2.5X depth, and greater than 6 GB of high-quality mapped de-duplicated bases per lane in paired-end sequencing runs with 120 bp reads. Sample tracking is accomplished by extending the well established database routines and web interfaces in use at the UMDSC: as a sample advances through the processing steps (sample QC, library prep, library QC, sequencing, data extraction, data QC, data transfer), a status code in the tracking database updates to maintain a clear indication of the location and progress of the sample through the process.

1858/T

A generic algorithm for calling classical HLA alleles from next-generation sequencing data. *P.I.W. de Bakker[1,2], X. Jia[1,2,3], E. Banks[2], R. Erlich[2], N. Lennon[2], M.A. DePristo[2].* 1) Dept Med, Brigham & Women's Hosp, Boston, MA; 2) Broad Institute of MIT and Harvard, Cambridge, MA; 3) Harvard-MIT Health Sciences and Technology, Boston, MA.

Background: Human leukocyte antigen (HLA) typing is an important tool for clinical diagnostics and biomedical research of autoimmune and infectious diseases. We have developed a generic algorithm for calling classical HLA alleles at 4-digit resolution at class I and II loci from next-generation sequencing data. **Methods**: The HLA caller algorithm, developed as part of the open-source Genome Analysis Tool Kit, examines sequence reads aligned to the classical HLA loci taking SAM/BAM formatted files as input, and calculates for each locus the posterior probabilities for all pairs of classical alleles based on three key considerations: (1) population-specific allele frequencies, (2) genotype calls at each base position, and (3) phase information of nearby variants. The output of the algorithm is a list of posterior probabilities for all possible pairs of HLA alleles. **Results**: We used sequence data from 454, Illumina, and SOLiD in two HapMap individuals (CEU and YRI) generated in pilot 2 of the 1000 Genomes Project (with known HLA types). No manual re-alignment or de novo assembly was attempted. The HLA caller determined all the correct alleles at 4-digit resolution in the two daughters with 454 data, except for HLA-A and HLA-DRB1 in the YRI daughter (NA19240). At HLA-A, one of the two alleles in this YRI individual was incorrect at 4-digit (but correct at 2-digit) resolution. At HLA-DRB1, we observed poor alignment (DRB3 reads aligning to DRB1), resulting in incorrect calls. Using the older Illumina and SOLiD data, the algorithm correctly determined 24/32 and 23/32 HLA alleles, respectively, at 4-digit resolution. Poor alignments across the HLA severely decreased the overall accuracy of the HLA calls. More recently, we also applied the HLA caller to Illumina GA-2 data (76, 101 and 151 bp paired-end at ~54X) as well as Illumina HiSeq data (101 bp at ~60X) in the CEU daughter (NA12878). The 4-digit HLA calls from the GA-2 and HiSeq data were completely concordant with the known types in this CEU individual. Although it was not possible from the sequence data alone to distinguish between DQB1*0201 and DQB1*0203, and between DRB1*0301 and DRB1*0311, the correct calls could still be made due to the population allele frequency priors. **Conclusion**: We have developed a generic HLA calling algorithm for next-generation sequencing data, which will be useful for studying diseases where the HLA is implicated.

1859/T

Integration of a Linear Isothermal Amplification Protocol with a Streamlined Method for Preparing DNA Libraries used in Next-Generation Sequencing. *S. Kain, H. Sethi, L. Turner, J. Magnus, V. Sementchenko, C. Raymond.* NuGEN Technologies, Inc., San Carlos, CA.

Recent advances in Next-Generation Sequencing (NGS) technology have increased both the throughput and capacity of sequencing platforms, calling for increased efficiency in sample preparation and the ability to work with smaller amounts of starting nucleic acid. The streamlined Encore™ library construction protocol, requiring only two purification steps and no agarose gels, results in amplified DNA libraries prepared from double-stranded cDNA or genomic DNA in as little as 3 hours. Further, a novel indexing capability to enable multiplex sequencing of up to 8 libraries per sequencing lane further improves sample throughput and reduces sequencing costs. These four-base index tags are intrinsic to the first sequencing read and are designed to provide equal representation across all nucleotide bases in the first several sequencing cycles. The index sequences are unambiguous, meaning that sequencing errors or deletion errors do not corrupt the code integrity. Data will be presented from the hybridization of a human genomic DNA library to a human genome CGH array, demonstrating the maintenance of relative stoichiometry across 43k+ coding and non-coding sequences. Other data (amplified cDNA expression array or Encore library sequencing data) will be presented that demonstrates the ability to achieve high quality differential expression analysis using NuGEN's linear isothermal amplification process from as little as 500 pg of total RNA using both RNA-Seq and Digital Gene Expression (DGE) systems. Differential expression data from MAQC A and B library samples, analyzed by RNA-Seq and quantitative PCR (qPCR), produce data concordant with the Taqman reference expression assay (R = 0.934) without significant data compression. Highly conserved differential expression data is observed between libraries from RNA-Seq amplified U937 and HeLa cell-line isolated total RNA. Lastly, data will be shown from deep sequencing of genomic DNA using several bacterial species to demonstrate unbiased mapping of reads as a function of coverage, and the ability to accurately segregate sequencing reads based on the index tags. In addition to genomic DNA, RNA-Seq and DGE samples, the Encore library construction method can be equally applied to the full range of NGS applications including ChIP-Seq, exome sequencing, and amplicon sequencing.

1860/T

SeqBayes: An adaptive Bayesian framework for calling genotypes from next-generation sequence data. *E.H. Powell, D.D. Kinnamon, M.A. Schmidt, E.R. Martin.* Dr. John T. Macdonald Foundation Department of Human Genetics, University of Miami Miller School of Medicine, Miami, FL.

Existing techniques for calling genotypes from next-generation sequence (NGS) data use estimated posterior genotype probabilities based on parameter estimates from previous data or the current sample. Because the degree of confidence in previous estimates and the amount of information in a given sample can vary greatly, we sought a principled approach for efficiently combining the two in genotype calling. We derived a binomial likelihood for the observed base read data from unrelated individuals at a single biallelic variant given a nucleotide-read error rate (r) and latent individual genotypes (G_i). Heterozygotes provide no information on r in this likelihood, so an informative hyperprior for r might be useful in some samples. After assigning a prior to G_i and hyperpriors to r and the variant allele frequency (p), posterior probabilities for G_i can be estimated using Markov chain Monte Carlo. The called genotype is then the one with the highest posterior probability. We estimated overall genotype call error in simulations with 10,000 replicates at read depths from 5 to 25, sample sizes from 10 to 500, p from 0.05 to 0.5, r from 0.01 to 0.10, and genotype frequencies both in and out of HWE. SeqBayes was applied assuming HWE, with a flat hyperprior for p, and with an informative scaled Beta(1, 9, 0, 0.5) hyperprior for r. This hyperprior reflects a vague expectation of r around 0.05 based on previous information. We compared these results to those obtained using sample-based maximum likelihood estimates (MLEs) of r and p. SeqBayes with an informative prior reduced the average genotype-call error rate by 20.13% compared to that obtained using the MLEs (0.049 vs. 0.061) for p=0.5 and r=0.01. In scenarios with excess heterozygosity, in which information on r is sparse, this reduction was 23.04% (0.072 vs. 0.093). Not calling variants for which identification of the MLEs was uncertain (18.87%) reduced the error rate in the called variants to below that of SeqBayes, but call rates were unacceptably low (e.g., 6%) in several scenarios with low read depths and excess heterozygosity. Incorporation of even vague prior knowledge can help to overcome identification problems with purely sample-based approaches, such as maximum likelihood, in sparse data. SeqBayes' ability to incorporate sample information also gives it greater flexibility than methods such as MAQ that rely on constants derived from previous data.

1861/T

Analysis of a South African genome using SOLiD error correction codes. *V. Tadigotla, C. Clouser, T. Weaver, J. Spangler, M. Sikora, J. Healy, C.C. Lee, T. Harkins.* Life Technologies, Beverly, MA.

The Bushmen of southern Africa are known to be genetically divergent from other humans. An individual from that community (KB1) has recently been sequenced using the 454 GS FLX platform to 10.2 fold coverage and a *de novo* assembly was created. Here we present the deep sequencing of KB1 using the latest developments with the SOLiD™ system and ligation-based sequencing. Specifically, recent improvements in paired-end sequencing have allowed us to achieve longer read lengths in both the forward and reverse direction. Novel error correction codes (ECC) for SOLiD™ are based on standard techniques used in modern communication and data storage systems. ECC works by transforming the valuable information and augmenting it with redundancy to make it more resistant to measurement error. Due to ligation based sequencing, the SOLiD system is unique and can employ ECC encoding by using specially-designed probe sets. This allows us to improve accuracy with minimal impact on sequencing time, as well as decode the original sequence using a Bayesian inference. In total, ~30 fold paired coverage of KB1 was achieved by combining longer paired end sequencing reads (75 bp forward, 30 bp reverse) with standard SOLiD™ mate pair libraries (2x50 bp). These libraries have median insert sizes of 250 bp and 1.5 kb respectively. When mapped against hg18 with ECC applied, there is a 40% reduction in uncovered regions in KB1 and a higher number of heterozygous SNPs are called, which are also present in dbSNP. Structural variations as well as the hybrid assembly of the KB1 genome using 454 and SOLiD data will also be discussed.

1862/T

Comparison of Sequence Alignment Algorithms for Targeted Sequencing Studies. *A.A. Van Zeeland[1], T. Phillips[1], M. Shaw[1], P. Hoover[2], W. Pfeiffer[2], M.A. Miller[2], N.J. Schork[1], S. Levy[1].* 1) Scripps Translational Science Institute, La Jolla, CA; 2) San Diego Supercomputer Center, San Diego, CA.

Accurate alignment to a reference genome is one of the fundamental steps in the analysis of human sequence data. All subsequent analyses, from calling single nucleotide polymorphisms (SNPs) and insertion/deletions (indels) to statistical analyses of variants, rely on accurate and high-quality alignment of the sequencing reads. Moreover, alignment results can provide the first measure of the success of the sequencing experiment by determining whether the library preparation, and if applicable, target enrichment, was effective. A multitude of alignment algorithms exist, and the choice of which to use may be influenced by both the sequencing technology and goals of the experimental study. Unlike previous algorithm comparisons that utilized simulated data (Li & Homer, 2010), here we compare the performance of a select number of both hash-based and Burrows-Wheeler transform (BWT) alignment methods using real data from a targeted sequencing study. Specifically, we targeted approximately 1.3Mb of coding sequence found in 150 candidate genes. In order to accurately assess the relative success of each of the alignment algorithms, we focused our analysis on the HuRef DNA sample, which has previously been Sanger sequenced (Levy et al., 2007). By mapping the HuRef reads back to the NCBI36 reference genome, we could compare the resultant variant calls to the known 'gold-standard' Sanger HuRef sequence. Regions were enriched using microdroplet-based PCR (RainDance, Lexington, MA) and subsequently subjected to sequencing by ligation (SOLiD, Life Technologies, Carlsbad, CA). We applied the default settings for each of the different alignment methods under investigation (BFAST, Bowtie, SHRiMP) and called variants with SAMtools (http://samtools.sourceforge.net/). Preliminary results indicate all mapping algorithms attained an acceptable level of mapped reads (range 56.2% - 69.0%), and achieved high levels of concordance (>90%) with the HuRef genome. The computational loads and speed-accuracy trade-offs will be discussed.

1863/T

Novel Peak region on 2q13 in Autism case control dataset. *J. Jaworski[1], D. MA[1], D. Salyakina[1], R. Martinez[1], I. Konidari[1], P. Whitehead[1], E. Martin[1], S. Williams[2], R. Menon[2], J. Gilbert[1], M. Cuccaro[1], J. Haines[2], M. Pericak-vance[1].* 1) Hussman Institute for Human Genomics (HIHG), University of Miami, Miller School of Medicine, Miami, FLMIHG, Univ Miami, Miami, FL; 2) Center for Human Genetics Research (CHGR), Vanderbilt University Medical Center, Nashville, TN.

Autism is one of the most heritable neuropsychiatric disorders. No single major gene has been identified conclusively suggesting a complex genetic etiology. Previously we have reported an association of common variants from 5p14.1 with autism risk in a family-based dataset of European ancestry. To validate and extend these results we aimed to take advantage of the case control study design. We selected a total number of 1314 ASD cases from our family dataset and performed association testing with a set of unrelated Caucasian pediatric controls (N=647. A subset of controls (310) were between the ages of 3-21 and were screened for birth defects, developmental and language delays, siezures, as well as ADHD and ASD. The remaining 337 controls were derived from newborn cord blood and screened for birth defects. Quality controls procedures were done at both the sample call rate and population stratification level. Analysis was carried out using the model trend test in PLINK. The previously reported region of 5p14.1 showed nominal p values for SNPs RS1330656 (0.001) and RS7704909 (0.03) although the effect size was much smaller. In addition, we identified an interesting region on 2q13 that has not been previously reported. The peak marker RS11123128 had a p-value of 1.54 * 107 and marked a region of 4 interesting SNPS; RS7600843 (p-value 6.67 * 10^7), RS10197919 (1.39 * 10^6) and RS7593053 (2.44 * 10^6). While not previously reported in autism, the region has been noted in a genome wide linkage study for Schizophrenia (Delisi, E et al, 2002). The peak SNP occurs within the TMEM87B gene while the other SNPS are within the FBLN7 gene. In conclusion, our results confirmed our previously established association at 5p14.1. It also suggests a novel candidate region at 2q13 for further validation.

1864/T

CD28 proximal promoter polymorphisms and systemic lupus erythematosus susceptibility. *A.J.L. Brambila-Tapia[1], I.P. Dávalos[1], J.I. Gámez-Nava[2], L. González-López[3], J. Medina-Díaz[1], A.G. Bernard-Medina[4], M. Salazar-Páramo[2].* 1) Doctorado en Genética Humana, Instituto de Genética Humana, CUCS, Universidad de Guadalajara (UdeG) y División de Genética, CIBO, Instituto Mexicano del Seguro Social (IMSS), Guadalajara, Mexico; 2) División de Investigación, UMAE, HE, UIEC, CMNO, IMSS y Depto. Fisiología, UdeG, Guadalajara, México; 3) Servicio de Reumatología, Hospital General de Zona, No. 110, IMSS; 4) Servicio de Reumatología, Hospital Civil de Guadalajara, Fray Antonio Alcalde.

Introduction: CD28 expression and serum levels are significantly increased in patients with SLE than in healthy controls (HC). Until now, there are not studies of proximal promoter polymorphisms of CD28 gene in SLE. Therefore, our objective was to investigate the polymorphisms present on the proximal promoter of CD28 in a group of SLE and HC and associate the polymorphisms present with the CD28 serum levels of 40 patients and 40 controls. Material and methods: One hundred and seven patients as well as 108 controls matched by age range and genders were included. The 11 ACR criteria were analyzed on the clinical files and the proximal promoter region of CD28 gene were analyzed by direct sequencing of a 489 base pairs fragment. C28 serum levels (sCD28) were measured by ELISA technique in 40 patients and 40 controls. Results: Only two of the 8 reported polymorphisms were found, they correspond to rs35593994 (-372 A/G) and rs56156157 (-145 -/C). The first had a prevalence of 41% and 36% in patients and controls respectively and the second of 1.4% in both groups. None of these polymorphisms were associated to SLE and the polymorphism the -372 A/G was not associated with the clinical features of disease. Likewise, the association with the sCD28 and the genotypes of -372 A/G polymorphism was not significant. Conclusions: The polymorphisms of the proximal promoter of CD28 are not associated with SLE and the polymorphism -372 A/G is not associated with the diagnostic criteria of SLE or the sCD28.

1865/T

High Throughput Genotyping: Massively parallel sequencing for Hereditary Spastic Paraplegia on the Roche 454 GSFLX System. *N. Schlipf[1], R. Schüle[2], O. Riess[1], L. Schöls[2], P. Bauer[1].* 1) Department of Medical Genetics, Institute of Human Genetics, Tübingen, Germany; 2) Clinical Neurogenetics, Department of Neurology and Hertie-Institute for Clinical Brain Research and German Center of Neurodegenerative Diseases, University of Tübingen, Tübingen, Germany.

The hereditary spastic paraplegias (HSPs) are a genetically heterogeneous group of neurodegenerative diseases characterized by progressive spasticity and weakness of the lower limbs. At least 46 different loci have been mapped, associated with autosomal dominant, autosomal recessive and X-linked mode of inheritance, 17 responsible genes have been identified. For correct diagnosis molecular testing is essential since clinical parameters by itself are not reliable to differentiate HSP forms. The 454 GSFLX System with its massively parallel pyrosequencing promises high resolution HSP genotyping for multiple individuals at multiple amplicons in a single run. The purpose of this study was to determine the sensitivity and specificity of array based amplicon library generation and massive parallel sequencing of pooled DNA samples for the identification of pathogenic mutations. 47 DNA samples from index cases with sporadic or recessive paraplegia were amplified using Fluidigm 48.48 Access Array System. To achieve high throughput genotyping, we have used PCR primers with multiplex identification tags to amplify *CYP7B1* (SPG5) and *SPG7* exons from individual samples, thereby pooling of the amplicons generated from different individuals prior to the emulsion PCR step. The average sequence read length was 250bp which is in accordance with an average PCR fragment length of 310bp. Also sufficient sequence coverage for full length reads was obtained. We identified eight (including two novel) heterozygous mutations in the *CYP7B1*- and *SPG7* gene in 4 patients. Three of these mutations were nonsense mutations (R63X in CYP7B1, and R247X and R398X in *SPG7*), two were missense mutations (R486C in CPY7B1, and G349S in *SPG7*), one variant (A759T), one a splice site mutation (c.1552+1 G>T) and one was a 29bp deletion (353-384X385) in *SPG7*. Additionally, several known polymorphisms were identified in the *SPG7* gene. All mutations, variants and known polymorphisms were detected by the analysis software and got validated by direct Sanger sequencing. In this study, we were able to establish a high throughput genotyping for sporadic and recessive HSP by an array based amplification strategy to generate amplicon libraries followed by sequencing with 454 GSFLX Titanium chemistry. Further studies will implement the GSFLX Titanium chemistry and genotyping software for higher throughput, longer sequence reads, and greater genomic coverage to provide higher resolution in HSP genotyping.

1866/T

Mapping antioxidant response elements and their variants in the human genome by ChIP-seq. *X. Wang, B. Chorley, M. Campbell, S. Kleeberger, D. Bell.* National Institute of Environmental Health Science, National Institutes of Health, Research Triangle Park, NC 27709.

The antioxidant response element (ARE) is a cis-acting enhancer sequence found in the promoter region of many genes encoding antioxidant and Phase II detoxification enzymes/proteins. In response to oxidative stress, the transcription factor NRF2 (nuclear factor erythroid-derived 2-like 2) translocates to the nucleus and dimerizes with other basic leucine zipper proteins such as small Maf proteins to form a transactivation complex that binds to AREs. To date, NRF2 is known to mediate a transcriptional network of approximately 50 responsive genes that modulate in vivo mechanisms against oxidative damage and reactive electrophiles. Our previous studies on Nrf2-knockout mice and sequence analysis suggest that hundreds of genes may be regulated by NRF2. To get an extensive survey of NRF2 binding to AREs and their variants, we have used chromatin immunoprecipitation with parallel sequencing (ChIP-Seq) technology to identify genome-wide NRF2 binding in human lymphoblastoid cell lines in response to the dietary isothiocyanate, sulforaphane (SFN). Using 12 million uniquely mapped 36-base sequence reads of ChIP DNA from 7 SFN-treated cell lines, we found 2647 NRF2-bound genomic regions. *De novo* motif discovery confirmed the top enriched motif matched the core NRF2 binding motif, and 1302 (49%) regions contained one or more putative AREs. Mapping these regions to nearby genes, we observed 2126 (80%) and 732 (28%) regions were within 100-kb and 5-kb of a gene transcriptional start site, respectively. Based on sequence tag count, we designated 328 regions with high confidence and 98% of them contained putative AREs. Many known AREs were identified in those high confidence regions, however the majority were novel. To determine NRF2 dependence, we selected 6 known, 7 putative, and 30 candidate genes, and measured gene expression in two NRF2-silenced cell lines. Expression of all but 2 known/putative and 20 of 30 candidate genes was reduced in both cell lines. We also identified SNPs within ChIP-seq peak regions and explored the impact of SNP on NRF2 binding and transcription by analyzing genotype and gene expression association in HapMap CEU individuals. This study greatly expands the NRF2 transcriptional landscape and reveals new insights into the role of NRF2 in stress response, environmental susceptibility, and disease etiology.

1867/T

GFM1 is not a major gene of hepatic failure associated with mitochondrial respiratory chain deficiency. *M. Beinat[1], L. Galmiche[1,2], V. Vedrenne[1], A. Slama[3], O. Bernard[4], V. Serre[1], A. Munnich[1], A. Rotig[1].* 1) INSERM U781 and Department of Genetics, Hôpital Necker-Enfants Malades and Assistance Publique-Hôpitaux de Paris, Faculty of Medicine, Université Paris Descartes, Paris, France; 2) Department of Pathology, Hôpital Necker Enfants-Malades and Assistance Publique-Hôpitaux de Paris, Faculty of Medicine, Université Paris Descartes, Paris, France; 3) Department of Biochemistry, Hôpital Kremlin-Bicêtre and Assistance Publique-Hôpitaux de Paris, Paris, France; 4) Department of Paediatric Hepato-gastroenterology, Hôpital Bicêtre and Assistance Publique-Hôpitaux de Paris, Paris, France.

GFM1 gene encodes mitochondrial elongation factor G1 required for elongation phase of mitochondrial translation as it catalyzes ribosome translocation during peptide elongation. GFM1 mutations have been rarely reported in human. Indeed, only four patients from two unrelated families presenting liver insufficiency and multiple respiratory chain (RC) deficiency were found to have GFM1 mutation and one patient with infantile encephalopathy also harbored GFM1 mutation. We have studied a large series of 18 patients with liver insufficiency related to multiple RC deficiencies with no quantitative or qualitative mitochondrial DNA (mtDNA) anomalies. In order to establish the frequency of GFM1 mutations in this group of patients, we have sequenced exons and exon-intron boundaries of GFM1 gene in 15 patients. A genome wide scan was performed in the three other patients using the GeneChip Human Mapping 10K 2.0 Array (Affymetrix) and allowed to exclude GFM1 as a causative gene. Only one patient, born to consanguineous parents, presented a homozygous mutation in exon 9 changing a highly conserved leucine into a proline (L398P). Hepatic failure is frequently associated with multiple RC deficiencies. Most of the patients are sporadic cases but few patients born to consanguineous parents suggest that this disease can be autosomal recessive. Indeed, some of these patients also presented severe mtDNA depletion related to mutations in DGUOK, POLG, MPV17 or PEO1 nuclear genes. The genetic origin of the disease in patients with normal mtDNA content is rarely identified. Mutations of TRMU encoding a mitochondrial tRNA 5-methylaminomethyl-2-thiouridylate methyltransferase allowing thiolation of mitochondrial tRNALys, tRNAGlu and tRNAGln were found in several patients of Arab, Maghreb and Yemenite Jewish origin. However, TRMU mutations were never found in European patients. This study therefore confirms the genetic heterogeneity of mitochondrial hepatic insufficiency and highlights the diversity of abnormal mitochondrial functions in these diseases.

1868/T

Whole exome sequencing analysis in siblings with optic atrophy and suspected mitochondrial disease. *M.J. Falk[1], E. Place[1], S. Dingley[1], J.C. Perin[1], E. Pierce[2], E. Rappaport[1], X. Gai[1].* 1) Department of Pediatrics, The Children's Hospital of Philadelphia and University of Pennsylvania, Philadelphia, PA; 2) Department of Ophthalmology, The Children's Hospital of Philadelphia and University of Pennsylvania, Philadelphia, PA.

Next generation sequencing technologies hold the potential to substantially improve the diagnostic yield for heterogeneous disorders, such as mitochondrial disease. Here, we report results of whole exome sequencing in 2 siblings who presented with optic atrophy, chronic fatigue, GI dysmotility, and motor delay. Genome-wide SNP microarray analysis as well as sequencing of *OPA1* and their mtDNA genome revealed no abnormalities. **METHODS:** 3 ug of fibroblast gDNA (Qiagen) was sheared with a Covaris S2 sonicator, end-repaired, and ligated to SOLiD-specific adapters (Agilent SureSelect Whole Exome bead-based kit). 150-200 base pair fragments were isolated and purified. Following emulsion PCR and bead-enrichment, libraries were sequenced on a SOLiD 3plus system using 50 base pair read length. Average whole exome depths of coverage of 100.3X and 41.9X were achieved in a single NGS run. Short reads were aligned by Burrows-Wheeler Algorithm (BWA). SNPs and indels were called with samtools and custom programs to assess variant calls for depth of coverage, variant percentage, affected gene, amino acid changes, previous population reports (dbSNP), and pathogenicity predictions in PolyPhen and SIFT. Variants were flagged by Human Gene Mutation Database annotation and gene set affiliation. Only shared variants were further considered. **RESULTS:** 7,821 total shared coding single nucleotide variants (SNVs) or small indels were identified in the whole exome, of which 3,713 were non-synonymous (including 43 nonsense) mutations. 97 non-synonymous SNVs were identified in the MitoCarta-defined mitochondria gene set. 29 genes had 31 mito SNVs having "probable", "possible", or "uncertain" deleterious predicted effects, half of which were implicated in fatty acid oxidation (10 genes) or mitochondrial translation (4). Although all 9 homozygous mito SNVs were previously reported in dbSNP, 7 of the heterozygous mito SNVs were predicted to be novel in both dbSNP and SeattleSeq within genes involved in fatty acid oxidation (3), mitochondrial translation (2), calcium transport (1), or unknown (1). Functional analyses are underway to assess FAO and translation in the siblings' fibroblasts. Mutation confirmation is underway in the larger kindred. **CONCLUSIONS:** Comprehensive evaluation of the entire exome in affected siblings, with particular focus on mitochondria-associated genes, permits evaluation of a wide range of potential pathogenic etiologies while minimizing expense and bias.

1869/T

Severe neonatal encephalopathy and Leigh syndrome is related to mutations in GFM1 gene encoding a mitochondrial elongation factor. *L. Galmiche[1,2], M. Beinat[1], V. Vedrenne[1], A. Slama[3], Z. Assouline[1], M. Rio[1], V. Serre[1], A. Munnich[1], A. Rotig[1].* 1) INSERM U781 and department of Genetics, Hôpital Necker-Enfants Malades and Assistance Publique-Hôpitaux de Paris, Faculty of Medicine, Université Paris Descartes, Paris, France; 2) Department of Pathology? Hôpital Necker-Enfants Malades and Assistance Publique-Hôpitaux de Paris, Faculty of Medicine, Université Paris Descartes, Paris, France; 3) Department of Biochemistry, Hôpital Kremlin-Bicêtre and Assistance Publique-Hôpitaux de Paris, Paris, France.

Multiple respiratory chain deficiencies represent a common cause of mitochondrial diseases as it accounts for one third of these disorders. These deficiencies are genetically heterogeneous and can be related to various causes such as abnormal mtDNA maintenance, abnormal mitochondrial translation or cardiolipin synthesis. We have studied a patient born to consanguineous parents of North African origin. He presented intrauterine growth retardation, neonatal feeding difficulties, seizures, encephalopathy, microcephaly and developmental delay. He also had hyperlactatemia and hyperlactatorachia. Brain MRI revealed abnormal basal ganglia. Respiratory chain (RC) analysis showed a complex IV deficiency in muscle and complex I and complex IV deficiency in cultured skin fibroblasts. No quantitative or qualitative mtDNA abnormalities could be identified. A genome wide scan was performed using the GeneChip Human Mapping 10K 2.0 Array (Affymetrix) for the two consanguineous parents and the patient. This revealed 12 regions of homozygosity ranging from 9 to 35 Mb. These regions encompassed 68 known genes encoding mitochondrial proteins, 14 of them being involved in mitochondrial translation. The multiple respiratory chain deficiency observed in fibroblasts of the patients could be related to an abnormal mitochondrial translation. Therefore, we sequenced the GFM1 gene included in a homozygous region of chromosome 3 and identified a homozygous mutation in exon 16 changing a highly conserved arginine into a cysteine (R671C). Structural modeling of the mutant protein showed that the new cysteine is located near to another cysteine and could therefore induce the creation of an abnormal sulfur bridge. This mutation was heterozygous in the two parents and absent from a series of 100 controls of the same ethnic origin. Mitochondrial translation defects of nuclear origin represent a growing cause of multiple RC deficiencies. GFM1 gene encodes a mitochondrial translation factor and mutations of this gene have been rarely reported. Two unrelated patients presented neonatal liver insufficiency whereas a third patient had infantile encephalopathy. Identification of a new GFM1 mutation in our patient confirms the clinical heterogeneity associated to mutations in this gene.

1870/T

Whole exome sequencing of autosomal recessive acute necrotizing encephalopathy. *D.E. Neilson, M. Keddache, P. Putnam, D. Fletcher.* Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

The most intriguing aspect of acute necrotizing encephalopathy (ANE) lies in its gene-environment interaction. In children genetically predisposed to this disorder, mild or non-neurotropic viruses produce a fulminant encephalopathy, resulting in damage to the thalamus and brainstem. Three previously healthy brothers developed episodes of ANE; two died from the disorder. These brothers, born of consanguineous parents, did not have mutations in *RANBP2* (which causes autosomal dominant ANE) and likely represent an autosomal recessive form of ANE. Defining the genetic cause in consanguineous families may allow the identification of a predisposing gene for the "sporadic" cases. DNA was available from only one of the three brothers from the kindred. Previously, this would not be enough to pursue further analysis, but the advent and success of high throughput exome sequencing allows the possibility of identifying novel mutations in regions of identity-by-descent (IBD). **METHODS**: DNA from the surviving proband was tested by whole exome sequencing. A DNA library was enriched by hybridization to a Nimblegen Exome SeqCap array and subsequently sequenced using an Illumina GAIIx next generation sequencer. To supplement marker density, data from a 10K Affymetrix SNP chip were merged with data from exome variants. Regions of IBD were identified through runs of consecutive homozygous markers greater than 1Mb. **RESULTS**: 17,637 total variants were identified. 1303 variants (7% of total) defined 239 regions of suspected IBD. The aggregate size of the regions was 461Mb, or 15% of the haploid genomic size. The largest sized segments were 17 and 18 Mb, whereas the average size was 1.9Mb and the median size 1.4Mb. From these regions, 449 known coding variants and 4 novel missense variants were revealed. **DISCUSSION**: Whole exome analysis reduced IBD candidate regions from 15% of the genome to 4 candidate variations. Although this method does not take into account the contribution of rare known SNPs or intronic variants, this process allows for streamlined candidate gene identification. Exome analyses in additional ANE patients may allow better refinement of candidate genes, but potential locus heterogeneity could complicate the process. For rare, idiopathic reactions such as ANE in which pedigree information and patient populations are limited, exome analysis may provide the best insights into pathogenesis for the near future.

1871/T

Multiplexed Massively Parallel Sequencing for the Prenatal Detection of Fetal Aneuploidy from Maternal Plasma. *T. Zwiefelhofer[1], J. Tynan[1], L. Cagasan[1], D. van den Boom[2], L. Tang[1], Y. Chen[1], C. Deciu[1], M. Ehrich[2].* 1) Sequenom Center for Molecular Medicine, San Diego, CA; 2) Sequenom Inc, San Diego, CA.

Prenatal diagnosis of fetal chromosome abnormalities such as Trisomy 21 currently relies on invasive procedures which may pose a significant risk to the fetus. Analyzing circulating cell free fetal nucleic acids derived from maternal plasma is a potential non-invasive alternative for aneuploidy detection. Multiple groups have shown high sensitivity and specificity in detection of trisomy 21 using a next-generation sequencing approach. Although the results of these initial studies suggest a superior performance to established screening methods, the expense and low throughput nature of the described technologies currently limits their application in a routine analytical setting. Here we explore an implementation of the sequencing approach that relies on multiplexed sample analysis to address these limitations. In multiplexed sequencing, sample-specific barcode tags are incorporated into each molecule along with a universal adapter sequence. Several tagged samples are then combined for analysis with the output sequences later assigned to their respective parent sample using the barcode tag. In this paper we describe the use of 4-plex sequencing. Due to the low quantity of fetal DNA in maternal plasma the sensitivity and specificity of the sequencing method for aneuploidy detection is dependent upon the total number of uniquely aligned sequences per sample. Increasing those uniquely aligned sequence reads heightens the discriminatory power of the assay as a result of the concomitant decrease in statistical noise. Though the analysis of 4 samples in a single reaction decreases the number of sequences aligned per sample, by adopting the latest biochemistry and software updates, our unique counts per sample have only decreased by half. Thus, we maintain the statistical power of the assay and gain a 4-fold increase in throughput. We studied the initial performance of multiplexed sequencing for T21 detection using a sample set of 88 euploid and 8 T21 samples. Libraries were prepared from each sample and then sequenced in 4-plex format using Illumina's Genome Analyzer IIx. A total of 3 flow cells were completed within 1.5 weeks. All 8 trisomy samples and 86 euploid samples were correctly identified (there were 2 false positives). With the increase in sample throughput and associated reduction in cost, these modifications can make the application of next-generation sequencing for chromosomal aneuploidy detection feasible in a routine analytical environment.

1872/T

Functional Genomic Approaches Unravel Candidate Genes Associated with Mortality of Critical Illness among African Americans. *L. Gao[1], T. Murray[2], N. Rafaels[1], R.A. Mathias[1], J.P. Maloney[3], M. Moss[3], G. Martin[4], C. Shanholtz[5], D.L. Herr[6], J.G. Garcia[7], J. Sevransky[1], P.M. Hassoun[1], T.H. Beaty[2], R. Brower[1].* 1) Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; 2) Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD; 3) University of Colorado Health Sciences Center, Denver, CO; 4) Emory University School of Medicine, Atlanta, GA; 5) University of Maryland School of Medicine, Baltimore, MD; 6) Washinton Hospital Center, Washington, DC; 7) University of Illinois at Chicago, Chicago, IL.

Rationale: Both acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are devastating clinical syndromes affecting over 200,000 patients in the United States each year. ALI is a complex pathophysiologic process involving a variety of molecular pathways. Using high-throughput expression profiling, we prioritized a total of 85 candidate genes that showed significant differential expression in mechanical ventilation or LPS-induced ALI models across different species (mouse, canine and human), and subsequently validated these markers in tests of genetic association using patients with severe sepsis and sepsis-associated ALI. Methods: We genotyped a panel of 1536 SNPs using the Illumina GoldenGate platform in a population of 759 African American subjects (195 severe sepsis patients, 158 ALI patients, and 406 controls). Association between outcome of ALI and SNPs were tested with a logistic regression model using PLINK. Pathway analysis was performed using the program Ingenuity Pathways Analysis (Ingenuity Systems: www.analysis.ingenuity.com). Results: Of the candidate genes tested, 11 genes (ABCB1, ADAM28, FGF10, FGFR3, FKBP5, GADD45G, HDAC9, IL8, NFKB1, PIK3CB, TGFBI) were significantly associated with 60-day mortality among African Americans (P = 0.04-0.0003). Variants in the histone deacetylase 9 (HDAC9) gene provided the most compelling evidence (P = 0.016-0.0003). These 11 genes were clustered into a major pathway related to (i) 'cell death'; (ii) 'organismal injury and abnormalities'; (iii) 'cellular growth and proliferation'; and (iv) 'carbohydrate metabolism'. Conclusions: Our findings using functional genomic approaches offer new insights into ALI pathogenesis and could explain to a certain extent the ethnic disparity (i.e., higher morbidity and mortality) noted among African American patients.

1873/T

Sample Quality Control and Troubleshooting for GAIIx Paired-End Sequencing. *B. Craig[1], B. Marosy[1], K. Hetrick[1], D. Mohr[1,2], M. Barnhart[1], S. Griffith[1], J. Goldstein[1], A.F. Scott[1,2], N. Pankratz[3], T. Foroud[3], K.F. Doheny[1].* 1) Center for Inherited Disease Research (CIDR), Johns Hopkins Univ School of Medicine, Baltimore, MD; 2) Genetic Resources Core Facility (GRCF), High Throughput Sequencing Center (HTS), Johns Hopkins Univ School of Medicine, Baltimore, MD; 3) Department of Medical and Molecular Genetics, Indiana University Medical Center, Indianapolis, Indiana, USA.

The Center for Inherited Disease Research (CIDR) provides high quality genotyping services and statistical genetics consultation to investigators working to discover genes that contribute to common disease. During CIDR's evaluation of the Illumina® GAIIx and Agilent SureSelect™ Target Enrichment systems for a production service, we have developed a workflow to monitor quality control metrics for samples being sequenced following enrichment for regions of interest. These metrics include capture specificity, efficiency of the target capture reaction at 10X coverage, percentage of duplicate reads, percentage of reads mapped to a reference, percentage of variants called in dbSNP129, transition/transversion ratio and concordance of the sequencing data with GWAS SNP genotypes. Following paired-end sequencing, image and base calling analysis are performed using Illumina's real-time analysis software. Base call files for each lane are analyzed via a CIDR developed software package, CIDRSeqSuite. This software converts the base call files to fastq format, aligns the data using BWA, and generates pileup files, variant call files, and reports for these metrics. Using this workflow, we evaluate the metrics to determine sample quality across GAIIx flowcell runs as well as quality across samples within a project. For our initial pilot project, we designed a custom 3.5Mb SureSelect™ bait library and prepared sample libraries to be indexed four per lane. The majority of the results analyzed thus far demonstrate consistent performance. For all pilot samples, the mean percent of duplicated reads is 10%, mean percent of mapped reads using BWA is 98%, mean target capture specificity of 85%, a mean depth of reads captured by the targeted regions of 175X, a mean percent of SNPs in dbSNP129 of 91% and a mean percent of targeted bases captured by 10 or greater reads of 99%. We have used these metrics to determine wet bench processing issues relating to failure of the capture reaction, insufficient yield from the sequencing run, and library complexity issues. When comparing our sequencing SNP calls to GWAS SNP calls, there is a mean concordance rate of 99.7%. A decrease in sequencing SNP call to GWAS call concordance was crucial in identifying a sample that had a low level of contamination from a neighboring sample during library preparation. With this workflow, we can process samples, determine and troubleshoot sample failures and quickly begin reprocessing those samples.

1874/T

Expanding Second-Generation Sequencing Applications Using the Agilent Technologies SureSelectTM Target Enrichment System. *S. Joshi[1], A. Giuffre[1], C. Pabón-Peña[2], B. Novak[2], M. Visitacion[2], B. Buehler[3], J. Ong[1], H. Ravi[1], E. Lin[2], D. Roberts[2], S. Happe[1], E. Leproust[2].* 1) Agilent Technologies, Cedar Creek, TX,78612, USA; 2) Agilent Technologies, Santa Clara, CA, 95051, USA; 3) Agilent Technologies, La Jolla, CA, 92037, USA.

Second-generation DNA sequencing has revolutionized the discovery of rare polymorphisms, structural variants, and novel transcripts. To meet the demand for fast, cost-effective, and accurate genome analysis methods from small scale studies to large sample cohorts, Agilent Technologies has developed the SureSelectTM Target Enrichment System. SureSelect is a highly robust, customizable, and scalable system that focuses analyses on specific genomic loci by hybrid capture of targets of interest. Agilent is continuing to expand its portfolio by increasing the number of applications available to users. We show high performance across Illumina, SOLiD, and 454 platforms, as measured by capture efficiency, uniformity, reproducibility, and SNP detection using DNA derived from cell lines, flash-frozen tumors, or FFPE samples. We highlight the utility of SureSelect across a wide range of target sizes and genome complexity using pre-designed catalog libraries targeting cancer gene sets, sequences encoding the kinome, and both human and mouse All Exon content. We propose a novel approach for variant discovery - using SureSelect catalog designs to uncover candidate variants, followed by the design of smaller focused custom libraries for SNP validation and region profiling. Custom content design is accomplished using the Agilent eArray software with candidate variant coordinates as input. By pooling many samples together per lane or slide, SureSelect kits for Illumina indexing and SOLiD barcoding enables the validation to be performed in large sample cohorts with substantial cost savings. In addition, accurate sample pooling is facilitated by the Agilent Bioanalyzer and QPCR NGS Library Quantification kits to ensure equal representation across samples. Further efficiencies are realized using the Bravo Automated Liquid Handling Platform to meet the need for parallel preparation of multiple indexed libraries. Lastly, we also describe a unique application of SureSelect for depletion of ribosomal RNA from RNA preparations to facilitate transcriptome analyses.

1875/T

SOLiD™ ChIP-seq kit for ChIP and ChIP-Sequencing from low number of cell or tissue samples. *Z. Chen, V. Anest, L. Pickle, K. Clancy, D. Krissinger, N. Mulakken, D. Leon, R. Bennett.* Life Technologies, 5791 Van Allen Way, Carlsbad, CA 92008.

Chromatin Immunoprecipitation (ChIP) assay is the most widely used and powerful method to identify regions of the genome associated with specific proteins. Combined with massively parallel next-generation sequencing technology, ChIP-sequencing provides a high resolution digital solution for genome-wide survey of protein-DNA interactions. We developed a SOLiD™ ChIP-seq kit, which offers an optimized ChIP workflow and an efficient ChIP-seq library construction from relatively low number of cell or tissue samples. ChIP procedure is usually laborious, time consuming, and typically requires large starting cell numbers or large amount of tissue. We use MAGnify™ Chromatin Immunoprecipitation System, which is suitable for fast enrichment of chromatin complexes and DNA recovery, for ChIP workflow in the kit. It is able to use lower starting cell numbers (10,000-300,000 cells) for ChIP thus preserving precious samples such as primary cells, stem cells, biopsies. We also developed a novel sample preparation method for a variety of tissues, such as brain, heart, kidney and liver. This new approach allows faster throughput to investigate different chromatin and transcription time-course events as well as enable antibody screening to determine ChIP compatibility. In addition, we develop a sensitive ChIP-seq library construction procedure which enables users to construct a complex library using as low as 1 ng ChIP DNA. Combining with SOLiD's ultra high sequencing throughput, SOLiD ChIP-seq offers a highly sensitive, hypothesis-neutral approach to accurately characterize protein-DNA interactions at genome-wide scale. Using SOLiD™ ChIP-seq kit, we characterize histone H3 methylation profile in the RSC-1 embryonic stem cells on the SOLiD system. The data demonstrate that SOLiD ChIP-seq kit provides a streamlined, optimized and reproducible assay for the enrichment of chromatin/protein complexes, DNA recovery using magnetic bead capture technology and construction of sequencing library.

1876/T

Single-tube targeted capture of 524 nuclear-encoded mitochondrial genes for DNA sequencing and copy number detection. *P. Shen[1], W. Wang[1,2], S. Krishnakumar[1], C. Palm[1], A. Chi[1], G. Enns[3], R. Davis[1], T. Speed[2], M. Mindrinos[1], C. Scharfe[1].* 1) Genome Technology Ctr, Stanford Univ, Palo Alto, CA; 2) Department of Statistics, University of California, Berkeley, CA; 3) Department of Pediatrics, Stanford University, Stanford, CA.

DNA sequencing costs have decreased rapidly, while applying high quality genome resequencing to many individuals with a suspected mitochondrial disorder has remained impracticable. As an alternative strategy, targeting genomic regions of higher interest, such as nuclear-encoded mitochondrial genes, allows for more cost effective resequencing of a larger number of clinically affected individuals. We developed a robust and reproducible single-tube assay of multiplex amplification of 524 nuclear-encoded mitochondrial genes (5,471 exons) using long capture circularization (LCC) probes. The LCC probes hybridize to sequences flanking a region of interest and capture these sequences of up to 600 bp in length by extension of the probes, followed by ligation to form circular molecules. The captured targets are multiplex-amplified from the common LCC priming sites. We evaluated the sequence capture efficiency through hybridization of a captured PCR sample to custom-designed Affymetrix resequencing arrays, which contain the coding sequences (0.82Mb) of the 524 candidate genes. We reproducible detected 92% of the captured sequences for DNA variant base-calling, while the efficiency increased to 98% for exons with a GC content of 20-65%. In addition, we found that our method preserved the copy number information of the genome in comparative sample analysis. Based on the analysis of array intensity signals, in a child with ornithine transcarbamylase (OTC) deficiency we identified a deletion of 9 out of 10 exons of this gene (the protein product localizes to mitochondria). In summary, the LCC technology uses only a small amount of ~300ng DNA starting material and enables both targeted resequencing and copy number detection in conjunction with array-based data analysis.

1877/T

Identification and analysis of DNaseI hypersensitive sites using tiling arrays and DNaseI-MLPA. *S.J. White, T. Ohnesorg, S. Eggers, F. Martin, A.H. Sinclair.* Molecular Development, Murdoch Children's Research Institute, Melbourne, Australia.

Open chromatin is a characteristic of genomic loci with regulatory functions, such as promoters, enhancers and silencers. These regions are preferentially digested by DNaseI, and DNaseI hypersensitivity assays are frequently used to identify and analyse regulatory regions. Many currently available methods, however, are either laborious, limited to short stretches of DNA, or require large numbers of cells. We aimed to develop a fast and simple method to overcome these limitations and facilitate the discovery of regulatory regions in the genome. We designed tiling arrays covering ~ 6 Mb of genomic DNA containing genes involved in gonad differentiation and development. DNA obtained from intact nuclei of ~250,000 cultured cells and treated with DNaseI was directly labelled and hybridised to the arrays. Our recently published method of DNaseI-MLPA was used to confirm the identified hypersensitive sites. In a preliminary study using two different testis cell lines (TM3 and TM4), we could identify a number of DNaseI hypersensitive sites, many of which were located close to transcriptional start sites. Interestingly, the few differences in DNaseI hypersensitive sites between the cell lines correlated with variable expression levels of associated genes. We are also using this method to identify and analyse regulatory regions of all members of the SOX gene family in 6 different human cell lines as well as a range of embryonic mouse tissues. We present here a simple and rapid method to identify and analyse DNaseI hypersensitive sites. Using only 250,000 cells we were able to identify many potential regulatory elements in the tested cell lines and tissues. Due to its simplicity, this method should facilitate the discovery and analysis of regulatory regions in the genome.

1878/T

Single Molecule Real-time DNA Sequencing on the Surface of a Quantum-dot Nanocrystal. *J.M. Beechem.* Genetic Systems, Life Technologies, Carlsbad, CA.

A single molecule, long read-length, real-time sequencing-by-synthesis technology has been developed by building a sequencer directly on the surface of a ~ 10 nm quantum-dot nanocrystal. Five-color fluorescence resonance energy-transfer technology (FRET) is utilized for DNA sequence detection, in which signals from the quantum-dot labeled DNA polymerase plus 4 DNA-base-specific acceptor dyes are simultaneously detected. Acting as the FRET donor, the Qdot™-polymerase generates a correlated "photon-dip" for every inserted based (termed the "quantum-correlation-signal"), allowing for more accurate base-calling. Because the sequencer is not physically bound to any solid substrate, it can be exchanged (like a reagent) during mid-sequence runs, effectively replacing damaged non-functioning polymerases mid-reaction. Each exchange cycle lengthens the effective read-length of the sequencer. In this manner, the read-length can be continuously extended without "gaps". Expanding upon this flexibility, after sequencing a particular length of DNA, the newly synthesized strand can be selectively removed. The original genomic DNA strand is then re-primed, Qdot™-polymerase sequencers are rebound, and the identical genomic DNA strand can be sequenced again, greatly increasing the net accuracy and not requiring circularization of genomic templates. In combining these features, the desired accuracy and read-length can be "tuned" by adjusting the number of reagent exchange cycles. Because each sequencing reaction can be completed in minutes, multiple exchange experiments can be performed per sequencing hour. Precisely engineered sequencing-grade Qdot™ nanocrystals are smaller than current commercially available materials (to increase FRET signals), and have an exctinction coefficient ~100X greater than organic-dyes, allowing for very low levels of excitation power to be used while sequencing, yielding an environment more biologically favorable to polymerase activity and template integrity . These Qdot™-polymerase sequencers can also bind to ultra-long DNA segments (>10kb) at multiple positions along the length of the DNA and sequence while moving "horizontally" (parallel to TIRF field), enabling the possibility of "ordered-reads" for long-phased haplotype sequencing. Examples of real-time sequencing of homopolymeric, patterned, and complex templates will be shown.

1879/T

High quality FFPE tumor sample analysis by a simple single-tube exon enrichment technology and high-throughput sequencing. *O. Ericsson[1], H. Johansson[1], M. Isaksson[1], M. Röjmyr[1], F. Roos[1], P. Eriksson[1], J. Stenberg[1], F. Dahl[1], M. Nilsson[2].* 1) Olink Genomics, Uppsala, Sweden; 2) Department of Genetics and Pathology, Uppsala University, Uppsala, Sweden.

We present data describing the application of Selector Technology to successfully enrich and sequence large sets of exons in both fresh frozen and formalin fixed paraffin embedded (FFPE) tumor samples. We also show preliminary data where the Selector Technology has been adapted to incorporate sequencing primers and barcodes during amplification thereby replacing the laborious sequencing sample preparation step with a single PCR. This enables streamlined analysis of large sets of samples. The technology has been scaled to amplify as many as 17,000 specific targets in one single reaction tube. Our data demonstrates an unbiased enrichment of exons for resequencing with more than 95% of reads mapping to amplified regions. Concordance with HapMap genotypes is 99% and the reproducibility (R2) between replicates is 0.98 at the single nucleotide level. High reproducibility provides the possibility to score copy number variations in tumor samples as well as calling copy number neutral loss of heterozygosity. These features are demonstrated using matched tumor-normal breast cancer samples. Conclusively, the Selector Technology is a convenient, high performance exon enrichment technology for analysis of large sets of cancer samples.

1880/T

Development of an automated, high-throughput exome capture and sequencing protocol. *C. Igartua, S.R. Austin, A. Gracien, M. Mynsberge, D.J. Roach, B.W. Paeper, J.D. Smith, E.H. Turner, J. Shendure, M.J. Rieder, D.A. Nickerson.* Department of Genome Sciences, University of Washington, Seattle, WA.

Exome sequencing provides a cost-effective method for the analysis of coding variation across large sample sets. Traditional shotgun library and exome capture methods have several production bottlenecks, reducing scalability and requiring manual sample manipulation. These methods, particularly those relying on a size-selection step, also often result in a low-complexity library that requires additional sequencing to cover the entire target at a depth sufficient for variant calling. We have developed an automated exome capture protocol that generates 96 sequencing libraries per week with no manual sample handling required. 3 ug of input genomic DNA undergoes a quality control test, including a gender check, concentration reading, PCR assay, and genotyping to establish sample quality and identity. Samples are then processed on liquid-handling robots in 96-well plates through shotgun library construction and in-solution exome capture with a custom pool of 2.1M Nimblegen SeqCap probes. No size-selection is required in this process. Throughout the protocol, the bulk of each sample is carried forward to maximize library complexity. Library quality is evaluated after shotgun library construction and after exome capture. With a single lane of Illumina paired-end 76bp sequencing, the 28 Mb exome target is covered >70x, with >90% of the target covered at 8x or greater; at least 80% of the target is covered at >20x. Approximately 15000 SNVs are detected in each sample, with >93% present in dbSNP (build 129); approximately 900 novel SNVs are called. Compared to high density genotyping of the input genomic DNA, the sequencing data shows >99% concordance.

1881/T

Using Lateral Ultrasonic Thrust™ for High-Throughput DNA Fragmentation. *B. Jamieson, K. Dev, V. Vivek, J. Shieh.* Microsonic Systems Inc.

With the continuing advancements in sequencing technologies, next-generation sequencers perform single molecule sequencing in real time and eliminate the need for DNA amplification. This dramatically reduces the sequencing cycle time. This reduction, however, creates a new bottleneck in the sample preparation process prior to sequencing. For either de novo sequencing or re-sequencing, DNA fragmentation is the first step in preparing DNA libraries. Shearing by ultrasonic energy has become a preferred method due to the large dynamic range of the technique, random fragments and the tight distribution of fragment lengths, but the throughput of current ultrasonic shearing methods is limited to one or few samples at a time and can take many hours to shear enough samples to feed next-generation sequencers. To ensure that up-stream DNA fragmentation does not slow down the next-generation sequencing process, Microsonic Systems Inc. is currently investigating the use of its patented Lateral Ultrasonic Thrust (LUT™) technology for rapid and effective DNA shearing. Currently used for compound solubilization, thawing frozen samples, assay mixing and bead and magnetic particle suspension, LUT technology works by using a Micro-Electrical-Mechanical Systems (MEMS) based transducer, which when excited with RF power generates ultrasonic waves. Microsonics has invented new techniques that use ultrasonic waves to enable rapid DNA fragmentation. To evaluate the effectiveness of LUT technology for DNA fragmentation, Lambda phage DNA (48 Kb) was fragmented at different energy settings. These fragmented samples were then analyzed for various lengths using gel electrophoresis. The results showed successful shearing of the whole genome DNA into fragments ranging from 500 bp to 12 Kb in minutes. This finding confirms that our MEMS based LUT technology can be applied to DNA fragmentation, and with its unique parallel sample processing capability, high-throughput DNA fragmentation is now possible at a speed compatible with the speed of next-generation sequencing.

1882/T

Evaluation of Three Targeted Enrichment Techniques on the ABI SOLiD Platform. *S. Linker, T. Guettouche, W.F. Hulme, A. Andersen, A. Diaz, M.A. Pericak-Vance, G. Bademci, J.M. Vance, J.R. Gilbert, D.J. Hedges.* Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL.

Despite the ever-increasing throughput and steadily decreasing cost of next generation sequencing (NGS), whole genome sequencing of humans is still not a viable option for the majority of genetics laboratories. This is particularly true in the case of complex disease studies where large sample sets are often required to achieve adequate statistical power. To fully leverage the potential of NGS technology on large sample sets, several methods have been developed to selectively enrich for regions of interest. Enrichment significantly reduces both monetary and computational costs compared to whole genome sequencing while allowing researchers to take advantage of NGS throughput. Several targeted enrichment approaches are currently available, including molecular inversion probe ligation sequencing (MIPS), oligonucleotide hybridization based approaches, and PCR-based strategies.The majority of data examining the efficiency of these approaches, however, has been obtained from the Illumina GAII NGS platform. To assess how these methods performed when used in conjunction with the ABI SOLID3+, we investigated three enrichment techniques: oligonucleotide hybridization array-based capture, oligonucleotide hybridization solution-based capture, and a multiplexed PCR-based approach. Target regions were selected from exons and Phast-17-way conserved areas throughout the human genome, covering 5 Mb in total. Probe and primer pair design was carried out for all three methods using their respective informatics pipelines. The hybridization approaches were able to target 89-91% of the desired sequence, while the PCR-based strategy achieved 97%. For the capture experiment, hybridization approach A targeted the entire 5Mb and methods B and C (PCR-based) targeted subsets of the 5Mb total. In all, approximately 1.5Mb of target space was identical for all 3 methods. Targeted regions from 16 human individuals were independently enriched with each method and separately sequenced on a single SOLID octet “spot.” The resulting reads were analyzed for several metrics, including consistency of coverage depth, on-target versus off-target efficiency, and genotype concordance with array-based genotyping data. Preliminary results indicate comparable overall peformance from both hybridization-based approaches, with reduced on-target efficiency for the PCR-based method. In contrast, the PCR-based approach could target a significantly larger percentage of desired sequence.

1883/T

Automated Library Construction Using A Digital Microfluidic Device For Next Generation Sequencing Platforms. *N.R. Mushero, M. Weiand, T.K. Sparrow, P.B. Cahill.* Genome Sequencing Platform, Broad Institute, Cambridge, MA.

The emergence of next generation DNA sequencing technologies has shifted most of the manual labor to the sample preparation portion of the process. The potential to run multiple samples together and ability to gain sufficient coverage from minimal sequencing runs has created a potential bottleneck at library construction. In addition to the considerable amount of time and energy needed to process samples, the sample preparation fraction of total sequencing costs becomes larger as sequencing instrument associated costs are reduced. Automating the sample preparation process allows for higher throughput, less sample to sample variation, minimal chance of contamination or sample mix-up, and significant reduction in reagent costs. Here we report on experiments done in collaboration with Advanced Liquid Logic (A.L.L.) using their microfluidic device as an automated method of next generation sequencing library creation. A.L.L.'s technology uses electrowetting to create tiny droplets in a chip, which can be manipulated electronically. The typical droplet size of 300nL allows all library construction reactions to be substantially scaled down, drastically reducing reagent costs. The chip design also makes sample indexing and pooling possible. DNA shearing and fragment size selection take place upstream of this device, but all other library construction steps occur on chip, including PCR and magnetic based reaction cleanup. Recent experiments examine sample input versus yield, bias, and coverage using A.L.L's device in comparison with standard manual library construction.

1884/T

Pair End Sequencing of Human Genomes on the SOLiD™ Platform. *L. Zhang, S. McLaughlin, E. Dimalanta, K. Eusko, D. Dhingra, C. Lee, C. Clouser, J. Spangler, T. Weaver, M. Lyons, H. Peckham, A. Blanchard, K. McKernan.* Life Technologies, Beverly, MA.

The SOLiD DNA sequencing system utilizes stepwise ligation of oligonucleotide probes and enables high fidelity, high throughput sequencing. Previous sequencing protocols for the SOLiD system have only been available in the forward direction (3' to 5'). However, fragment library paired end sequencing (in both forward and reverse directions) is highly desired to maximize sequencing capacity and to meet special research interests such as whole transcriptome and translocation studies. To achieve this using the SOLiD platform, novel ligation chemistries were developed to support 5' to 3' read lengths of up to 35 bases. Utilizing this new chemistry, we sequenced an anonymous Caucasian male to an average depth of coverage of 22.4x (2 SOLiD system sequencing runs) covering 96.55% of the genome. The purpose of any resquencing project is to measure variants against a known reference sequence, so we analyzed this data using our in-house variant detection algorithms to assess data integrity. Approximately 2.97M SNPs were discovered: 1.21M homozygotes (94.4% in dbSNP v129) and 1.76M heterozygotes (73.7% in dbSNP v129). Also detected were 103,027 small indels (73.8% in dbSNP v129). Paired-end sequencing with this novel chemistry presented here is effective at variant-detection in a human genome and these values are similar to variant totals from other large-scale human resequencing.

1885/T

The Association of Biomolecular Resource Facilities (ABRF): Advancing Human Genetics through Research, Communication, and Education. *D. Baldwin[1], T. Hunter[2], M. Detwiler[3], G. Grills[4], K. Sol-Church[5] for the ABRF Research Groups.* 1) Penn Molecular Profiling Facility, Univ. Pennsylvania, Philadelphia, PA; 2) University of Vermont; 3) Roswell Park Cancer Institute; 4) Cornell University; 5) Center for Pediatric Research, NCC-Delaware.

Improvements in cutting edge and emerging technologies, such as the introduction of massively parallel sequencing to biomedical research, have dramatically changed the landscape of genetics and the way genetic research is performed. Today, genetic research is often a collaborative effort that is performed in association with shared resource biotechnology (core) laboratories. These cores represent centers of technological excellence that bridge discovery and translational research by integrating state-of-the-art molecular genetics and proteomics technologies into current research programs. The Association of Biomolecular Resource Facilities (ABRF) is an international society dedicated to advancing biotechnology laboratories through research, communication, and education. ABRF Research Groups conduct multi-institutional studies that focus on comparison and optimization of the state-of-the-arts biotechnologies and methodologies available to biomedical researchers and geneticists. An overview of the aims and results of ABRF Research Group (RG) studies will be presented to illustrate the challenges and opportunities of genetic technologies. For example, the results of an RG gene expression study, based on reverse transcription quantitative real time PCR, underscores the importance of sample quality, optimal priming strategies, qPCR assay location and data troubleshooting. An RG study on microRNA expression highlights the strengths and weaknesses of some of the most popular array platforms and compares data from conventional microarray platforms with Taqman Low Density Arrays (TLDA) and two deep sequencing technologies (Illumina GAII and ABI SOLiD). An RG study on SNP discovery and validation also provides performance comparisons between these two sequencing platforms, and highlights the strengths and weaknesses of these platforms in dealing with allelic discrimination in a diploid organism. This presentation will provide a forum for discussion of cutting edge methodologies and approaches in molecular genetics and will illustrate how they can be used to facilitate human genetics research.

1886/T

Direct determination of molecular haplotypes by chromosome microdissection. *Q. Song[1], L. Ma[1], Y. Xiao[1], H. Huang[2], Q. Wang[1], W. Rao[1], Y. Feng[3], K. Zhang[4].* 1) Cardiovascular Res Inst, Morehouse Sch Med, Atlanta, GA; 2) Clinical Cytogenetics Laboratory, Department of Pathology, Vanderbilt University Medical Center, Nashville, TN; 3) Department of Pharmacology, Emory University School of Medicine, Atlanta, GA; 4) Section on Statistical Genetics, Department of Biostatistics, University of Alabama at Birmingham, Birmingham, AL.

Haplotype refers to a group of alleles inherited on a single chromosome. Haplotype analysis plays an important role for mapping disease genes, elucidating the population histories, studying the evolutionary genetics, and exploring the cis-interactions in the gene expression regulation. Direct observation of haplotypes is technical challenging. Here we report a method for determination of haplotypes through chromosome microdissection. We demonstrate this technology by accurately (> 98.85%) observing human haplotypes with 24,245 heterozygous single-nucleotide polymorphism (SNP) loci in genome-wide chromosome-range phasing distance. Chromosomal haplotypes will be important for functional interpretations of SNPs in cis-interactions and the integration of genetic and epigenetic datasets along the chromosomes.

1887/T

SyTE, a novel bioinformatics tool for efficient discovery of lens disease genes: Application in identification of a new cataract gene *TDRD7*. *S. Lachke[1], F. Alkuraya[2], G. Kryukov[1], S. Choe[1], A. Tsai[3], S. Al-Fadhli[4], A. AL-Hajeri[4], H. Shamseldin[2], S. Sunyaev[1], A. Drack[5], R. Maas[1].* 1) Division of Genetics, Brigham & Women's Hospital and Harvard Medical School, Boston, MA, USA; 2) Department of Genetics, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia; 3) Department of Pediatrics, University of Colorado-Denver, Aurora, CO, USA; 4) Department of Molecular Biology, Kuwait University, Kuwait City, Kuwait; 5) Department of Ophthalmology and Visual Sciences, The University of Iowa, Iowa City, IA, USA.

Identification of candidate mutant genes within a mapped interval in patients with developmental disorders remains a challenge. We aimed to devise a novel strategy that allows prioritization of candidate genes within a mapped interval. As a proof of principle, to efficiently identify lens cataract genes, we developed Systems Tool for Eye gene discovery (SyTE) that uses a developmental profile of the mouse lens transcriptome generated by microarray analysis of the lens as it transitions from the stage of placode invagination to that of vesicle formation. Using a novel *in silico* subtraction protocol with a developmentally matched microarray dataset representing whole embryo minus ocular tissue, we assigned lens enrichment *p*-values to individual probes, thus identifying candidate lens cataract genes. When SyTE was tested on mapped intervals of 23 previously identified non-syndromic cataract genes, it identified the correct cataract gene in >90% cases by ranking it within the top 5 candidates from around 50 or more genes in each locus. To test its ability to detect novel cataract genes, we studied a juvenile cataract patient who carries the balanced paracentric inversion 46,XY,inv(9)(q22.33q34.11). SyTE predicted *TDRD7* as the most probable candidate among 50 genes within a 5 Mb interval around the q22.33 breakpoint. *TDRD7* disruption and haploinsufficiency in the patient was confirmed by FISH and Western blot analysis. We then recruited a consanguineous family with recessive congenital cataract in which the causative mutation also mapped to 9q22.33. Upon sequencing *TDRD7*, we found a novel three base pair deletion mutation that results in deletion of Valine 618, which is conserved from fish to humans. Furthermore, in chick, *TDRD7* knockdown results in cataract formation, corroborating the assignment of *TDRD7* as the gene responsible for the cataract phenotype in human. Finally, section *in situ* analyses show high lens expression of eight candidate cataract genes, further establishing the validity of SyTE, and human orthologs of two of these (*SlPA1l3* and *PTPRU*) fall within mapped human cataract loci. In conclusion, we report a novel systems tool SyTE, which serves to prioritize candidate genes in mapped intervals and demonstrate its use in the identification of a novel cataract gene *TDRD7*. We anticipate that modified versions of SyTE can be used to efficiently identify causative genes in developmental disorders affecting other organs.

1888/T

Comparison and application of whole exome and genome sequencing on an individual with high risk for atherosclerosis. *J.K. Teer, N.F. Hansen, P.F. Cherukuri, L.L. Bonnycastle, P. Cruz, P.S. Chines, H. Ozel Abaan, E.H. Margulies, E.D. Green, J.C. Mullikin, L.G. Biesecker.* NHGRI, NIH, Bethesda, MD.

Massively parallel sequencing has allowed broader interrogation of genomes for variants that cause disease. Continuing improvements now allow whole human genome sequencing in a relatively short period of time. However, targeted sequencing requires fewer bases than a whole genome, and therefore allows more targeted samples than whole genome samples. We have compared coverage of the CCDS exome in NA18507 (HapMap Yoruba) using three different exome capture kits. We find that genotype sensitivity (% of the CCDS regions covered with high-quality genotype calls) is similar between all methods: 86%-88%. In comparison, previously reported 30x whole genome coverage of the CCDS regions in NA18507 was ~73%. We have also compared a more recent 60x whole genome sequence of a ClinSeq™ individual with exome capture. 60x whole genome sequence covered 86% of the CCDS using 192Gb total sequence, whereas exome capture covered 89% with 6.7Gb total. Both methods showed >99.9% overall concordance with genotype chip calls.

We have implemented a secondary analysis pipeline to realign reads using a gapped aligner, cross_match, and to call genotypes using a Bayesian based program, Most Probable Genotype (MPG). Genotypes are then annotated for coding status and potential detriment using CD_Pred. We have also developed a graphical java tool, VarSifter, to view, sort, and filter the resulting data, allowing investigators to focus on interesting variants. Using these tools we have sequenced and analyzed more than 70 exomes as part of the ClinSeq™ program.

We have performed both whole genome shotgun and exome sequencing on a ClinSeq™ individual at high risk for atherosclerosis. To get the highest coverage, we have merged the two data sets, resulting in 94.9% genotype coverage of the CCDS. Using our analysis tools, we identified 3,719,419 total variants, 22,264 of which were coding. We have examined the variants, and have limited the list by removing variants previously observed in 8 HapMap samples, by including non-synonymous single-nucleotide variants and frame-shifting deletion/insertion variants, and by examining variants within a linkage region. Many variants fit these criteria, and we are currently evaluating which are most likely to be causative.

1889/T

DNA microarray intensity prediction. *Y.A. Jakubek, D.J. Cutler.* Human Genetics, Emory University, Atlanta, GA.

There are several types of DNA microarrays including SNP, re-sequencing, CNV, and expression-profiling arrays. The principle behind these arrays is to hybridize labeled target DNA to probes that are attached to a solid surface. The intensity readings form the probe-target duplexes are used to infer genotype and/or copy number for the target DNA. However, often intensity data from certain probes and from entire chips is difficult to interpret, decreasing the information yield for the experiment. Known problems with microarrays include batch effects, high intensity variance between probes, high background intensity variance between chips, and cross-hybridization. In order to minimize the amount of data discarded and to increase the data quality, we created a detailed model of the probe-target DNA hybridization experiment that includes probe specific and experiment specific parameters. We first estimate expected hybridization kinetic values (Kd's) for all possible probe-target DNA duplexes that can form on the array. Then for each chip we estimate: probe nucleotide incorporation rates, mean fragmentation size, intensity detection curve shape, wash stringency, and target DNA concentrations. We use this information to estimate probe intensity in a model that incorporates cross-hybridization, chip-specific parameters, and probe-target DNA thermodynamics. The algorithm can be used to calculate probe intensity data for any type of DNA microarray experiment. Data from a re-sequencing array experiment consisting of 62 chips was analyzed using this approach. The mean correlation between expected and observed probe intensities for the chips (80,408 probes/chip) ranges from 0.59 to 0.85, with a mean correlation of 0.74 across all the chips. The mean of the estimated incorporation rates for A, C, G, and T nucleotides for the chips are 0.97, 0.92, 0.89, and 0.96 respectively. This intensity prediction model allows for discrimination between the effects of probe sequence, experimental conditions, and genotype on probe intensity.

1890/T

Detecting Mutant Allele Fractions as low as 0.01% by combining Digital PCR, LunaProbes™ and Mutant Allele Amplification Bias (MAAB) Methods. *M.D. Poulson, J.T. McKinney.* Idaho Technology, Inc., Salt Lake City, UT.

Detection of low level mutations has driven development of several intriguing methods. We have combined aspects of three prior techniques: digital PCR, LunaProbes, and Mutant Allele Amplification Bias (MAAB). Digital PCR (dPCR, Vogelstein and Kinzler, 1999) requires many replicates of a sample using template diluted to 1 copy/reaction. A variation on the original dPCR technique was developed for Hi-Res Melting (HRM) using template dilutions of 5 copies/reaction (McKinney, 2007). This dictates the mutant allele, if present, will comprise at least 20% of alleles in a reaction and enable detection of heteroduplexes. Sensitivity of dPCR is 2% or less, depending on number of replicates assayed. LunaProbes enable genotyping by using an unlabeled oligonucleotide in the PCR reaction (Zhou, 2005). Sensitivity has been established at approximately 5% for LunaProbes (Wall, 2007). MAAB utilizes the stability of the LunaProbe to preferentially amplify the mutant allele. MAAB has demonstrated sensitivity of <1% (McKinney, 2009). We hypothesized these methods could be integrated with additive benefits. A SNP in the PAH gene was targeted with a standard LunaProbe assay. Samples homozygous for both alleles were identified, standardized for concentration, and mixed to generate serial dilutions of the mutant allele down to 0.01%. Template dilutions (5 to 100 copies) were made, and 96 replicates of each were run on mutant allele dilutions of 1%, 0.1%, and 0.01% using dPCR, MAAB, and dPCR + MAAB (dMAAB) protocols. Results were analyzed to determine optimal PCR protocols, template dilutions, and replicates/dilution required to achieve the greatest sensitivity. A template dilution of 5 copies was optimal for the dPCR assay. For the MAAB protocol, 100 copies/replicate was optimal. All mutant allele dilutions were successfully detected using the dPCR and dMAAB protocols. The MAAB protocol used 3000 copies/replicate and detected the 1% and 0.1% dilutions. Fewer replicates were required to detect the 1% dilution relative to the 0.1% and 0.01% dilutions. The ability to detect low levels of mutant DNA has multiple applications. Some of these include drug resistant pathogens, fetal DNA, low level somatic mutations, and detection of residual disease from peripheral blood post-therapy. Combining previous methods and adding the use of a LunaProbe increases sensitivity 10-100 fold and allows mutant allele confirmation via specific probe melting profiles.

1891/T

Next Generation Multiplexed Small RNA Sequencing Reveals Differential miroRNA Expression Patterns. *M. Toloue.* Bioo Scientific, Austin, TX.

The demand for preparative steps that deliver fast and unbiased small nucleic acid sequence for investigation cannot be overemphasized. Current isolation, fragmentation, ligation and purification methods lead to bias, require significant preparatory time and are highly inefficient. To circumvent these obstacles we have developed several strategies that not only eliminate small RNA circularization and linker dimers, but allow for cross genome analysis using multiplexed barcodes, improving next gen. sequencing and experimental design. We simultaneously examined microRNA (miRNA) expression from 10 organ tissues by multiplexing the sequencing of these samples on a single flow cell lane. This experiment normally requires ten flow cell lanes. Multiplexed samples were tested for sequence bias and compared to individually prepared samples. The results not only identified the highest expressed miRNAs per sample but also demonstrated that within one sample preparation there was a strong expression correlation, however between different tissues, expression levels of miRNA significantly varied. For the first time, miRNA expression levels of normal versus diseased tissue can be studied in a single sequencing reaction lane. This indexing procedure reduces the number of lanes required for a multiplex experiment, eliminates sequence bias associated with ligation and allows drug and time course studies to be performed using an Illumina sequencing platform. It has become obvious that the use of the same preparatory methods to identify and quantify rare transcripts and perform large scale comparative evolutionary studies is not ideal and that there are clear advantages for particular applications over others. Our recent efforts directed towards the development of new template preparations illustrate this.

1892/T

Genotyping SNPs for Native American Y haplogroup assignment. *A.E. Justice[1,3], N.F. Baldi[1,3], C. Phillips-Krawczak[2,3], J. Gerst[4,5], P. Williams[5], M.H. Crawford[1,3].* 1) Anthropology, University of Kansas, Lawrence, KS; 2) Genetics, University of Kansas, Lawrence, KS; 3) Lab of Biological Anthropology, University of Kansas, Lawrence, KS; 4) Medical Center, University of Kansas, Kansas City, KS; 5) Evogen, Inc., Kansas City, MO.

Efficient single nucleotide polymorphism (SNP) genotyping methods are becoming increasingly common in anthropological genetics for haplogroup assignment and identification of ancestry specific markers. SNPs offer a statistical advantage over STRs, are appropriate for typing Native American populations when performed in a hierarchical fashion, and are more cost effective. As such, there is a need for affordable, efficient and reliable techniques for SNP analysis that are appropriate for both high throughput and smaller laboratories to increase consistency and comparability of data. One such method is developed here using a new probe technology, Hybeacons® (Evogen, Inc.), which addresses these goals. The technique involves a single primer pair to amplify the region of interest, normal PCR reagents, and Hybeacons® in a single PCR reaction. HyBeacons® are high definition fluorescently labeled PCR probes with two flourophores. When a probe binds with the target DNA sequence, the level of fluorescence from the probe intensifies, and is measured through melt curve analysis. The difference among melt curves allows the researcher to differentiate among the wild type and the polymorphic SNPs. Hybeacons® are appropriate for high-throughput laboratories as automated genotyping requires only one reaction per SNP. This new method was authenticated using four common Y chromosome SNPs used for haplogroup assignment (Q-M242, Q-M3, R-M269, Q-P36) in Native American populations from the Caribbean, Guatemala, Nicaragua, and Peru. The results were consistent with those from an independent sequencing of a subset of samples from each population. The Hybeacons® have the potential to be a robust typing tool for both high throughput and smaller labs with a wide range of applications in anthropological and population genetics.

1893/F

LRP4 is associated with fracture and bone phenotypes and interacts with other genes in the Wnt- and BMP signaling pathways. *J. Kumar[1], M. Swanberg[1], F. McGuigan[1], M. Callreus[1], P. Gerdhem[2], K. Åkesson[1].* 1) Clinical and Molecular Osteoporosis Research Unit, Department of Clinical Sciences, Lund University, and the Department of Orthopedics, Malmö University Hospital, Malmö, Sweden; 2) Department of Orthopedics, Karolinska University Hospital, Department of Clinical Science, Intervention and Technology, Karolinska Institute, Stockholm, Sweden.

Osteoporosis is a complex disorder leading changes in the micro-architecture of bone and on increased risk of fracture. It is common in postmenopausal women with an estimated 40 percent of women sustaining at least one osteoporotic fracture during their lifetime. Members of the low-density lipoprotein receptor-related protein (LRP) gene family regulate bone development and physiology through the Wnt signaling pathway. The LRP4 gene has been shown to be important in osteoporotic phenotypes through candidate gene and genome wide association studies. Cross-talk between Wnt and BMP signaling pathways is also known to play a role in the regulation of bone. In the current study, we have analyzed the association of three single nucleotide polymorphisms (SNPs) from the LRP4 gene for their association with bone mineral density (BMD), fracture and other bone related phenotypes. Additionally, we have analyzed interactions of LRP4 with three other genes (LRP5, G protein-coupled receptor 177 (GPR177) and Bone Morphogenetic Protein 2 (BMP2)). LRP5 and GPR177 can modify Wnt signaling while BMP2 is part of the BMP signaling pathway. Analyses were performed in two cohorts of Swedish women differing in age: OPRA (1002 elderly women all aged 75 years) and PEAK-25 (1005 young women all aged 25 years) to determine if age influences the possible associations and interactions of these genes. SNPs from the LRP4 gene were found to be significantly associated with increased BMD (p=0.05), decreased bone loss (p=0.02), improved bone quality (p=0.02) and geometry (p=0.007) along with decreased incidence of fractures (p=0.001) in the elderly women. LRP4 polymorphisms were also found to be significantly associated with increased BMD in young women (p=0.007). Significant gene-gene interactions were observed for LRP4 with the GPR177, BMP2 and LRP5 genes. LRP4-GPR177 and LRP4-BMP2 was associated with BMD and bone loss (p=0.05 to <0.001), while LRP4-LRP5 and LRP4-BMP2 were associated with bone quality in the elderly women (p=0.05-0.02). In the young women, interactions for BMD were observed for LRP4-BMP2 (p=0.03-0.004). In conclusion, SNPs from LRP4 gene showed significant association with several bone phenotypes and fracture in elderly women. Additionally, interactions between genes from the Wnt and BMP signaling pathways contribute to the modulation of bone phenotypes contributing to fracture risk, the clinical endpoint of osteoporosis.

1894/F

Identification of novel mutations in the U87 glioblastoma cell-line by examining exomic multi-nucleotide polymorphisms. *J. Rosenfeld[1,2,4], A. Malhotra[1,2,3], T. Lencz[1,2,3].* 1) Zucker Hillside Hospital, Glen Oaks, NY; 2) The Feinstein Institute for Medical Research, North Shore - Long Island Jewish Health System; 3) Dept. of Psychiatry and Behavioral Sciences, Albert Einstein College of Medicine; 4) American Museum of Natural History, New York, NY.

Genomic sequence comparisons between individuals are usually restricted to the analysis of single nucleotide polymorphisms (SNPs). While the interrogation and analysis of SNPs is efficient, they are not the only form of divergence between genomes. We expand the scope of polymorphism detection by investigating the occurrence of double nucleotide polymorphisms (DNPs) and triple nucleotide polymorphisms (TNPs), in which consecutive nucleotides are altered compared to the reference sequence. These mutations are missed by traditional re-sequencing analysis, since the underlying reads would be rejected for having too many mismatches compared to the reference genome. Before looking at the U87 exome, we first analyzed two complete human genomes and exomes to identify DNPs and TNPs. We observed that, although the overall abundance of DNPs and TNPs in exomes is much less than SNPs, these mutations are more likely to affect two amino acids and could contribute to disease risk. In U87 exome, we identified 53 DNPs and 8 TNPs within the coding regions. The most potentially pathogenic mutations that we identified were a TNP causing a single amino acid change in the LAMC2 oncogene and a TNP causing a nonsense mutation in the HUWE1 gene. The mutation in the LAMC2 gene, L952D was predicted by to be damaging by computational analysis. The HUWE1 nonsense mutation causes the protein to be mRNA to be truncated from 4,374 residues to 1,668 residues in length. Neither of these mutations were identified by the conventional SNP-based exome analysis.

1895/F

Convergence of *CXCL12* and *PHACTR1*: Novel Genome Wide Signals for Myocardial Infarction Regulate Plasma CXCL12 Levels. *NN. Mehta[1,2], D. William[1], M. Li[2], A. Khera[1], L. Qu[2], S. DerOhannessian[1], L. Pruscino[1], L. Shaikh[1], C. Hinkle[1], J. He[2], I. Stylianou[1], H. Hakonarson[3,4], DJ. Rader[1], MP. Reilly[1,2].* 1) Dept Cardiology, Univ Pennsylvania, Philadelphia, PA; 2) Center for Clinical Biostatistics and Epidemiology, Univ Pennsylvania, Philadelphia, PA; 3) Center for Applied Genomics, Univ Pennsylvania, Philadelphia, PA; 4) Children's Hospital of Philadelphia, Philadelphia, PA.

Recent genome wide association studies have shown association between coronary artery disease (CAD) / myocardial infarction (MI) and common variation at locus 10q11 ($8.1x10^{-9}$) downstream from *CXCL12*, an inflammatory cytokine; and at locus 6p24 ($3.1x10^{-8}$) near gene *PHACTR1*, a phosphatase-related enzyme. Genetic variation at 10q11 also relates to plasma levels of CXCL12. However, other factors involved in regulation of CXCL12 are poorly understood. Therefore, we performed a genome-wide association study of plasma CXCL12 levels to better understand the biology and relationship to MI. **Methods and Findings:** We measured plasma CXCL12 levels (ELISA, R&D) in a subset of the angiographic study, PennCath (n=1182, 406 with acute MI, 386 with chronic CAD, 390 angiographically normal controls). Genotyping was performed using the Affymetrix 6.0 chip. All samples & SNPs passed stringent QC; there was no significant population stratification (lambda=1.00085). There were multiple SNPs strongly associated (p value $<10^{-5}$) with plasma CXCL12. These SNPs mapped to chromosome (chr) 6p24.1 (*PHACTR1*, top SNP rs202071, $p=5.14x10^{-7}$, beta-coefficient -0.145), chr 6p21.3 (*HIST1H2BC*, rs13206443, $2.19x10^{-7}$, 0.322), chr 5q11.2 (*ITGA1*, rs10044180, $p=2.29x10^{-6}$,-0.123), and 11q23.2 at (*ZW10*, rs2279996, $p=3.42x10^{-6}$, 0.169). These associations remained robust after adjustment for age, gender, CAD, DM, and HTN. **Conclusions:** We show a number of independent genetic associations with CXCL12 plasma levels, and reveal *PHACTR1* in potential trans-regulation of CXCL12 as well as CAD/MI. Ongoing studies for replicating these associations and to examine how these novel loci relate to CAD/MI and each other may reveal novel regulatory pathways in CAD and MI.

1896/F

dbRIP, a database of retrotransposon insertion polymorphism in humans --- a report of major updates. *P. Liang[1], W. Tang[1], B. Abraham[2].* 1) Department of Biological Sciences, Brock University, St. Catharines, Ontario, Canada L2S 3A1; 2) NIH Graduate Partnerships Program with Boston University, Boston University, Boston, MA, USA 02215; National Heart, Lung and Blood Institute, NIH, Bethesda, MD, USA 20892.

Retrotranspons contribute to almost 50% of the human genome, and they play important roles in shaping the evolution of the genomes. Since members of many retrotranspons families are still active, they have generated genome diversity and continue to do so by transposition-mediated insertions, and such Retrotransposon Insertion Polymorphisms (RIPs) can impose a variety of impacts on gene function. The database, dbRIP, was developed in 2006 as the first database dedicated to the documentation of such types of genetic polymorphism in humans. Since then dbRIP has been recognized as a valuable resource in the retrotransposon community because of its easy-to-use interface, which integrates with the UCSC Genome Browser, and high quality of detailed and manually curated data, and easy access to the full dataset. We have recently performed major updates to dbRIP, which include the addition of substantial new RIP data sets and coverage for HERV polymorphism data, migration to the hg18 and hg19 UCSC genome versions, implementing a stable database identification schema, and addition of a position mapping utility. As of writing, dbRIP contains a total of more than 2,600 cases of manually curated retrotransposon insertion polymorphisms, covering Alus, L1s, SVAs, and HERVs. Future maintenance of dbRIP will be focused on timely updating of newly available RIP data, a lot of which will likely come from analysis of personal genomes. The database is freely accessible at **http://dbRIP.brocku.ca**. (This work is in part supported by grants from the Canada Research Chair program, CFI, OMRI, NSERC and Brock University to PL.).

1897/F

ALFRED: rapidly growing resource for research and teaching. *H. Rajeevan[1,2], U. Soundararajan[1], A.J. Pakstis[1], J.R. Kidd[1], K.K. Kidd[1].* 1) Dept Genetics,School of Medicine, Yale Univ, New Haven, CT; 2) Center for Medical Informatics, School of Medicine, Yale University School of Medicine, New Haven, CT.

ALFRED (http://alfred.med.yale.edu) is a free, web-accessible and actively curated compilation of gene frequency data for molecularly defined polymorphisms on anthropologically defined population samples with active links to ethnographic and molecular databases, and to the relevant literature. Data sources include published literature, collaborators, high throughput data sources and the host laboratory. In the past year the number of allele frequency tables has increased from 500,000 to 1,452,000, largely through the addition of large datasets. Two such additions are: Illumina 370CNV chip data typed on an Estonian population sample (PMID: 19424496, data contributed by Andres Metspalu) and Illumina HumanHap650Y Beadchip data typed on a Korean population sample. Data currently in curation (650K data on HGDP and Kidd Lab populations) will bring the total number of populations to 77 for this panel of 650,000 markers. ALFRED now contains data on several defined SNP panels (e.g PMID: 16586411, PMID: 19083773) that are searchable through a recently added search function "SNP sets" available from ALFRED's homepage. The markers in each of these SNP sets are annotated with relevant information including the locus name, rs#, fst, average heterozygosity and the number of populations for which there are data available in ALFRED. The SNP sets can be sorted by all these functions as well. Another new feature is the ability to restrict the Fst search by "minimum number of populations" option in addition to the already available options. A new addition to the tabular summaries is the "Populations List" displaying the list of all populations in ALFRED with the geographic region and the number of samples for that population. This table can be sorted by Geographic region/Population/# samples option. Among multiple graphical display modes for allele frequencies for a polymorphism, pie-charts can be viewed on Google Map and Google Earth. These display modes allow users to utilize all the functions available from the Google Map, Google Earth applications. ALFRED is supported by US NSF BCS0840570.

1898/F

Confirmation of Genetic Associations from a Genome-wide Association Scan of the GoKinD Collection Identifies Common Type 1 and Type 2 Diabetic Nephropathy Loci on Chromosomes 11p and 13q. *M.G. Pezzolesi[1,2], G.D. Poznik[1], J. Skupien[1,2], A. Smiles[1], J.C. Mychaleckyj[3], S.S. Rich[3], J.H. Warram[1,2], A.S. Krolewski[1,2].* 1) Section on Genetics and Epidemiology, Joslin Diabetes Center, Boston, MA; 2) Department of Medicine, Harvard Medical School, Boston, MA; 3) Center for Public Health Genomics, University of Virginia School of Medicine, Charlottesville, VA.

Objective: Diabetic nephropathy (DN) is a complication that affects 30% to 40% of all patients with either type 1 (T1D) or type 2 diabetes (T2D). In a genome-wide association (GWA) scan of the Genetics of Kidneys in Diabetes (GoKinD) collection, we identified four novel susceptibility loci on chromosomes 7p, 9q, 11p, and 13q that were significantly associated with DN in T1D. To follow-up on our initial findings, we examined whether the leading single nucleotide polymorphisms (SNPs) from each of these loci were associated with the risk of DN in patients from the Joslin T2D DN collection. Research Design and Methods: Six SNPs (rs39075 on chromosome 7p, rs1888747 and rs10868025 on chromosome 9q, rs451041 on chromosome 11p, and rs1411766 and rs9521445 on chromosome 13q) across four distinct susceptibility loci were genotyped in 692 normoalbuminuric (control) and 751 proteinuric or end-stage renal disease (case) subjects of European ancestry from the Joslin T2D DN collection. Results: Significant associations were identified at both the 11p (rs451041: OR=1.21, $P=7.5x10^{-3}$) and 13q loci (rs9521445: OR=1.24, $P=2.5x10^{-3}$) in the Joslin T2D collection. A meta-analysis combining these data with that from GoKinD supported these results (rs451041: OR=1.30, $P=3.3x10^{-7}$ and rs9521445: OR=1.31, $P=9.7x10^{-8}$). SNPs on chromosomes 7p and 9q were not associated with DN in the Joslin T2D DN collection. Conclusions: Our analysis of SNPs identified in a GWA scan of the GoKinD collection confirmed associations at two loci; one on chromosome 11p (near the *CARS* gene) and one on chromosome 13q (in an intergenic region between *MYO16* and *IRS2*). These findings implicate the 11p and 13q regions as common diabetic nephropathy loci in both type 1 and type 2 diabetes.

1899/F

Decoding the Information Content of SNP Haploblocks Associated with Innate Immunity in African Americans. *T.E. Mason[1], L. Ricks-Santi[1,2], P. Kurian[3], W. Hercules[3], J. Lindesay[3], G.M. Dunston[1,3,4].* 1) National Human Genome Center, Howard University, Washington, DC; 2) Howard University Cancer Center, Washington, DC; 3) Department of Physics, Howard University, Washington, DC; 4) Department of Microbiology, Howard University, Washington, DC.

Background: Decoding the functional significance of natural variation in the human genome is at the forefront of translational genomic research. Various studies have shown an association between single nucleotide polymorphisms (SNPs) and common disease which lead to the "common- variant-common-disease" hypothesis. We hypothesize that information encoded in the structure of common variants, such as SNP haploblocks, can elucidate molecular pathways and cellular mechanisms involved in the regulation of host adaptation to the environment. Specifically that the regulation of candidate genes underlying innate immunity are essential to the host's adaptation to pathogens in the microbial environment. We utilized the normalized information content (NIC), a derived metric which is based on SNP haploblock structure for interrogating the biology of candidate genes in the immune response. Methods: SNP haploblocks were constructed (Haploview v. 4.2) for the human Major Histocompatibility Complex (MHC) in the African American population from HapMap Phase III data. NIC scores were derived for these haploblocks and analyzed for functional significance of common variants in the MHC, the master regulatory region of the host immune system. Initial attention was given to an assessment of NIC scores relative to potential regulatory elements using ConSite and miRBase. Results: We found that all 10 of the SNP haploblocks with NIC scores in the lower bound (<0.40 on a scale of 0 to 1; $p<0.05$) contained putative transcription factor binding sites (TFBS) and microRNA (miRNA) motifs, thus leading to the identification of common variants involved in the regulation of candidate gene pathways underlying host adaptive mechanisms, such as the immune response. Conclusion: Using the NIC scores, we were able to translate a biophysical mathematical measure of common variants into a deeper understanding of biochemical patterns in SNP haploblocks. We submit that this new metric, the NIC score, may be useful in decoding the functional significance of natural variation and analyzing the regulation of genes involved in host adaptation to environmental pathogens and positive selection.

1900/F

Variation in Transcription Factor Binding Among Humans. *M. Kasowski[1], F. Grubert[2], C. Heffelfinger[1], M. Hariharan[2], A. Asabere[1], S. Waszak[3], L. Habegger[1], J. Rozowsky[1], M. Shi[2], A. Urban[2], M. Hong[1], K. Karczewski[2], W. Huber[3], S. Weissman[1], M. Gerstein[1], J. Korbel[3], M. Snyder[1,2].* 1) Yale Univeristy, New Haven, CT; 2) Stanford University School of Medicine, Stanford, CA; 3) European Molecular Biology Laboratory, Heidelberg, Germany.

Differences in gene expression may play a major role in speciation and phenotypic diversity. Although variations in gene expression among individuals have been documented, the origins of these differences are not clear, and studies that directly measure differences in transcription factor binding sites among humans have not been performed. We have examined genome-wide variation in transcription factor binding in different individuals and a chimpanzee using chromatin immunoprecipitation followed by massively-parallel sequencing (ChIP-Seq). The binding sites of RNA Polymerase II (Pol II) as well as a key regulator of immune responses, NFκB, have been mapped in ten lymphoblastoid cell lines derived from individuals of African, European, and Asian ancestry, including a parent-offspring trio. Using a stringent threshold, approximately 7.5% and 25% of the respective NFκB and Pol II binding regions exhibit differences between any two individuals. To understand the underlying basis of the variations, we examined the effect of SNPs and genomic structural variations (SVs) on binding differences among individuals. We find that many binding differences are associated with SNPs and SVs. Comparison of the binding data with gene expression data generated by RNA-Seq revealed that differences in binding often correlate with gene expression differences. Furthermore, comparison of the Pol II human sites with binding sites identified in the chimpanzee suggests a high level of divergence in binding relative to our closest evolutionary neighbor. Our results indicate that many differences in individuals occur at the level of TF binding and provide insight into the genetic events responsible for these differences.

1901/F

Developing Ethnic-Based Reference Sequences: *CFTR* as a Model. *P.G. Ridge Jr[1], E. Lyon[1,2], K.V. Voelkerding[1,2].* 1) ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT; 2) Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT.

Background: Publically available human genome reference sequences may not be representative of multiple ethnic groups. This creates interpretive challenges in diagnostic resequencing studies as many ethnic specific variations are likely missing or not identified as to ethnicity from current reference sequences. Using the *CFTR* gene as a model, the goal of the current study is to develop an expanded *CFTR* reference sequence that incorporates ethnic specific variant information.

Methods: The database for the current study is comprised of approximately 1,000 *CFTR* full gene Sanger sequences from patients referred for clinical testing for suspicion of cystic fibrosis. Sequencing trace files were sorted by self-reported ethnic background (African-American, Ashkenazi Jewish, Caucasian, Hispanic, Middle-Eastern, Other, and Mixed ethnicity). Traces were aligned and variants called according to *CFTR* transcript (NM_ 000492.3) using the CLC Genomics Workbench. Using custom built Java programs we determined which variants were present in dbSNP and/or the Cystic Fibrosis Mutation Database (CFMD).

Results: In a first subset analysis, we have examined 82, 15, and 15 individuals each for Caucasians, African-Americans, and Hispanics, respectively. In this subset, 569 unique variations were identified; 412 in Caucasians, 127 in African-Americans, and 45 in Hispanics. Between 57.8% and 96.6% of these variants were ethnic specific. In Caucasians and African Americans, respectively, 10.1% and 12.7% of variants were present in at least one of the databases (dbSNP and CFMD). No Hispanic-specific variants were found in either database. In total there were 16 missense variants identified, 14 of which were ethnic specific. Only two variants of these 14 ethnic specific variants were found in CFMD (none in dbSNP), both of which were Caucasian specific. Additionally, 2 nonsense mutations were identified.

Conclusions: In 112 patient samples analyzed to date, multiple unique, ethnic specific variants in the *CFTR* gene were identified. A majority of the identified variants were not present in publically available databases. A complete analysis of the 1,000 patient sample set is ongoing in which identification of additional unique variants is expected. From this, an expanded, ethnic specific *CFTR* database will be developed.

1902/F

Flip-flop associations at common SNPs in HIV-1 controllers of European and African ancestry are due to differing LD patterns with protective class I HLA alleles. *P.J. McLaren[1,2], X. Jia[1,2], S. Ripke[2,3], A.L. Price[4], S. Pollack[4], F. Pereyra[1,5], N. Gupta[2], D.W. Haas[6], B.D. Walker[5], P.I.W. de Bakker[1,2], Intl HIV Controllers Study.* 1) Brigham and Women's Hospital, Boston, MA; 2) Broad Institute of MIT and Harvard, Boston, MA; 3) Massachusetts General Hospital, Boston, MA; 4) Harvard School of Public Health, Boston, MA; 5) The Ragon Institute of MGH, MIT and Harvard, Boston, MA; 6) Vanderbilt University School of Medicine, Nashville, TN.

Purpose: Localization of causal variants can be confounded by apparent heterogeneity in SNP associations in different populations. Understanding the nature of so-called "flip-flop" associations (with effects in opposite directions) should help pinpoint causal variants. Methods: We genotyped a cohort of 857 HIV-1 controllers defined as maintaining viral load <2,000 per mL for at least 1 year, and 2,088 progressors, with median viral load >50,000 per mL, from two ethnic groups (European ancestry and African American). Using data from HapMap and the Type 1 Diabetes Genetics Consortium, we imputed 1.4 million SNPs and classical HLA alleles. For both ethnic groups, we tested SNPs and HLA alleles for association by logistic regression correcting for population structure, and performed meta-analysis. In the combined sample, we also performed an association test (2-df chi-squared) that allows for different odds ratios for African vs. European local ancestry as estimated by HAPMIX. Results: We observed >300 SNPs in the European ancestry sample and >30 SNPs in African Americans with genome-wide significance (p<5E-8), all located in the MHC region. Meta-analysis across the two samples demonstrates substantial effect heterogeneity for a range of SNPs that reached genome-wide significance in either sample. The most extreme example was for SNP rs2596503 that reached genome-wide significance in both samples but with opposite direction (p= 1.3E-9, OR=2.1 in Europeans; p=1.9E-11, OR=0.43 in African Americans; p=0.6 in meta-analysis). For many of the SNPs with apparent heterogeneity, the differences in effect estimates for European vs. African ancestry was statistically significant. A close look at the LD structure reveals that the apparent heterogeneity at rs2596503 can be explained by protective HLA alleles in Europeans (B*57, B*27 and B*14) segregating with the G allele, and protective alleles in African Americans (Cw*18 and B*81) segregating with the A allele. Conclusions: Flip-flop associations can be reconciled by differences in LD patterns between common SNPs and causal variants. For diseases where the MHC is implicated we expect such "flip-flop" associations to occur frequently in this region due to the highly polymorphic nature and broad LD.

1903/F

ELF1 is associated with systemic lupus erythematosus in Asian populations. *J. Yang, W. Yang, Y.L. Lau.* University of Hong Kong, Hong Kong, Hong Kong.

Great progress has been made in revealing the genetic variants that predispose individuals to complex diseases through genome-wide association studies (GWAS). However, the genes discovered so far only explain a small portion of heritability for complex disease such as systemic lupus erythematosus (SLE). Imbalance in the populations studied may have prevented us from identifying the population-specific susceptibility genes. This may be especially true for SLE, which has an apparent population difference in both disease prevalence and severity. Through a multi-stage study including GWAS and replication in several Asian populations, which included a total of 3164 patients and 4482 matched controls, we identified ELF1, an Ets family transcription factor that is involved in T cell development and function, associated with SLE (rs7329174, OR = 1.26, joint P = 1.47X10-8). The SNP also showed more association in patients with renal nephritis comparing to patients without (OR = 1.14, joint P = 0.059). The risk allele for this SNP and other SNPs in LD with rs7329174 has extremely low allele frequencies in populations of European ancestry. Bioinformatics analysis of the ELF1 gene revealed evidence for 3 alternative first exons in a 40 kb region in this gene, indicative of a complex control for its transcription, and SNP rs7379174 was located upstream of the third exon1, E1C. The usage of the 3 distinct first-exons was confirmed in PBMC in all patients and healthy controls examined. Furthermore, a prominent alternative splicing variant coding for a 56 amino acid deletion form of the protein was detected also in both patients and controls, both with abundant expression level. Although direct association of rs7329174 with exon 1 usage or alternative splicing was not established, these findings suggest that ELF1 may play a role in SLE susceptibility and the gene is tightly regulated in expression and alternative splicing.

1904/F

The Human Variome Project - Pilot Projects and Progress. *R. Cotton[1], F. Macrae[2], M.J. Sobrido[3], A. Lo[4], H. Howard[1].* 1) Genomic Disorders Res Ctr, Melbourne, Australia; 2) The Royal Melbourne Hospital, Melbourne, Australia; 3) Fundacion Publica Galega de Medicina Xenomica, Santiago, Chile; 4) Victorian Partnership for Advanced Computing, Melbourne, Australia.

Cotton R.G.H., Macrae Finlay, Sobrido Maria Jesus, Lo, Alan, Howard Heather Convenor, Human Variome Project; Head, Genomic Disorders Research Centre, Howard Florey Institute; Melbourne, Australia Email: cotton@unimelb.edu.au The Human Variome Project (2007) (www.humanvariomeproject.org) initiated in June 2006 (Ring, Kwok et al. 2006) focuses on the importance of collection and its phenotype, and to develop programs to put this into practice. The project builds on work and concepts of the HGVS over the years (www.hgvs.org) to focus variations associated with disease. It includes those discovering mutations, their effectsand collect the data and make it instantly available to clinical decision-makers and researchers. The HVP and InSiGHT (www.insight-group.org) has developed a major pilot study to develop procedures and systems that allow effortless flow of de-identified data from the patient/clinic/diagnostic laboratory via curated locus or gene specific databases to central databases/genome browsers such as NCBI, UCSC and EBI. The system will be easily adaptable to other genes and to multiple laboratories, states and countries worldwide. A country specific collection pilot is underway in Australia and an International Confederation of these countries has been initiated with Korea and China in the application process. Other pilot studies developed include specific ethical studies on mutation collection, loading of LSDB content to dbSNP, funding of curation of LSDBs, a system of Microattribution/reward for mutation submission. References:- (2007). "What is the human variome project?" Nat Genet 39(4): 423. Cotton, R. G., W. Appelbe, et al. (2007). "Recommendations of the 2006 Human Variome Project meeting." Nat Genet 39(4): 433-6. Cotton, R. G., A. D. Auerbach, et al. (2008). "GENETICS. The Human Variome Project." Science 322(5903): 861-2. Kaput, J., R. G. Cotton, et al. (2009). "Planning the Human Variome Project: The Spain report." Hum Mutat 30(4): 496-510. Ring, H. Z., P. Y. Kwok, et al. (2006). "Human Variome Project: an international collaboration to catalogue human genetic variation." Pharmacogenomics 7(7): 969-72.

1905/F

Allele Frequency Spectrum Based Analysis Suggest Sequencing Bias in Pilot 1 Data of 1000 Genomes Project. *z. hou[1,2], H. Siu[1,2], K. T[3], L. Jin[1,2,3], M. Xiong[1,2,4].* 1) Laboratory of Theoretical Systems Biology, School of Life Science, Fudan University, Shanghai, China; 2) State Key Laboratory of Genetic Engineering and MOE Key Laboratory of Contemporary Anthropology, School of Life Sciences and Institutes of Biomedical Sciences, Fudan University, Shanghai, China; 3) CAS-MPG Partner Institute for Computational Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China; 4) Human Genetics Center, University of Texas School of Public Health, Houston.

The 1000 Genomes Project is the first project to sequence the whole genome of a large number of people, which is expected to provide a comprehensive resource on human genetic variation. Pilot 1 data, whole-genome sequencing of 180 samples (60 samples in each of the three populations, YRI, CEU and ASI), in this project have been released in several versions, of which the latest two were published in December, 2009 and March, 2010 and were named as Data I and Data II by us respectively. We analyzed the two data sets by calculating two different estimations of the fundamental population genetic parameter θ (Tajima's statistic and Watterson's statistic), and several allele frequency spectrum- based statistics, namely Tajima's D, Fu and Li's F* and Achaz's Y*. We used a sliding window of 100 kb for calculations. Comparisons with previous published works show great differences in both the average of estimations of θ and the distributions of Tajima's D. To investigate their differences, forwards-in-time simulations were carried out with FREGENE, according to the widely used comprehensive demographic models under the condition of neutral evolution. Distributions of all statistics from simulations also illustrate great skew to the left, comparing with empirical data. To find out how the deviations occurred, we found that all the curves of the ratio of minor allele frequency (MAF) of the two data sets to simulated data under neutrality dramatically change with the number of minor alleles when the number of minor alleles is less than 7, which demonstrates that minor alleles of low frequencies are much less than expected. To confirm whether it is generated from sequencing bias or very recent serious population bottlenecks, we simulated a serious bottleneck for each population by slightly modifying the demographic model under neutrality, using a serious bottleneck during which the effective population size decreased to 1% of the previous from recovery of the second bottleneck to 50 generations ago, to instead of last expansion. Comparing with results of simulation under neutrality, such kind of bottleneck does skew the trend to the right, however, the deviation is much less than the one between Pilot 1 data and neutral simulations. This showed that bias is not caused by population bottleneck.

1906/F

A Modular Pipeline for Detecting Human Genetic Variations from Next-Generation Sequencing Data at NCBI. *C. Xiao[1], A. Ward[3], T. Blackwell[2], A. Mnev[1], W. Chen[2], P. Anderson[2], H. Kang[2], B. Li[2], X. Zhan[2], D. Stewart[3], W. Lee[3], M. Stromberg[3], W. Leong[3], A. Indap[3], D. Barnett[3], R. Agarwala[1], C. Marks[1], J. Paschall[1], T. Sneddon[1], J. Garner[1], D. Church[1], L. Phon[1], M. Shumway[1], D. Preuss[1], E. Yaschenko[1], M. DiCuccio[1], G. Abecasis[2], G. Marth[3], S. Sherry[1], the 1000 Genomes Project.* 1) National Center for Biotechnology Information, National Library of Medicine, National Institute of Health, Bethesda, MD; 2) Department of Biostatistics, University of Michigan at Ann Arbor, 1420 Washington Heights, Ann Arbor, MI; 3) Boston College Biology Department, 140 Commonwealth Avenue, Chestnut Hill, Massachusetts.

With the extremely high throughputs and reduced costs, next-generation sequencing (NGS) technologies have revolutionized genome sequencing and provided researchers with unprecedented opportunities to address many important biomedical problems efficiently. Large-scale resequencing projects, e.g. 1000 Genomes, TCGA, TSP, and Exome Sequencing Project etc, have been initiated to extend our knowledge of single nucleotide polymorphisms (SNPs), short insertions/deletions (INDELs) and structural variations (SVs) and relate these variants to human diseases. The amount of NGS data submitted to public repositories such as the Short Read Archives (SRA) at the NIH National Center for Biotechnology Information (NCBI) is growing exponentially and submissions represent a wide array of technology platforms and sequence collection strategies. To process and analyze these data for variation detection in a uniform manner is a challenge requiring a standardized modular pipeline. In collaboration with investigators at Boston College and the University of Michigan, NCBI continues to develop a framework Variation Discovery and Annotation Pipeline (Gpipe). The pipeline generates quality input sequence data from SRA, checks sample identities, aligns the read data with the human reference genome sequences, refines the mapping of placed reads (base quality recalibration and duplicate mark etc), and calls SNPs, INDELs, and SVs according to data availability and project-specific policies. A centrally implemented pipeline streamlines the data processing workflow for the data generated by next-generation sequencing technologies. Currently, this NIH-based modular pipeline is serving as one of the two pipelines for processing the 1000 Genomes main project datasets.

1907/F

Pooled next generation sequencing to identify mutations and rare variants in diabetes. *W. Xie[1], AM. Patch[2], R. Caswell[2], S. Ellard[2], MI. McCarthy[3], AT. Hattersley[2], TM. Frayling[1], MN. Weedon[1].* 1) Peninsula College of Medicine & Dentistry, Exeter, Devon, United Kingdom; 2) Institute of Biomedical and Clinical Science, Peninsula Medical School, Exeter, United Kingdom; 3) Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Churchill Hospital, Headington, Oxford OX3 7LJ, United Kingdom.

Genome-wide association studies have identified ~30 loci where common variants are associated with risk of type 2 diabetes. However, these loci explain <10% of the heritability of the disease, and low-frequency and rare variants, which are not captured well by existing genome-wide association (GWA) studies, are thought to explain a substantial fraction of the "missing" heritability. Next generation sequencing (NGS) technologies provide an opportunity to study this type of variant, but whole exome/genome approaches are still not feasible in the large numbers of individuals required for polygenic disease. We therefore targeted 11 candidate type 2 diabetes genes using a pooled-NGS approach. We amplified these 11 genes plus 1kb of sequence 5' and 3' (a total of 95kb) by long range PCR in 4 overlapping pools of 24 and 48 individuals with diabetes. All these individuals had been part of the WTCCC-T2D GWA study, except for 5 that have diabetes due to a known mutation in one of the 11 genes. We performed 76bp sequencing of the PCR products on the Illumina GA2. After alignment, 95% of the targeted regions were covered at sufficient depth (>10-fold average coverage per individual) to call SNPs and estimate allele frequencies. Where we had sufficient coverage, all 13 of the genotyped SNPs from the GWA study were detected. All the known single nucleotide substitution mutations from the monogenic diabetes samples were also detected, even though the mutation represented just 1 heterozygous carrier in the pools. The correlation between observed and expected allele frequencies was >0.99 in all 4 pools. Of the >500 variants we identified in pools of 24 individuals >90% were also identified in the overlapping pools of 48 individuals suggesting a low false positive rate. Approximately 50% of the variants were not in dbSNP or current 1000 genomes data. We have selected 16 non-synonymous SNPs that had estimated variant frequency <5% and 8 novel non-coding SNPs that are highly conserved across species to genotype in additional case and control samples. This study shows that targeted pooled sequencing is an efficient way to identify low frequency variants for further study. The accuracy of the allele frequency estimates from the pools suggests that extending sequencing to additional pools of cases and controls should provide an efficient screen of candidate genes for low-frequency moderate-penetrance type 2 diabetes risk alleles.

1908/F

Genome-Wide Association Studies in Hong Kong identified Novel Susceptibility Genes for Systemic Lupus Erythematosus. *W. Yang[1], R.J. Li[2], J. Yang[1], D.Q. Ye[2], Y.L. Lau[1].* 1) Paediatrics & Adolescent Med, Univ Hong Kong, Hong Kong, Hong Kong; 2) Department of Epidemiology and Biostatistics, School of Public Health, Anhui Medical University, China.

Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease with complex genetic inheritance. GWAS have identified a number of susceptibility genes for SLE in the last two years. However, the imbalance of the populations studied may have hindered our ability to detect susceptibility genes that are either population specific, or show differences in allele frequencies or linkage disequilibrium. We have conducted GWAS on 620 SLE patients collected in Hong Kong and 2193 controls matched by ethnicity and geography. Replication on 200 selected SNPs on independent sample collections from Hong Kong, Anhui, China, and Bangkok Thailand identified novel susceptibility genes for SLE include ELF1, CD247, and others. We present detailed data on CD247 below. Recently, CD247 (CD3Z) was found to be associated with SLE in populations of Caucasian in associated studies of small sample size. Our GWAS data from Chinese living in Hong Kong identified SNPs in and around CD247 to be associated with SLE risk. Two most significant SNPs were selected for further replication in 3180 Asian patients from Hong Kong, Mainland China, and Thailand, as well as 4626 ethnically- and geographically-matched controls. We confirmed the association of CD247 with SLE in Asian populations (rs704853, odds ratio (OR) = 0. 82, P = 7. 31 x 10-6; rs858543, OR = 1. 104, P = 0.0048). Subphenotype analysis showed that rs704853 is also linked to oral ulcer (OR = 0. 78, P = 0. 047), hematologic disorder (OR = 0. 78, P = 0. 033) and anti-ds-DNA antibody (OR = 0. 76, P = 0. 028). Understanding of the involvement of CD247 in the disease could shed new light on SLE mechanisms and help with development of new treatment paradigms.

1909/F

Characterizing Genetic Patterns of Insertion and Deletion Polymorphisms in 1000 Genomes Project data. *F. Yu, D. Challis, L. Bull, M. Bainbridge, R. Gibbs.* Human Genome Sequence Ctr, Baylor College Med, Houston, TX.

Insertion-deletion polymorphisms (INDELs) are important both because of their abundance in the human genome and their significant association with many diseases. The "1000 Genomes Project" aims to resequence 2500 human individuals collected from more than 20 ethnicities world wide, using second generation sequencing platforms. It has already made unprecedented progress in uncovering a massive number of novel SNPs. Yet many genomic characteristics around INDELs remain unexplored, primarily because INDELs have remained difficult to accurately identify due to the relatively short read length and low accuracy of the sequencing platforms. We have developed an algorithm, Atlas-Indel2, to differentiate between true INDELs and sequencing or alignment errors using a logistic model of pertinent variables, tuned with training data. Our results indicate the p-value successfully separates true INDELs from errors within coding regions. The density for p-values between 0.95 and 1 is 0.28, and 0.11 between 0.5 and 0.95, while the density is 1.88 for p-values below 0.5. Using a 0.95 p-value cutoff which requires at least 5 variant reads, on average 1.5 insertions and 2 deletions are discovered per 10,000 exonic bases per individual. For INDELs above the p=0.95 cutoff, 63% have a non-frameshift length of 3-12 (3, 6, 9, or 12), 36% have a length of 1 and about 1% are a frameshift length 2-11base pairs. Most of the INDELs causing a frameshift are with lower population frequency, possibly reflecting a stronger purifying selection effect. In this study, we apply Atlas-Indel2 to discover INDELs from 1000 Genomes Project data, characterize the linkage disequilibrium patterns around INDELs, and further examine the power of imputing known and novel INDELs. We find the level of single nucleotide variation diversity is elevated around INDELs. The long term goal is to enable applications that incorporate small INDELs in genome wide association studies for integrative disease mapping efforts.

1910/F

Genome-wide association study identifies three ancestral loci shared with Neandertal as important for skeletal muscle volume in young males. *J.M. Devaney[1], E.F. Orkunoglu-Suer[1], B.T. Haron[1], H. Heather Gordish-Dressman[1], P.M. Clarkson[2], P.D. Thompson[3], T.J. Angelopoulos[4], P.M. Gordon[5], N.M. Moyna[6], L.S. Pescatello[7], P.S. Visich[8], R.F. Zoeller[9], E.P. Hoffman[1].* 1) Department of Integrative Systems Biology, Research Ctr Genetic Med, Childrens Natl Medical Ctr, Washington, DC; 2) Department of Kinesiology, University of Massachusetts, Amherst, MA; 3) Division of Cardiology, Henry Low Heart Center, Hartford Hospital, Hartford, CT; 4) Center for Lifestyle Medicine and Department of Health Professions, University of Central Florida, Orlando, FL; 5) Department of Physical Medicine and Rehabilitation, School of Medicine, University of Michigan, Ann Arbor, MI; 6) School of Health and Human Performance, Dublin City University, Dublin 9, Ireland; 7) Department of Kinesiology and Human Performance Laboratory, University of Connecticut, Storrs, CT,; 8) Human Performance Laboratory, Central Michigan University, Mount Pleasant, MI; 9) Department of Exercise Science and Health Promotion, Florida Atlantic University, Davie, FL.

Skeletal muscle is extremely important in many physiological and disease processes, including mobility limitation, osteoporosis, higher risk of fracture, dyslipidemia, obesity, insulin resistance, overall frailty, and increased mortality. Skeletal muscle size is under strong genetic determination with heritability ranging from 52% to 84%. However, the location of gene or regions of the genome associated with muscle size has been limited to candidate genes. Recently, we completed a genome wide association study (GWAS) in 144 males (age=24) that participated in the Functional Polymorphisms Associated with Human Muscle Size and Strength (FAMUSS) study. The FAMUSS study is a cohort of young, healthy individuals that undertook a 12-week resistance-training program on the non-dominant upper arm. Before individuals started the training program, we measured skeletal muscle size of the upper arm using magnetic resonance imaging. We selected six variants from the GWAS results for genotyping in the rest of the males and females that participated in FAMUSS study (n=173 males and n=466 females; age= 24 yrs old). Three of the six variants were confirmed in the larger set of males but none of the loci were significant in the females. Two of the variants are located in the ZNF609 gene, a novel zinc finger protein located on chromosome 15 and the other SNP is located in a non-genic region on chromosome 2. Interestingly, all three alleles associated with skeletal muscle size are ancestral in nature and were the same alleles present in the Neandertal sequence recently published in Science (Green et al. A draft sequence of the Neandertal genome. Science. 2010 May 7;328: 710-22). Neandertals were physically diverse, but in general they were larger boned and more heavily muscled than most modern humans. These genomic regions may be the result of the interbreeding of early modern humans and Neanderthals that occurred 50,000 and 100,000 years ago. In This work needs to be replicated and may have significance in the understanding of the basic biology of muscle.

1911/F

Creation of a comprehensive SNP imputation dataset from individuals of European descent. *A.A. Hutchinson[1,2], K.B. Jacobs[1,2], Z. Wang[1,2], M. Yeager[1,2], M.A. Tucker[2], S.J. Chanock[2].* 1) Core Genotyping Facility, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD 21702, USA; 2) Division of Cancer Epidemiology and Genetics, NCI, NIH, Bethesda, MD 20892, USA.

The NCI Division of Cancer Epidemiology and Genetics (DCEG) has performed numerous genome-wide association studies (GWAS) to detect heritable genetic risk factors for numerous cancer sites. Primarily, genotypes have been generated using Illumina HumanHap BeadChip SNP arrays based on the International HapMap project phases I and II. These products are being superseded by a new "Omni" series of arrays that are based on phase III of the HapMap project and variants discovered from preliminary analysis of data from the 1,000 Genomes Project. While these newer arrays provide additional SNP content that may aid in mapping common diseases, all of the content from the older HumanHap series of arrays was not carried into the newer products. In addition, GWAS data from individuals scanned using the Affymetrix 500K and 6.0 arrays are routinely combined with our Illumina data. As we plan future GWAS projects, we must determine the extent data from subjects genotyped on HumanHap arrays can be combined with those that will be genotyped on the new Omni arrays and Affymetrix arrays. Further, we aim to generate a comprehensive dataset to serve as the basis for genotype imputation across these platforms and is of sufficient size to accurately infer variants with relatively low population frequency (>~2.5%). This dataset is being constructed using 750 well-characterized individuals of European descent from three cohort studies. Genomic DNA samples from these individuals are being genotyped using multiple SNP arrays from Illumina (each on the Human Hap1M-Duo, Human Omni1-Quad, and Human Omni2.5M) and Affymetrix 6.0 arrays. We shall use this dataset to evaluate the extent and accuracy of several genotype imputation algorithms and to combine data across all platforms. The resulting genotype data will be combined with data from the HapMap and 1,000 Genomes projects and formatted for use in several popular SNP imputation programs, including IMPUTE, MACH, and Beagle. Once ready, we shall post these datasets to the NCBI dbGaP portal for use by the broader scientific community. Funded by NCI Contract No. HHSN261200800001E.

1912/F

Korean Genome wide association study for pulse rate identifies 6q22.31c loci. *N. Kim, Y. Kim, Y. Cho.* Center for Genome Science, Seoul, Korea.

Pulse rate has been widely known to be related to cardiovascular diseases, lifespan, arrhythmia, hypertension, lipids, diabetes and menopause. Despite the given impact of this trait on several complex diseases/phenotyes, there have been fewer attempts to identify genetic influence on pulse rate. Here we report the associated loci responsible for the variation of pulse rate in Korea population. We performed a genome-wide association study (GWAS) with 352,228 SNPs typed in 8,842 subjects from KARE (The Korea Association REsource) project and replicated the significant signals in 3,703 subjects from independent population. We identified significant associations between pulse rate and genetic loci in four regions near on chromosome 6q22.31c (p=1.1×10-5, in GJA1), 7p15.1b (1.2×10-5, C7orf41), 1q32.2a (3.8×10-7, in CD46), and 6q22.13c (0.3×10-6, in LOCL644502). CD46 and LOCL644502 were previously reported as candidate genes for pulse rate. In present study, we propose a novel locus related to pulse rate on 6q22.31c (GJA1). The encoded protein by GJA1 is the major protein of gap junctions in the heart that is thought to have a crucial role in the synchronized contraction of the heart and in embryonic development.

1913/F

Genome-wide association study to identify the genetic basis of alcohol consumption in population-based cohorts. *J. Lee, M. Go, Y. Kim, Y. Cho.* Center for Genome Science, Seoul, Korea.

Alcohol dependence, which is related to high alcohol consumption, causes various common public health problems. Previous studies indicated that individual's alcohol consumption is influenced by not only environmental but also genetic factors. To gain insight into genetic basis of individual's alcohol consumption, we conducted genome-wide association study (GWAS) from population-based cohorts. The amount of daily alcohol consumption was calculated based on the second Korean National Health and Nutrition Examination Survey. Our GWAS was conducted to test 352,228 single nucleotide polymorphisms (SNPs) from 3,951 subjects in population-based cohorts of Korean Genome Epidemiology Study I, (KoGES I). We tested genetic association between SNPs and alcohol consumption by performing linear regression analysis after adjustment of 4 variables (age, sex, area, BMI). We identified significantly associated SNPs with alcohol consumption (P-value < 10-6) from initial GWAS. The replication of these associations will be carried out in the independent population (KoGES III samples of about 5,000 samples). The progress and future works of this study will be presented.

1914/F

IGOR: new software for calling genotypes from Illumina BeadArrays. *W. Zhou[1,2], Z. Wang[1,2], Q. Chen[1,2], M. Yeager[1,2], S.J. Chanock[2], K.B. Jacobs[1,2].* 1) Core Genotyping Facility, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD 21702; 2) Division of Cancer Epidemiology and Genetics, NCI, NIH, Bethesda, MD 20892.

Most algorithms used to call genotypes from BeadArray data have difficulty calling rare alleles and handling outliers, and require complex heuristics or questionable assumptions (such as fitness for Hardy-Weinberg proportion) to compensate. We propose a new software suite to process data from the Illumina BeadArray platform to estimate genotypes states. Our new software, Illumina Genetype Probabalistic Caller (IGOR, pronounced 'ahy-gohr'), begins by reading raw Illumina intensity data files (IDAT), normalized or unnormalized Illumina genotype final reports or binary HDF5 files. For unnormalized intensity data, IGOR uses an algorithm similar to the Illumina normalization method. Probe intensity data can be processed further with optional variance stabilization, local GC/CpG sequence context adjustment, and quantile normalization. Genotype clusters are estimated by fitting two multivariate mixture models: one to probe intensities within each array to estimate prior homozygote and heterozygote probabilities for all SNPs; and the other uses the within-array prior probabilities to stabilize cluster assignments for each SNP across all arrays. This procedure provides accurate and consistent genotype assignments, even for monomorphic loci and for those with extremely rare alleles. A variety of output formats are supported, including those that report the most likely genotype (GLU text and binary, PLINK text and binary, PHASE, VCF, etc.), as well as those that support probabilistic genotype assignments (GLU, WTCCC, VCF, etc.). IGOR is designed to run on modern multi-core CPUs as well as computing clusters, enabling high-throughput and scalable processing. IGOR also has the advantage of not relying on the Illumina GenomeStudio software, since it can process native Illumina data files and includes algorithms for probe normalization and genotype calling. Once validated, IGOR will be released as free and open source software. Funded by NCI Contract No. HHSN261200800001E.

1915/F

High throughput single molecule analysis of genomic DNA integrity in nano-channel arrays. *W. Stedman, M. Akana, S. Das, M. Austin, P. Deshpande, D. Bozinov, H. Sadowski, H. Cao, M. Xiao.* BioNanomatrix Inc, 3701 Market St, 4th Floor, Philadelphia, PA 19104.

Nucleotides in DNA and RNA are frequently modified by exogenous and endogenous damaging factors, leading to single strand, double strand DNA breaks, genomic rearrangements and a broad spectrum of chemical modifications (e.g. formations of cyclobutane pyrimidine dimer, oxidation, etc). These changes can alter gene expression, resulting in genome instability and protein malfunction, that often correlates with diseases. Currently, there are limited available high-throughput and sensitive methods to detect these events without the need of DNA amplification, which can conceal those modifications. We have developed a high throughput assay that can directly image the size distribution of genomic DNA molecules and nucleotide modifications of these molecules in massively parallel nano-channel arrays. Our method starts with enzymatic labeling of specific nucleotide modifications (single strand breaks or chemical modifications) on long genomic DNA molecules with fluorophores. The labeled DNA molecules are then linearized inside the nano-channel array and imaged with high resolution fluorescence microscopy. Double-strand breaks can be detected directly by determining the size distribution of the genomic DNA molecules. By localizing fluorescent labels on the DNA backbone, the structural information of the genome and the distribution of modified nucleotides on individual DNA molecule can be inferred with great accuracy and reproducibility. This highly miniaturized nano-array device together with the flexible and efficient labeling chemistry enables direct image analysis of the whole genome at the single molecule level.

1916/F

How significant is the human genome in determining disease phenotypes. Are post-DNA events the key to understanding disease ontogeny? *B. Gottlieb[1,2], L.K. Beitel[1,2,3], M. Trifiro[1,2,3].* 1) Dept Cell Genetics, Lady Davis Institute for Medical Research, Montreal, QC, Canada; 2) Dept Human Genetics, McGill University, Montreal, QC, Canada; 3) Dept Medicine, McGill University, Montreal, QC, Canada.

Since the beginning of the Human Genome Project and throughout subsequent follow-up studies, it has been assumed that the most important factor governing the ontogeny of both common and rare diseases is the nature of an individual's genotype. This assumption has been the basis for the huge amount of resources applied to projects such as the 1000 genomes, the cancer genome, as well as numerous genome wide association studies. While in a few cases such studies have identified genes that cause rare diseases, identification of significant genes in multifactorial diseases such as cancer and cardiovascular disease has not been that successful, mainly because it has been assumed for the most part, that the genes identified so far have not been rare enough to be truly significant. To test this assumption, we decided to examine the genomics of the androgen receptor (AR) gene, a locus-specific gene, in which mutations are responsible for the rare condition known as androgen insensitivity syndrome (AIS). We examined a total of 140 patients with a clear AIS phenotype, and found that in over 40% a mutation in their AR coding exons could not be identified. Further, similar results have been reported in two other AIS databases. In the absence of any other gene being identified as causing AIS to date, the question arises as to whether post-DNA events may play a critical role in determining AIS phenotype. Over the past few years a number of such events and factors have indeed been identified that can significantly affect the relationship between genotype and phenotype. These include DNA and RNA editing, and alternative splicing. They also include many epigenetic factors such as DNA methylation and interactions involving coactivators and corepressors. In light of all these possible sources of phenotypic variation and the apparent disconnect between genotype and phenotype in many diseased individuals, we will discuss a new model of the genotype to phenotype pathways that reflects the actual complexity of this relationship. We believe that understanding such pathways will be critical in determining the ontogeny of disease phenotypes and help bring about the era of personalized medicine in the treatment of disease, which will be critical to both curing and hopefully preventing multifactorial and rare diseases.

1917/F

Common genetic variants in the Nucleotide-binding Oligomerization Domain-1 (NOD1) gene are associated with asthma in a population of African descent. *C.S. Jackson[1,2], L. Santi-Ruiz[2], T.E. Mason[2], V. Apprey[2], G. Bonney[2,3], G.M. Dunston[1,2,3].* 1) Microbiology, Howard University, Washington, DC; 2) National Human Genome Center at Howard University, Washington, DC; 3) Department of Community Health and Family Medicine, Howard University, Washington, DC.

Background: Asthma is a chronic inflammatory disease affecting approximately 7.5 % of Americans 25 years of age and older. The greatest burden of hospitalization and mortality is mainly observed in ethnic minority populations, thus making asthma one of the leading health disparities in the United States. We hypothesize that genetic polymorphisms in the nucleotide-binding oligomerization domain-1 (*NOD1*) gene are associated with asthma susceptibility in an African American population. Our laboratory analyzes the functional significance of common variants in candidate genes associated with health disparities, like asthma. *NOD1*, which codes for an intracellular microbial pattern recognition receptor, is a candidate gene associated with the pathogenesis of asthma in European populations. **Methods:** We performed a genetic association study in a population of 400 African American asthmatic cases (200) and controls (200) from the Washington, DC metropolitan area to determine single-nucleotide polymorphisms (SNPs) associated with asthma. Eight SNPS (rs2075818, rs2235099, rs2075820, rs2075821, rs2907748, rs5743336, rs2906766, and rs2529444) located in both the regulatory and structural domains of *NOD1* were genotyped using PCR-RFLP and Pyrosequencing. **Results:** By using genetic modeling, the SNPs rs29007748 (p=0.039, OR=0.37, 95%CI 0.13-1.01) and rs5743336 (p=0.016, OR=0.50, 95%CI 0.28-0.89) were found to be associated with asthma in the dominant model when stratified by gender. SNP rs2907748 is located in intron 11, and rs5743336 is located in the 5'untranslated region. **Conclusion:** This study shows that common variants in *NOD1* are associated with asthma in an African American population. In addition, genotype-based diagnosis and targeted intervention may be instructive in the emergence of personalized genomic medicine in diverse populations.

1918/F

The European Genome-phenome Archive (EGA). *I. Lappalainen, J. Hinton, V. Kumanduri, M. Maguire, P. Marin-Garcia, P. Flicek.* European Bioinformatics Institute, Cambridge, United Kingdom.

The European Genome-phenome Archive (EGA), a service of the European Bioinformatics Institute, provides a permanent archive for all types of potentially identifiable genetic and phenotypic data. The EGA contains data collected from individuals for the purpose of medical or genetic research and whose consent agreements prevents open, public data distribution. The EGA follows strict protocols for information management, data storage, security and dissemination. Researchers with appropriate authorisation may access data from over 40 studies and 50000 individuals currently provided through the EGA web site at http://www.ebi.ac.uk/ega/. These studies include dense genotype experiments between cases and controls, population based studies or re-sequencing and RNA-seq data from various cancer genomes. The EGA has implemented a distributed data access policy whereby the data access decisions are made by a data access-granting organisation (DAO) and not by the EGA project. The DAO may be the same organisation that approved and monitored the initial study protocol or a designate of this approving organization such as a dedicated data access committee. In a typical case, the EGA will direct users to a project homepage where the user can apply for access that is then managed by the EGA. Accepted data types include genotypes, structural variants and whole-genome sequence which are stored in optimised data structures. In addition, manufacturer-specific raw data formats from array-based genotyping and raw DNA sequence data arising from re-sequencing, transcriptomics or other assays may be deposited for archiving and distribution. The EGA also accepts any phenotype data associated with the samples. All deposited data must have a DAO approved data release policy that provides data access in accordance with the original consent agreements. The EGA also provides an analysis infrastructure to add value to data submitted into our system. Our quality control applies to both samples and experimental data without altering the original data but allowing us to merge data collected using different technologies, phase submitted data or impute unobserved genotypes using public resources such as the 1000 Genomes project. The data are made available together with our partner DAOs in the most widely used formats to those users that have been granted access.

1919/F

Distribution of Deleterious Alleles Across Diverse Populations. *S. Musharoff[1], J. Kidd[1], B. Henn[1], MC. Yee[1], M. Snyder[1], F. De La Vega[2], C.D. Bustamante[1].* 1) Genetics, Stanford University School of Medicine, Stanford, CA; 2) Life Technologies, Foster City CA.

Distributions of deleterious alleles are relevant to the study of human disease prevention and treatment. The recent advancements in sequencing whole genomes have enabled the identification of genome-wide functional changes that can be examined in a population-genetic manner. Here we analyze the distribution of deleterious alleles in the diploid genomes of 12 HapMap Phase 3 human individuals from diverse populations which have been sequenced to high coverage using SOLiD technology and relate the differences in distributions of synonymous vs. benign, possibly damaging, and probably damaging non-synonymous coding variants to demographic history. As two of the individuals analyzed are “admixed” with respect to European, African, and / or Native American ancestry, their “admixture breakpoints” are first fine-mapped using admixture deconvolution methods. Then, functional changes are considered separately within tracts of unique ancestry. We extend this analysis to a select sample of individuals from the Human Genome Diversity Project (HGDP) whose genomes are fully sequenced on another next generation sequencing (NGS) platform and examine the retention of deleterious mutations considering population bottlenecks and other known migration events. Considering distributions of such functional changes in populations in the light of human demographic history is essential in the age of personalized genomic medicine.

1920/F

Factors influencing the success of exome sequencing for gene discovery in mendelian disorders. *S. Ng, obo the National Heart, Lung, and Blood Institute's Exome Sequencing Program (Lung Project Team).* Genome Sciences, University of Washington, Seattle, WA.

Several studies published in the last year have demonstrated the utility of massively parallel sequencing for the identification of genes underlying rare, monogenic diseases. We have shown that our approach - based on sequencing the exomes of a small number (i.e., 4-10) of unrelated individuals and identifying the intersection of genes containing rare, protein-altering variants - can be an efficient and successful strategy for discovering genes for both recessive and dominant disorders (Ng et al. 2009, Ng et al. 2010). However, the factors that influence the success of this approach have yet to be well defined. Using a large dataset of 75 human exomes, we analyze the impact of parameters including sample size, pedigree structure, mode of inheritance, genetic heterogeneity, missing data, and variant filtering based on frequency and function. Capture of these exomes was done using in-solution hybridization probes against a CCDS (2008) target, comprising about 28Mb over 160,000 exons, and sequencing was done to an average of 56X. These exomes are part of the U.S. National Heart, Lung, and Blood Institute's (NHLBI) Exome Sequencing Program, and are from a modifier study of cystic fibrosis (CF). We use the CF cohort to assess the effect of varying the threshold frequency for candidate mutations on the power and specificity to identify the major causal gene, CFTR. This is of interest because the carrier frequency for CF mutations is relatively high (up to 1:25 in populations of European ancestry), and these results will have implications for diseases with moderate prevalence and carrier frequencies. We also use permutation analyses of these exomes to estimate the effect of other factors - including the disease model and presence of heterogeneity - on the power of this approach. Our results identify the strengths and weaknesses of various study designs for exome analysis of mendelian disorders, including both rare syndromes and monogenic subsets of common disease, and may be informative for further analyses on more common, complex disease.

1921/F

Association of the functional CD226 307Ser variant with systemic sclerosis: evidence for a contribution of co-stimulation pathways in SSc pathogenesis. *P. Dieudé[1], M. Guedj[2], M.E. Truchetet[3], J. Wipff[4,5], G. Riemekasten[6], M. Matucci-Cerinic[7], I. Melchers[8], E. Hachulla[9], P. Airo[10], E. Diot[11], N. Hunzelmann[12], L. Mouthon[13], J. Cabane[14], J.L. Cracowski[15], V. Riccieri[16], J. Distler[17], Z. Amoura[18], G. Valentini[19], P. Camaraschi[20], I. Tarner[21], C. Frances[22], P. Carpentier[23], N.C. Brembilla[3], O. Meyer[1], A. Kahan[5], C. Chizzolini[3], C. Boileau[4,24], Y. Allanore[4,5].* 1) Rheumatology, Bichat Hosp, PARIS, France; 2) Laboratoire Statistique et Génome, UMR CNRS-8071 / INRA-1152 / Université d'Evry Val d'Essonne, France; 3) Immunology and Allergy, Geneva University Hospital and School of Medicine, 1211 Geneva 14, Switzerland; 4) Université Paris Descartes, INSERM U781, Hôpital Necker, Paris, France; 5) Université Paris Descartes, Service de Rhumatologie A, Hôpital Cochin, Paris, France; 6) Department of Rheumatology and Clinical Immunology, Charité University Hospital, Berlin, Germany; 7) Department of Biomedicine, Section of Rheumatology, Florence, Italy; 8) Clinical Research Unit for Rheumatology, University Medical Center, Freiburg, Germany; 9) Université Lille II, Médecine Interne, Lille, France; 10) Rheumatology and Clinical Immunology, Spedali Civili, Brescia, Italy; 11) INSERM U618, IFR 135, CHU Bretonneau, Tours, France; 12) Department of Dermatology, University of Cologne, Köln, Germany; 13) Université Pierre et Marie Curie, Service de Médecine Interne, Hôpital Saint-Antoine, APHP, Paris, France; 14) Paris Descartes Université, Médecine Interne, Hôpital Cochin, APHP, Paris, France; 15) INSERM CIC3, CHU Grenoble, France; 16) Department of Medical Clinic and Therapy "Sapienza" University of Rome - Italy; 17) Department for Internal Medicine 3 and Institute for Clinical Immunology Friedrich-Alexander-University Erlangen-Nuremberg, Germany; 18) Université Paris 6, Medecine Interne, Pitié Salpétrière, Paris, France; 19) Rheumatology Section, Department of Clinical and Experimental Medicine, Second University of Naples, Via Pansini 5, 80131, Naples, Italy; 20) University of Verona, Department of Clinical and Experimental Medicine - Rheumatology Unit, Verona, Italy; 21) University of Giessen, Department of Rheumatology and Clinical Immunology Kerckhoff-Klinik, Bad Nauheim Germany; 22) Université Paris 6, Dermatologie, Hôpital Tenon, Paris; 23) Clinique Universitaire de Médecine Vasculaire, Pôle Pluridisciplinaire de Médecine, Centre Hospitalier Universitaire, Grenoble, France; 24) Université Versailles Saint Quentin Yvelines, Laboratoire de Biochimie Hormonale et Génétique, Hôpital Ambroise Paré, AP-HP, Boulogne, France.

Background. The non-synonymous polymorphism rs763361 of the CD226 gene, which encodes the DNAX accessory molecule 1, involved in T cell co-stimulation pathways, has recently been identified as a genetic risk factor for autoimmunity. Objective. To test the association the CD226 rs763361 polymorphism with systemic sclerosis (SSc) in European Caucasian populations. Methods. CD226 rs763361 was genotyped in 3645 individuals comprising a discovery set (991 SSc patients and 1008 controls) and a replication set (999 SSc patients and 634 controls), all individuals being of European Caucasian origin. Expression of CD226 was assessed on PBMCs obtained from 14 SSc patients and from 10 healthy donors genotyped for CD226 rs763361. Results. The CD226 rs763361 T allele was found to be associated with SSc in both discovery and replication samples providing the following results in the combined populations: OR 1.22 95%CI[1.10-1.34], P=5.69x10-5. The CD226 T allele was also associated with various SSc subsets highlighting a potential contribution in disease severity. The most remarkable associations of the CD226 TT risk genotype were observed with diffuse cutaneous subtype, anti-topoisomerase I antibodies positive and SSc-related fibrosing alveolitis subsets: OR 1.86 95%CI[1.42-2.43], P=5.15x10-6, OR 1.82 95%CI[1.38-2.40], P=2.16x10-5 and OR 1.61 95%CI[1.25-2.08], P=2.73x10-4, respectively. The CD226 expression was significantly linked with CD226 rs763361 genotypes in CD3+, CD4+ and CD56+ cells. Conclusions. Our results establish CD226 as a new SSc genetic susceptibility factor and provide evidence for a functional consequence of CD226 rs763361 by demonstrating an association of the CD226 genotypes with CD226 expression in helper T cells and NK cells.

1922/F

Genome-Wide Association Identifies Novel Loci Impacting Common Variable Immunodeficiency. *J.T. Glessner[1], J.S. Orange[2], K.E. Sullivan[2], E.E. Perez[3], E. Resnick[4], M. Gray[5], C.E. Kim[1], R. Chiavacci[1], J.W. Sleasman[3], H. Chapel[5], C. Cunningham-Rundles[4], H. Hakonarson[1,6].* 1) Ctr Applied Genomics, Children's Hosp Philadelphia, Philadelphia, PA; 2) Division of Allergy and Immunology, Children's Hospital of Philadelphia Research Institute, Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA; 3) Division of Allergy, Immunology & Rheumatology, Department of Pediatrics, University of South Florida, Saint Petersburg, FL 33701, USA; 4) Mount Sinai School of Medicine, New York, NY, 10029 USA; 5) Nuffield Department of Medicine, University of Oxford and Oxford Radcliffe Hospital, Oxford, OX3 9DU, UK; 6) Division of Genetics, Department of Pediatrics, The Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, PA, 19104, USA.

Common variable immunodeficiency (CVID) is a generic term for a group of uncommon heterogeneous immune defects characterized by hypogammaglobulinemia, failure of antibody production, susceptibility to bacterial infections and an array of serious comorbidities. To address the underlying immunopathogeneses of CVID, we conducted the first ever genome-wide association study of patients with CVID. 363 patients were genotyped with 610,000 SNPs. Due to the relative rarity of this group of disorders, the study cohort was recruited at four sites. Patients were randomly divided into a discovery cohort of 179 cases in comparison with 1,917 disease-free controls and a replication cohort of 109 cases in comparison with 1,114 controls, controlled for population stratification. Our analyses detected strong association with the MHC region and uncovered a novel association with a cluster of ADAM genes that was replicated in the independent case cohort. Analysis of the same cases for copy number variation (CNV) revealed 28 disease-associated deletions and duplications, as well as 84 unique rare deletions and 98 duplications suggesting multiple novel genetic etiologies for individual CVID cases. Analysis of CVID comorbidities identified significantly associated SNP genotypes with the major CVID clinical phenotypes. Taken together, our integrative genome-wide analysis of SNP genotypes and CNVs has uncovered multiple novel susceptibility loci for CVID, both common and rare, consistent with the highly heterogeneous nature of CVID. These results may allow for improved diagnosis of CVID and prediction of the CVID clinical phenotypes based upon these unique genetic variations.

1923/F

Two missense variants in UHRF1BP1 are independently associated with systemic lupus erythematosus in Hong Kong Chinese. *Y. Zhang[1], W.L. Yang[1], P.W. Lee[1], J. Yang[1], P.C. Sham[2], Y.L. Lau[1].* 1) Paediatrics, The University of HongKong, HongKong; 2) Psychiatry, The Univeristy of HongKong, HongKong.

UHRF1BP1 is a highly conserved protein with unknown function. Previously, a coding variant in this gene was found to be associated with SLE in populations of European ancestry (rs11755393, R454Q, P = 2.22 x 10-8, OR = 1.17). In this study, by a combination of genome-wide study and replication involving a total of 1230 patients and 2193 controls, we confirmed the association of this coding variant in SLE susceptibility in Hong Kong Chinese, and also identified another coding variant in this gene that independently contributes to SLE susceptibility (rs13205210, M1098T, P = 4.44 x 10-9, OR= 1.49). Unlike rs11755393, rs13205210 association fits better to a dominant model and shows higher effect size than reported in the European populations for this gene. Cross population confirmation establishes the involvement of this locus in SLE and indicates that distinct alleles, possibly coding changes, are contributing to the disease.

1924/F

Validation Of Exome Sequencing Variation Using Orthogonal Methods. *M.J. Rieder, I.B. Stanaway, J.D. Smith, E.H. Turner, B.W. Paeper, D.A. Nickerson.* Dept of Genome Sciences, University of Washington, Seattle, WA.

We performed in-solution capture (NimbleGen SeqCap 2.1 M custom probe set) for the protein coding regions of the human genome (i.e. the exome). Sequences have been produced by Illumina GAIIx to identify variants in 50 population diverse samples derived from the HapMap sample set and/or Coriell collection. These samples were also resequenced using traditional Sanger sequencing methods across 231 candidate genes through the NIEHS Environmental Genome Project (EGP). Additionally, a subset of the samples (n=20) were genotyped using a 1M Illumina genome-wide array. All the GAIIx sequencing data was analyzed using a pipeline composed of the Burrows-Wheeler Aligner (BWA) followed by variant detection using the Bayesian, Unified Genotyper in multi-sample mode from the Genome Analysis Toolkit (GATK). We identified an average of 17,125 variants per exome with a mean coverage depth of 68x. To evaluate the robustness of variant calling, we compared the exome dataset to the 1M genotyping data for overlapping regions resulting in a concordance of 99.5% over an average of 8,390 variants per sample. By comparing discrepant sites, we find that for the remaining 0.5% of variants a significant proportion of the miscalls are due to genotyping errors. The false-negative call rate estimated based on the 1M variants to be approximately 3.4%. From a large Sanger based-sequencing dataset (n=5,340 variants), we determined the false discovery rate is 7.5% and the false negative rate is ~ 8%. By assessing filtering metrics and depth of coverage, false negative rates were reduced to ~2%. Using two orthogonal datasets against the exome sequencing data we have estimated the current error rates inherent in high-throughput human exome analysis using the Illumina platform.

1925/F

Validation of 1000 Genomes Project SNP calls using the Affymetrix Axiom™ Genotyping Solution. *J. Gollub, T. Asbury, J. Bleyhl, Y. Lu, G. Mei, M. Purdy, L. Weng, Y. Zhan, T. Webster.* Affymetrix, Inc., Santa Clara, CA.

We have validated approximately three million novel human SNPs discovered by the 1000 Genomes Project for use with the Axiom™ Genotyping Solution, and examined concordance of array-based genotypes to those in the pilot 1 and pilot 2 releases. This effort focused on a subset of SNPs not found in the HapMap Project or dbSNP (release 130). Polymorphism was observed for each validated SNP in at least two HapMap2 individuals, with very high call rates and concordance with 1000 Genomes Project reference genotypes for individuals genotyped in common.

The March 2010 release of 1000 Genomes Project data contains genotypes for approximately 15 million SNPs, based on low-coverage sequencing and genotype imputation for 180 individuals overlapping the HapMap 2 individuals (pilot study 1), and deep sequencing of two nuclear family trios (pilot study 2). Array-based genotypes of the deep-sequenced individuals are more than 99.5% concordant with the reference genotypes. We find noticeably lower concordance (around 98.4%) with the low-coverage sequencing-based genotypes. Concordance with shallow sequencing-based calls of heterozygous alleles (around 97%) is lower than concordance to homozygous calls (98.5%). We ascribe the difference in part to expected errors resulting from the low-pass sequencing and imputation used for SNP calls in the first pilot study.

1926/F

Genetic modifiers of disease severity in Duchenne muscular dystrophy. *A. Kesari[1], J. Devaney[1], H. Gordish[1], C. McDonald[2], E. Hoffman[1], Cooperative International Neuromuscular Research Group (CINRG), FAMuSS group.* 1) Center for Genetic Medicine, Children's National Medical Center, Washington, DC; 2) University of California, Davis, CA.

Background: Duchenne muscular dystrophy (DMD) is a monogenic condition caused by loss-of-function mutations in the dystrophin gene. Patients show variability in disease onset, progression, and response to glucocorticoids, suggesting genetic modifiers. Understanding genetic modifiers is particularly important as increasing numbers of potential therapies are moving into clinical trials, yet inter-patient variability complicates interpretation of drug efficacy. The Cooperative International Neuromuscular Research Group (CINRG) conducted a natural history study that included a goal of identifying genetic modifiers and comprised 348 participants. One approach to identify genetic modifiers is to use genome-wide association studies (GWAS) of relevant phenotypes in larger normal volunteer populations. Towards this end, we assessed response to muscle resistance training in 1,300 healthy college-age volunteers (FAMuSS study). Those participants that showed the least and greatest increase in strength after 12 weeks of unilateral upper arm training were studied using GWAS methods (1 million SNPs). The top loci were then utilized as candidates for genetic modifiers of DMD. Methods: 180 DMD participants (of 348 or 51.7%) from the CINRG Natural History Study were genotyped for 9 haptag SNPs from the FAMuSS GWAS study of male strength response. Sequence variations were genotyped by TaqMan allele discrimination assays. Analysis of covariance (ANCOVA) with age and current steroid use as covariates was used to identify statistically significant differences in mean measures of muscle strength among genotypes. Results: Of the 9 GWAS strength loci in healthy male volunteers, 4 showed statistical significance as genetic modifiers of DMD using grip strength as the clinical phenotype. In each case, the direction of change was consistent between normal healthy volunteers and DMD participants. Conclusions: The 4 loci showed a similar genotype/phenotype association in both healthy males and DMD boys (e.g. rare allele = increased strength). Inclusion of genetic modifiers in clinical trials holds the potential of increasing sensitivity and specificity of assessments of drug efficacy.

1927/F

Exploring Regions of Extreme Diversity in the Human Genome. *K.M. Steinberg[1], J.M. Kidd[1], F. Antonacci[1], P. Sudmant[1], T. Graves[2], R.K. Wilson[2], E.E. Eichler[1].* 1) Department of Genome Sciences and Howard Hughes Medical Institute, University of Washington, Seattle, WA; 2) Washington University Genome Sequencing Center, School of Medicine, St. Louis, MO.

Highly divergent regions of the human genome, defined as less than 99.5% identity to the reference genome, are often refractory to next generation sequencing technologies and represent a part of the genome where SNP assignment is questionable and annotation is limited. It is therefore critical to further interrogate and accurately annotate the sequence composition of these regions in order to fully understand the complete spectrum of genetic diversity. Utilizing clone-derived end-sequence data from 16 diverse human genomes, we have identified 385 loci greater than 100kb where four or more clones map to the region but the percent identity between the sample and reference is less than 99.5. The average sequence identity of these regions is 97.9%, and over 88% of these regions overlap with segmental duplications. Almost half of these regions overlap RefSeq genes representing potential sites of functional significance. Genes related to the immune system and response to stimuli are overrepresented in these regions, and, in some cases, these loci are also enriched for structural variants. One particularly dramatic and complex region of variation in the human genome is the 17q21.31 inversion locus. The H2 (inverted) haplotype is found primarily in Europeans where its nucleotide diversity is limited when compared to other H2 haplotypes but increased when compared to the H1 (direct orientation) haplotype suggesting an ancient coalescence. H2 haplotypes do exist in non-European populations; however, neither the distribution of this haplotype nor the nucleotide diversity has been explored. Using capillary-based sequencing and 454 technologies, we have directly resequenced 238 fosmid clones from 46 of these highly divergent regions, including the 17q21.31 locus. The average size of the genomic intervals is approximately 120Kb, and all but 4 of the selected regions overlap a RefSeq exon. Six of the regions were sequenced in 8 of the individuals, and the remaining 40 were sequenced in one or two individuals. We have annotated single nucleotide variants that have not been previously identified by the HapMap or 1000 Genomes projects. Additionally, we present data from genotyping assays used to assess the distribution of these variants across a diverse sampling of populations.

1928/F

Development and validation of genotyping assays for functional SNPs in the FC gamma receptor family genes. *S. Cottrell[1], C-P. Huang[1], L. Young[2], J. Dell'Aringa[1], Y. Pan[1].* 1) Molecular Sciences, Amgen, Inc., Seattle, WA; 2) Molecular Sciences, Amgen, Inc., Cambridge, MA.

Five highly homologous FC gamma receptor genes are located in a 200kb region of chromosome 1 (1q23). The products of these genes are receptors expressed on immune effector cells that bind the FC region of IgG antibodies to direct destruction of target cells. Several of these genes contain polymorphisms that affect receptor function, and the polymorphisms have been associated with incidence of diseases (including systemic lupus erythematosus, rheumatoid arthritis, coronary artery disease, asthma) and response to therapeutic antibodies (including rituximab, cetuximab, and trastuzumab). However, research on the phenotypic effects of the alleles has been hampered by the extremely high homology among the genes, linkage disequilibrium in the region, and a reliance on slow, labor-intensive assays. We have developed and validated allelic discrimination assays for SNPs in FCGR3A (rs396991) and FCGR2A (rs1801274), both of which have alleles encoding high and low affinity versions of the receptors. In both cases, primers were designed to specifically amplify the gene of interest by targeting the rare sequence difference between the gene of interest and family members, and the genotype is interrogated by the probes. The accuracy of the assays was confirmed by sequencing 24 donor samples. The call rate for both assays is greater than 95 percent, and we confirmed that copy number variations were the major cause of failed genotype determinations. Robustness, reproducibility, and sensitivity (DNA input amount) were also included in the assay validation in preparation for analysis of clinical trial samples. We tested the assays on a panel of 166 genomic DNA samples and found linkage disequilibrium between the loci (D'=0.39), with significant non-random association of the allele for the high affinity FC gamma receptor 3A with the allele for the high affinity FC gamma receptor 2A (chi square test, p value <0.0001). In conclusion, we have developed high throughput validated assays for SNPs in the FCGR2A and FCGR3A genes that are suitable for analysis of clinical trial samples. Furthermore, we have demonstrated incomplete linkage disequilibrium between the genes, suggesting that both genes need to be genotyped for assessment of association with the phenotype of interest.

1929/F

Korean Genome-Wide Association Studies for Type 2 Diabetes and its related traits identify two independent loci at 12q24. *M. Go[1], Y. Kim[1], J. Lee[1], B. Han[1], H. Kim[1,2], Y. Cho[1].* 1) Center for Genome Science, Seoul 122-701, Korea; 2) Department of Biochemistry, School of Medicine, Ewha Womans University, Seoul 158-710, Korea.

Type 2 diabetes (T2D) is one of major public health problems with its rapidly rising incidence and prevalence worldwide. Nineteen genetic loci showing strong evidence of association with T2D have been identified to date by linkage, candidate gene and GWA approaches. Most of T2D loci were detected from European samples except KCNQ1 which was from East Asian. In this study, we conducted Korea genome-wide association study (GWAS) to identify susceptibility loci to type 2 diabetes (T2D) in 1,042 cases and 2,943 controls in the Korea Association Resource (KARE) study. Nine promising GWAS signals were replicated in 1,216 cases and 1,352 controls from the independent population. Seven of nine signals have been reported for its association with T2D mostly in European populations, while two located in chromosome 12 are newly identified in our study. T2D association to these loci was corroborated by the strong evidence of association for increased fasting glucose levels and reduced beta-cell function as measure by homeostasis model assessment in combined analysis with a total of 14,232 non-diabetic individuals comprising 7,696 GWA and 6,536 replication study subjects. Interestingly, strong associations of two novel loci with T2D and its related traits were only detected in men but not in women, implying gender-specificity of these loci.

1930/F

A survey of the genetics of gene expression in four metabolic tissues from a morbidly obese cohort. *D.M. Greenawalt[1], R. Dobrin[2], E. Chudin[3], I.J. Hatoum[4], C. Suver[3], J. Beaulaurier[3], B. Zhang[3], V. Castro[4], J. Zhu[3], S.K. Sieberts[3], S. Wang[3], C. Molony[1], S.B. Heymsfield[2], D.M. Kemp[2], M.L. Reitman[2], P. Yee Lum[3], L.M. Kaplan[4], E.E. Schadt[3].* 1) Merck & Co., Inc., Boston, MA; 2) Merck & Co., Inc., Rahway, NJ; 3) Rosetta Inpharmatics, Seattle, WA; 4) Weight Center, Massachusetts General Hospital, Boston, MA.

For individuals who suffer from extreme obesity (BMI > 40 kg/m2) surgical interventions such as Roux-en-Y gastric bypass can have a great effect not only on weight but co-morbidities as well. To characterize the molecular components associated with morbid obesity and response to RYGB, we have performed high-throughput genotyping and gene expression profiling in 4 tissues; liver, omental adipose, subcutaneous adipose and stomach, from approximately 1000 patients undergoing Roux-en-y gastric bypass and collected clinical traits associated with weight loss. This data has not only allowed us to calculate the association between clinical weight traits and genetic polymorphisms from individuals suffering from extreme obesity to identify associations to BMI and leptin; but to also calculate the association between SNPs and gene expression of 4 metabolically relevant tissues, to identify expression SNPs (eSNPs). We successfully identified 24,531 eSNPs corresponding to ~10,000 distinct genes. This represents the greatest number of eSNPs identified to our knowledge by any study to date and the first study to identify eSNPs from stomach tissue. We then demonstrate how these eSNPs provide a high quality disease map for each tissue in morbidly obese patients to not only inform genetic associations indentified in this cohort, but in previously published genome wide association studies as well. eSNP data generated from this study of 4 metabolically relevant tissues will aid in informing current and future GWA studies, and provide a path forward to pinpointing genes and associated gene networks of interest responding to genetic perturbations associated with disease.

1931/F

Genome-wide association study identifies sequence variants associated with hematological traits in Asian population. *Y. Kim, J. Oh, Y. Kim, M. Go, Y. Cho.* Center for Genome Science, Seoul, Korea.

To identify genetic loci influencing hematological traits (such as platelet count, white blood cell (WBC) count, red blood cell (RBC) count, hematocrit and hemoglobin concentration), we conducted a genome-wide association study (GWAS) of 8,842 subjects recruited from population-based cohorts in Korea. Replication from independent population (N=7,861) to validate GWAS results revealed SNPs reaching genome-wide significance for selected hematological traits. We identified significant associations between platelet count and genetic variants in four regions on chromosome 4p16.1 (Pcombined = 1.46 × 10-10, in KIAA0232), 4q25 (Pcombined = 6.68 × 10-12, in or near EGF), 12q24.12 (Pcombined = 1.11 × 10-15, in SH2B3) and 6p21 (Pcombined = 1.69 × 10-7, in BAK1). GWAS for WBC showed strong evidence of genetic association on 17q21.1a (Pcombined = 1.1 × 10-16) which contains CSF3 gene. Blood hemoglobin concentration was significantly associated with one region on 22q12.3d (Pcombined = 2.2 × 10-8) localizing to TMPRSS6 that is required to sense iron deficiency. Two SNPs were detected for their association with RBC from GWAS (RBC data not available in replication subjects). One (P = 8.6 × 10-9) is located on 6p21.1f in MED20 encoding a component of the mediator complex. The other (P = 3.3 × 10-12) localizes to 4q12 surrounded with several genes (CHIC2/GSX2/PDGFRA/KIT). Interestingly, One locus located on 6q23.3a near MYB showed strong association with WBC (Pcombined = 5.7 × 10-7), RBC (P = 3.8 × 10-25) and hematocrit (Pcombined = 5.1 × 10-9). This locus is known to play an important role in proliferation and differentiation of hematopoietic progenitor cells. Our findings might enhance to unravel molecular mechanisms underlying these hematological traits.

1932/F

Novel genetic variants behind blood biochemical traits identified by a large-scale meta-analysis in Korean population. *Y. Kim[1], M. Go[1], C. Hong[1], J. Lee[1], B. Han[1], H. Kim[1,2], Y. Cho[1].* 1) Center for Genome Science, Seoul 122-701, Korea; 2) Department of Biochemistry, School of Medicine, Ewha Womans University, Seoul 158-710, Korea.

During the last decade, there were flood of published documents finding the evidence of genetic influence on the variation of phenotypes. Genome-wide association studies (GWAS) have been very successful for identifying susceptible loci influencing several traits in populations with European ancestry. However, GWAS in populations of Asian or African ancestry have been insufficiently represented. Here we present a large-scale effort to look for the genetic loci responsible for the phenotypic variation in blood biochemical traits. Identifying genes responsible for the variation of blood biochemical traits is beneficial to understand the underlying mechanisms of the complex diseases since the levels of biochemical measurements in blood are highly correlated with several complex diseases. Furthermore, the highly heritable nature of these traits revealed from numerous previous studies makes these traits attractive for genetic association studies of diverse phenotypes. Several SNPs revealed the evidence of association with one or more of 14 quantitative traits related to phenotypes such as plasma glucose level, lipid metabolism and hepatic and renal functions from discovery stage meta-analysis combining two independent GWASs of 12,500 samples. Those SNPs taken forward to replication stage analysis were mostly validated in the independent poplation of 7,500 samples. Replicated signals achieving genome-wide significance (P < 5 X 10-8) from overall meta-analysis comprising about 20,000 Korean individuals include previously known loci from European or Japanese GWASs for blood biochemical traits as well as ones newly identified in this study. Fifteen novel loci locate to CUX2 and C12orf51 for high density lipoprotein cholesterol, ABO for low density lipoprotein cholesterol, SIX2-SIX3 for fasting plasma glucose, PLCXD2 and PSMA1 for renal traits, and CUX2, OAS3 and HNF1A for hepatic traits.

1933/F

Genotype stability of highthroughput genotyping microarray using Epstein Barr Virus-Transformed B-Lymphocyte Cell lines. *J. Oh, Y. Kim, J. Jeon, Y. Cho.* Center for Genome Science, Seoul, Korea.

The highthroughput SNP (single nucleotide polymorphism) genotyping microarray has greatly contributed to genome wide association studies (GWAS) for identifying human disease susceptibility loci. Although primary cells or tissue samples are major resources to collect genomic DNA for genotyping, major problem arises when encounter its limited access to primary cells or tissue samples. Due to the insufficiency of the primary cells or tissue samples, the Epstein-Barr Virus (EBV)-transformed lymphoblastoid cell lines (LCL) are promising alternative choice for genomic DNA extraction. Previously, Herbeck et al. reported fidelity of SNP array genotyping using immortalized cell lines. In this study we aimed not only to confirm Herbeck's findings but also to test genotype stability of LCL at different propagation stages in terms of cell passages. We genotyped peripheral blood mononuclear cell (PBMC) and LCLs at 6 different passages of 20 different individuals using Affymetrix SNP 500k genotyping chips. Six LCL samples were generated from long-term subcultures up to a passage number of 160. We extensively monitored the concordance between PBMC and LCL at each propagation stage in terms of identity-by-state (IBS). Our finding shows no significant difference in genotypes between PBMC and all types of LCL samples, suggesting that LCL samples at any stage of propagation may be the trustworthy DNA source for SNP genotype analysis.

1934/F

Initial results from dense SNP genotyping of the 9q22-q31 CRI in ASP from the NIMH and NCRAD cohorts. *R. Perry[1], H. Wiener[1], R.C.P. Go[1,2].* 1) Dept Epidemiology, Univ Alabama at Birmingham, Birmingham, AL; 2) Pacific Health Research Institute, Honolulu, HI.

Background: Linkage scans of Alzheimer's Disease (AD) families by us and others have identified the 9q22 region as a candidate region. Next to the 19q13 peak where *APOE* is located, the 9q22 signal was the most suggestive from the linkage scan of the NIMH Alzheimer's Disease Genetic Initiative (ADGI) family cohort. Follow-up confirmation of this signal was demonstrated when we genotyped additional microsatellite markers, narrowing the 1 LOD region to 11.5 cm. Four candidate genes located between 2-6 Mb proximal to this peak have been reported from candidate gene and genome wide association studies to be significantly associated with AD. We have confirmed the association of one of these genes, *NTRK*2, in a separate set of families from NCRAD. Methods: In order to confirm and more precisely determine the nature and location of these signals, we have performed dense SNP genotyping of the 9q21-9q31 region. This covers a total of 18 Mb which includes the 9q22 candidate region and the proximal 6 Mb where these four candidate genes are located. Haplotag SNPs and unblocked SNPs were chosen from HapMap. Additional SNPs located in reported CNVs from this region were also identified and chosen, resulting in a total of ~ 5800 SNPs. These were genotyped using the iSelect platform from Illumina. Results: Using Family Based Association Testing, more than one signal reached the level of significance. We are currently validating these signals and determining their functional significance. Additionally, we are investigating suggestive evidence for the presence of CNVs. Conclusion: Dense SNP genotyping of the 9q22 candidate region and six Mb of genome proximal to the candidate region identified signals with significant association to AD in ASP from NIMH and NCRAD cohorts. There may also be suggestive evidence of CNVs present. We are in the process of determining if these signals are valid or if they are in LD with additional variants. Significance of these results and follow-up strategy will be presented at the meeting.

1935/F

dbSNP and dbVar: NCBI Databases of Simple and Structural Variations. *L. Phan, M. Ward, G. Yu, H. Zhang, A. Vinokurov, M. Quintos, M. Kholodov, D. Shao, E. Shekhtman, R. Maiti, J. Lopez, T. Hefferon, J. Garner, A. Mardanov, D. Church, L. Forman, D. Maglott, M. Feolo, S. Sherry.* NCBI, National Institutes of Health, Bethesda, MD.

The National Center for Biotechnology Information (NCBI) creates and maintains a set of databases that archive, process, display and report information related to germline and somatic variants from multiple species. These databases, primarily the Database of Single Nucleotide Polymorphisms (dbSNP), the Database of Genomic Structural Variations (dbVar), and the Database of Genotypes and Phenotypes (dbGaP) are integrated with NCBI resources including Gene, GeneTests, OMIM, PubMed, and Nucleotide. This presentation focuses on dbSNP and dbVar, summarizing current function and highlighting recent improvements. The primary roles of both databases are the archival and processing functions. Each submission is assigned a database identifier (dbSNP ss# or dbVar nsv#/esv#) based either on flanking invariant sequence or locations asserted on reference sequences. These submissions are then processed to aggregate information from multiple submitters (assign rs# in dbSNP) and to calculate locations on each version of a genome, RefSeqGenes (LRG), and other NCBI Reference Sequences (RefSeqs). Because these stable public accessions are citable in publications, they facilitate aggregation of information as diverse populations are tested for variation. Researchers and genetic testers are encouraged to submit their variation data and to cite their submissions in manuscripts and on the web. Once data are accessioned, they are made available in diverse ways: Entrez searches, study-specific reports, annotation on the genome, human gene-specific displays such as Variation Viewer, and ftp transfer. This presentation will highlight recently added search and display options and new variation FTP downloads such as for the Variant Call Format (VCF) reports and human gene-specific reports. dbSNP and dbVar represent millions of human variants, with more than 7 million having minor allele frequency > 0.05 in at least one population and more than 5 million validated by genotyping. They also contain thousands of records with possible 'clinical significance'. Data are integrated from large scale international projects such as the Human Genome Project, International HapMap Consortium, DGV/DGVa, 1000 Genomes Project, and from highly-curated Locus Specific Databases (LSDBs), OMIM, the literature, or other gene-specific resources. Given our ever-increasing need for understanding of human variation, maintenance and effective use of centralized variation databases is critical.

1936/F

Confirmation of *CNTNAP2* as an autism susceptibility gene. *S. Sampath, S.S. Bhat, B. Doan, D.E. Arking, A. Chakravarti.* McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD.

The pathophysiology of autism, a childhood neuropsychiatric disorder, remains largely unknown despite a strong genetic component to disease susceptibility. In a previous genomic study of autism we identified a common sequence variant (rs7794745) in intron 2 of contactin associated protein-like 2 (*CNTNAP2*) that was linked and associated with autism susceptibility. Other independent studies have also pointed to the role of common, rare, and structural variants in *CNTNAP2* in autism susceptibility. To further characterize the role of *CNTNAP2* in autism, we studied polymorphisms at a high density across the »3 Mb of the *CNTNAP2* locus. We genotyped 400 trios each from multiplex and simplex families using a custom genotyping array that included 3,150 SNPs. Because the custom array was designed for allelic rather than dosage discrimination, it precluded the efficient use of raw intensities from each SNP for copy number analysis. For statistical analysis we removed SNPs based on missing data (> 0.10), minor allele frequency (< 0.05), Hardy Weinberg fit ($p < 0.001$), and Mendelian errors (> 0.10). We analyzed 2100 SNPs using a transmission disequilibrium test (TDT). Additionally, the presence of contiguous Mendelian errors within each family was evaluated to identify deletions. TDT analysis performed in either multiplex or simplex families alone did not yield any significant finding. However, combining the data from both multiplex and simplex families yielded two SNPs (rs2710093, $p = 1.1 \times 10^{-5}$; rs2253031, $p = 2.5 \times 10^{-5}$) with significant effects at *CNTNAP2*. Both SNPs were located in intron 14 and are highly correlated with each other ($r^2 = 0.99$). The overtransmission of the rs2710093 'C' allele ($\tau = 0.61$) had a significant parent-of-origin effect from the paternal than maternal side ($\tau_{pat} = 0.66$, $\tau_{mat} = 0.56$, $p = 0.0043$). rs2710093 is »57 kb downstream of, and in a different LD block from, SNPs rs2710102 and rs17236239 that have shown significant effects in two previous studies of association to the autism endo-phenotypes of language QTL (age at first word) and specific language impairment. We are currently genotyping additional autism families to confirm this finding. Analysis of large contiguous Mendelian errors within each family did not indicate any segregating deletions or duplications. Cumulative evidence from this and previous autism studies now confirm that multiple common, rare, and structural variants in *CNTNAP2* play a significant role in autism.

1937/F

Evaluation of the performance of genomic DNA from saliva collected with Oragene®•DNA for the purpose SNP discovery on various Illumina technologies. *M.A. Tayeb[1], R.M. Iwasiow[1], P. Hu[2], R.F. Wintle[2], S. Scherer[2].* 1) DNA Genotek Inc., Ottawa, ON, Canada; 2) The Centre for Applied Genomics, The Hospital for Sick Children, Toronto, ON, Canada.

Genome-wide association studies allow for rapid scanning of markers across entire genomes. The Illumina Human610-Quad and Human1M-Duo BeadChips include more than 600,000 and 1.1 million markers for single nucleotide polymorphism analysis per sample, respectively. The current study evaluates the use of gDNA from saliva collected with Oragene•DNA on the Illumina Human610-Quad Human1M-Duo BeadChips. Oragene•DNA simplifies sample collection by providing an alternative to blood, eliminating phlebotomy cost and complexity and facilitating collection from subjects dispersed throughout the general population. Oragene•DNA is a non-invasive, self-collection device intended for collection of large quantities of high-molecular weight genomic DNA (gDNA) from saliva. DNA is stabilized at room temperature for extended periods enabling sample collection and transport via regular mail and flexibility to process in batches. Two saliva samples were collected from each of four donors on two separate days using the Oragene•DNA kit and the gDNA extracted from each of these collections was analyzed. Performance of the gDNA from saliva was assessed by evaluating the SNP calls from each sample. Reproducibility of the data was determined by comparing the results from two separate collections from the same donor (Human1M-Duo BeadChip only). We report QC call rates >98.8% on the Human610-Quad and >99.8% on the Human1M-Duo. The standard deviation of log R ratio (SDLRR) of all saliva samples was <0.4 for the Human610-Quad and <0.2 for the Huaman1M-Duo. Concordance between separately-collected saliva samples was >99.8% (Human1M-Duo). Concordances between blood and saliva samples from the same donor were >99.9% and >99.8% on the the Huaman610-Quad and Human1M-Duo, respectively. The results indicate that gDNA isolated from saliva collected with Oragene•DNA is a suitable substitute for gDNA from blood for use on Illumina SNP discovery technologies.

1938/F

A common variant on chromosome 5q33.3 is associated with susceptibility to systemic lupus erythematosus in Chinese Han population. *Y. Ren[1,2], Y. Li[1,2], Z. Zhang[1,2], J. Han[1,2], L. Sun[1,2], S. Yang[1,2], X. Zhang[1,2].* 1) Key Laboratory of Dermatology (Anhui Medical University), Ministry of Education, China, Hefei, Anhui 230032, China; 2) Institute of Dermatology and Department of Dermatology at No.1 Hospital, Anhui Medical University, Hefei, Anhui, China.

Background: Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by the presence of autoantibodies and by the anatomical and functional damage of multiple organs. The pathogenesis of SLE is complex and involves a combination of multiple genetic and environmental factors. During the past 20 years, many studies have identified multiple genetic factors related to SLE. However, these studies were mainly conducted on European popu=AClations and less performed in other ethnic populations. In fact, the genetic heterogeneity between ethnic populations has been suggested to be important in SLE risk. For example, the single nucleotide polymorphism (SNP) rs2431697T on chromosome 5q33.3 has been shown to be associated with SLE in European populations, but not been studied whether being a risk factor in other ethnic populations. Objective: To investigate whether the SNP rs2431697 on 5q33.3 is also a risk factor for SLE of Chinese Han. Method: EDTA anticoagulated venous blood samples were collected from all partici=ACpants (1,993 SLE cases and 4,770 controls). Genomic DNA was extracted from peripheral blood lymphocytes by stand=ACard procedures using FlexiGene DNA kits and was diluted to concentrations of 15-20ng/ul for the validation study. The SNP was genotyped using the Sequenom MassArray system. The SNP rs2431697 was tested for significant deviation from Hardy-Weinberg equilibrium in controls and it passed the test with P-values >0.05.The association between the SNP rs2431697 and disease susceptibility to SLE was assessed using the Chi-square test with 2×3 and 2×2 contingency tables. Result: The SNP rs2431697T on 5q33.3 was found to be associated with SLE (P=6.27×10-11, odds ratio (OR) = 1.43 on the major allele, 95% confidence interval (95% CI): 1.28-1.59). Conclusion: We confirmed that the SNP rs2431697T on 5q33.3 was also associated with SLE in Chinese Han population, which suggested that it might be a common susceptibility factor for SLE within different populations.

1939/F

Follow up study identifies two novel susceptibility loci for systemic lupus erythematosus in Chinese Han population. *S. Yang[1,2], Y. Li[1,2], Z. Zhang[1,2], J. Han[1,2], L. Sun[1,2], X. Zhang[1,2].* 1) Key Laboratory of Dermatology (Anhui Medical University), Ministry of Education, China, Hefei, Anhui 230032, China; 2) Institute of Dermatology and Department of Dermatology at No.1 Hospital, Anhui Medical University, Hefei, Anhui, China.

Background: Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by dysregulated immune responses mediated by T and B cells, leading to increased production of pathogenic autoantibodies, against several self antigens. The etiology of SLE might include genetic and environmental factors. In past two years, six genome wide association study (GWAS) of SLE have been published, which identified more than 30 susceptibility genes or loci for SLE and confirmed some of genes found by candidate gene study. In 2009, we have completed a GWAS of SLE in Chinese Han population, and identified 9 new susceptibility loci, as well as confirmed 8 reported ones in previous study. Objective: To investigate additional potential genetic variants for SLE in Chinese Han population. Method: We re-analyzed the GWAS data and selected 72 SNPs, which were genotyped in 3,152 cases and 7,050 controls of Chinese Han using the Sequenom MassArray system. For the each replication study, 72 SNPs were analyzed using the logistic regression (gender as a covariate). The joint analysis of the combined GWAS and replication samples was performed by using logistic regression (gender and sample cohorts as covariates). Results: Two susceptibility loci were validated and surpassed genome-wide significance in combined analysis with GWAS data, which located at 16q11 (P=1.352×10-9,) and 2p23 (P=3.91×10-8). Conclusion: By follow up GWAS dataset, this study identified two novel loci (16q11 and 2p23), which should expand the catalog of genetic factors for SLE and throw new insights into the pathogenesis to the molecular level.

1940/F

Follow up study identifies six novel susceptibility loci for psoriasis in Chinese Han population. *X. Zhang[1,2], L. Sun[1,2], Z. Wang[1,2], H. Cheng[1,2], S. Yang[1,2].* 1) Key Laboratory of Dermatology (Anhui Medical University), Ministry of Education, China, Hefei, Anhui 230032, China; 2) Institute of Dermatology and Department of Dermatology at No.1 Hospital, Anhui Medical University, Hefei, Anhui, China.

Background Psoriasis is a chronic inflammatory hyperproliferative skin disease influenced by multiple genetic factors. The exact etiology of psoriasis is not fully clear so far. In the past several years, genome-wide association study (GWAS) plus linkage and association studies have implicated many genomic regions in the pathogenesis of psoriasis, not all of which have been confirmed. Previously, we have completed a first large GWAS of psoriasis in Chinese Han population, in addition to validating the established susceptibility loci major histocompatibility complex (MHC) and IL12B, we identified a novel susceptibility locus within the late cornified envelope (LCE) gene cluster. Objective To further explore additional susceptibility loci for psoriasis and investigate disease heterogeneity. Methods A follow-up study of psoriasis was performed within multiple populations including 6,634 cases and 10,868 controls in Chinese populations, 539 cases and 824 controls in Chinese Uygur, and 823 cases and 1840 controls of European population. Results Six new susceptibility loci (Pcombined Chinese Han £ 3.78 × 10-8) were identified and one previously reported one for psoriasis (P < 4.55 × 10-18) was validated in Chinese Han. Conclusion Our findings increase the number of genetic risk factors of psoriasis, some of which have also been implicated in other autoimmune diseases. Our results also highlight novel and plausible biological pathways in psoriasis, suggest additional genetic factors that may contribute to its age at onset, and provide insight into genetic heterogeneity of psoriasis across different ethnic populations.

1941/F

Lack of association of telomere length and type-2 diabetes(T2D). *Y. Liu[1], Q. Shen[2], Z. Zhang[2], L. Cao[1], L. He[1].* 1) Institutes Biomedical Sci, Fudan Univ, Shanghai, China; 2) Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, PR China.

Telomeres are structures at the ends of eukaryotic chromosome and engaged a lot in genomic stability.High oxidative stress potentially leads to accelerated telomere shortening and consequent premature cell senescence,which implicated in type 2 diabetes (T2D) development. Therefore, we conducted association study of leukocyte telomere length (LTL) with the presence of T2D in Chinese Han population.We measured mean telomere length in 1936 patients and 2080 controls using an established and validated quantitative PCR-based technique. In this study, we analysed the LTL adjusted by age,gender,smoking and drinking, telomere length declined by 0.00673 T/S per year (r2 = 0.036, P<0.0001), and is longer in woman than man(r2=0.01,p<0.0001),while no association of T2D presence with telomere length when adjusted (p=0.151). In conclusion, telomere length is unlikely to play a major role in the susceptibility of T2D.

1942/F

High-throughput resequencing of human LINE-1 insertion sites suggests both inter- and intra-individual genetic variation in L1 content. *A.D. Ewing[1], S.R. Richardson[2], J.L. Garcia-Perez[3], J.V. Moran[2,4], H.H. Kazazian, Jr.[1].* 1) Department of Genetics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104, PA; 2) Departments of Human Genetics and Internal Medicine, University of Michigan Medical School, Ann Arbor, Michigan 48109, USA; 3) Andalusian Stem Cell Bank, Center for Biomedical Research, Avda Conocimiento s/n, University of Granada, 18100, Spain; 4) Howard Hughes Medical Institute, Chevy Chase, Maryland 20815, USA.

We previously have reported a high level of inter-individual variation of dimorphic L1 insertions in human genomes, using a technique that locates human-specific L1 insertions by selectively resequencing their 3' genomic flanking regions. Further analysis, aided by cross-referencing with the results of an algorithm that identifies L1 insertions from whole-genome resequencing data, now reveals more extensive L1 variation than recognized previously. We have identified ~500 non-reference L1 insertions present in a collection of 25 individuals (15 of whom are unrelated), after including insertions generated via both techniques. These data represent a substantial addition to a previous report of 367 non-reference L1 insertions in the same cohort.

In addition to inter-individual variation in L1 content, we report evidence for intra-individual somatic variation due to L1 retrotransposition. These data come from two lines of evidence: analysis of cultured cells, including human embryonic stem cells (hESCs), and analysis of trios consisting of a proband and both parents. In cultured cells, we thus far have discovered six L1 insertions that appear to be present in only one clonal line from pairs that were established and propagated from the same parental cell line. Thus, the L1 retrotransposition events likely occurred from endogenous L1 progenitor loci while the cells were in culture. Three L1 insertions are present in clonal PA-1 ovarian embryonic carcinoma lines, whereas the other three insertions are present in clonal H13B hESCs. Two of the three insertions in H13B cells likely are present at less than one copy per genome, as nested PCR was required to amplify the 'filled' insertion sites. Moreover, we uncovered evidence for a sub-genomic insertion in placental tissue derived from a stillborn proband. This L1 insertion was identified by nested PCR in placental DNA derived from the proband, but not from blood-derived genomic DNA samples from either parent. This putative somatic insertion was identified from one of only two studied stillborn trios. Thus, these data suggest that L1 retrotransposition contributes to both inter- and intra-individual human variation.

1943/F

Comparison of tagging and imputation for HLA allele prediction and association testing. *J. Shen[1], S. Leslie[2], S. Bacanu[1], J. Whittaker[3], G. McVean[2], M. Nelson[1].* 1) Genetic, R&D, GlaxoSmithKline, Research Triangle Park, NC, 27709; 2) Department of Statistics, University of Oxford, Oxford Ox1 3TG, UK; 3) Genetic, R&D, GlaxoSmithKline, Harlow, UK.

The major histocompatibility complex (MHC) includes the genes that encode the human leukocyte antigens (HLA) involved in adaptive immune response. HLA genotyping by sequencing is the gold standard, but is expensive and requires relatively large quantities of DNA. Making inferences about HLA associations using SNPs offers a promising alternative. Both tagging and imputation methods have been proposed for HLA allele prediction from SNPs. However, there remains considerable uncertainty regarding the reliability with which these methods can infer HLA alleles from SNPs and the extent to which power to detect HLA associations is eroded when imputation or tagging is used. We compare the ability of single-SNP tagging, single-SNP imputation and multi-SNP hidden Markov model (HMM) imputation to predict high resolution alleles of HLA-A, B, C, DQA1, DQB1 and DRB1 in subjects of European background and the potential power loss in a case-control association testing framework. We find that multi-SNP HMM imputation generally performs better on both common and rare HLA alleles than single-SNP methods. As expected, rare alleles are more difficult to predict by all three methods, requiring standard methods of high resolution typing when such are of pivotal research interest. For some HLA alleles, lower tag SNP predictive values may be offset by relatively high HMM imputation no-call rates, suggesting that a mixed approach to analysis may be most powerful. Overall, we conclude that when sufficient training data are available, exploratory HLA analysis can be carried out using HMM imputation on SNP genotypes with little loss of power. This has considerable importance for the design and analysis of association studies where HLA alleles are believed likely to be important, for instance in pharmacogenetics.

1944/F

Linkage Disequilibrium mapping: why coverage is so much more important than sample size. *W. Lau, N. Maniatis.* Genetics Evolution & Environment, Univeristy College London, London, United Kingdom.

Over the recent years, association mapping of disease genes has developed into one of the most dynamic research areas of human genetics. Establishing the Linkage Disequilibrium (LD) structure of the Human Genome was extremely important for the effective application of association mapping. It was also instrumental for the advancement of genome wide association scans. From the International HapMap Project and other sources, millions of SNP genotypes are now available for constructing high resolution LD maps. Here we compare the maps in LD units that were constructed from the 60 CEU individuals of the Phase II HapMap data and the combined 17,000 individuals from the Welcome Trust Case Control Consortium (WTCCC). We investigate the importance of high resolution and sample size on the genome wide patterns of LD and find that SNP resolution is the main factor in obtaining smaller error variance and finer localization of LD hot and cold spots. These results may have important implications in association mapping.

1945/F

Relationship between fine-scale recombination and Linkage Disequilibrium maps in the human genome. *N. Maniatis, W. Lau.* Genetics Evolution & Environment, University College London, London, United Kingdom.

Recombination is essential for correct chromosome segregation and for increasing haplotype diversity within populations. It is the main determinant of Linkage Disequilibrium (LD) patterns in the genome, which influences our ability to map disease-related loci. The small number of informative meioses in pedigree studies has limited the resolution of linkage maps. A recent study has mapped crossovers in nuclear families using a high-density SNP typing. However, even a much higher resolution profiles of recombination can be obtained from linkage disequilibrium maps based on SNP data from the International HapMap Project. In this study we compare two linkage maps from previous studies that vary on resolution and family size with the maps in LD units (LDU). We investigate the nature of recombination hotspots and the quantitative relationship between cross-over frequency and LDU distance.

1946/F

Admixture mapping of fasting glucose: Identification of a candidate locus associated with T2D. *G. Chen[1], D. Shriner[1], J. Zhou[1], A. Doumatey[1], H. Huang[1], N. Gerry[3], A. Herbert[4], M. Christman[3], G. Douston[2], M. Faruque[2], Y. Chen[2], A. Adeyemo[1], C. Rotimi[1].* 1) CRGGH/NHGRI/NIH, NIH, Bethersda, MD; 2) National Human Genome Center, Howard University, Washington DC 20060 USA; 3) Department of Genetics and Genomics, Coriell Coriell Institute for Medical Research, Camden, NJ 08103 USA; 4) Department of Genetics and Genomics, Boston University School of Medicine, Boston, Massachusetts 02118 USA.

Elevated fasting blood glucose is a feature of type 2 diabetes and insulin resistance. We have carried out a search for novel loci associated with fasting blood glucose (FBG) among African Americans using the mapping by admixture linkage disequilibrium (or “admixture mapping”) approach. Using an ancestry informative marker (AIM) panel comprising 1800 SNPs, the genomes of 619 non diabetic and non hypertensive unrelated African Americans from the Howard University Family Study (HUFS) were screened for regions with elevated ancestry proportions from either of the ancestral populations (reference HapMap YRI and CEU). The mean proportion of African ancestry in the study sample was 0.81 ± 0.10. An increased fasting glucose was associated with increasing proportion of European ancestry with β = 0.087 and p value = 0.044. Using ADMIXMAP, we identified a ~12.36 Mb region (46.49 - 59.85 Mb) on Chromosome 14q22 - 23 associated with FBG with the best p value 6.4x10-5 (rs12895262). Ten AIMs in this region showed Bonferroni corrected p values < 0.05. Repeating the analysis with the STRUCTURE program yielded the similar results. In a follow up fine mapping study, 800 SNPs in this region (average density 9.8 kb) present on the Affymetrix 6.0 SNP array were tested for association with FBG. The best p values were 3.38x10-6 (rs10146136, position 51079426bp, ATL1) and 3.23x10-5 (rs11570807, 50830141bp, CDKL1).In summary, we found that increasing percent of European ancestry was associated with increasing FBG among African Americans. We identified a new locus on chromosome 14q22-23 that influences FBG levels and fine mapping confirmed this association.

1947/F

Direct Assessment of Multiple Testing Correction in Case-Control Association Studies with Related Individuals. *Z. Wang.* Division of Biostatistics, Yale University, New Haven, CT.

Genome-wide association studies typically test large numbers of genetic variants for association with trait values. It is well known that linkage disequilibrium (LD) between nearby markers tends to introduce correlation among association tests. Failure to properly adjust for multiple comparisons can lead to false positive results or miss true positive signals. The Bonferroni adjustment, which ignores dependence among test statistics, is generally conservative in the presence of LD. The permutation procedure, although has been widely employed to adjust for correlated tests, is not applicable when related individuals are included in case-control samples. When relatives are sampled, the dependence among relatives’ genotypes can contribute to the correlation between tests. We present a new method P_{norm} to correct for multiple hypothesis testing in case-control association studies with related individuals. The adjustment with P_{norm} simultaneously accounts for two sources of correlations of the test statistics: (1) LD among genetic markers (2) dependence among genotypes across related individuals. Through simulation studies, we demonstrate that it is more accurate in terms of error rate and more powerful than some of the recently developed methods. We apply the method to a genome-wide association study of alcoholism in the GAW 14 COGA data set and detect genome-wide significant association.

1948/F

Global exon expression analysis of alternative splicing during aging in mice. *S. Rodriguez, M. Eriksson.* Karolinska Institutet, Department of Biosciences and Nutrition, Karolinska University Hospital, Novum, SE-141 83 Huddinge, Stockholm, Sweden.

The severe segmental premature aging disease Hutchinson-Gilford progeria syndrome (HGPS or progeria) is caused by a *de novo* point mutation in exon 11 of the *LMNA* gene. The mutation leads to the activation of a cryptic splice site that removes 150 nucleotides from the prelamin A mRNA and thus results in a truncated prelamin A protein, commonly known as progerin. Recent reports have identified low levels of progerin in normal tissues and that these levels increases during cell aging in cells from both progeria patients and in unaffected controls. We hypothesize that during normal aging, mis-splicing events such as the cryptic splicing seen in progeria could become more widespread all over the genome possibly as a result of a general decline in the stringency of the splicing machinery. To test this hypothesis we have used GeneChip® Mouse Exon 1.0 ST Arrays which provide a unique ability to interrogate global gene expression at the exon level to analyze alternative splicing during aging of C57BL/6 wild-type mice. RNA was extracted from several tissues including skin, skeletal muscle, bone, thymus, and white adipose tissue. Samples were obtained from three different ages of mice, 4, 18, and 28 months. Hybridization data was analyzed using the Partek® Genomic Suite™. Preliminary results have identified a considerable number of genes displaying significant differential alternative splicing with age in all mouse tissues analyzed. Our results suggest that alternative splicing might be affected during tissue aging. As far as we know, this is the first analysis of alternative splicing to be performed on normal aging of animal tissues and highlights the importance of global genome analysis to increase the understanding of genetic mechanisms during aging.

1949/F

Using mouse models for Potocki-Lupski and Smith-Magenis syndromes to study the impact of CNV on behavioral phenotypes. *M. Heney[1], W. Gu[1], C. Spencer[1], R. Paylor[1], J.R. Lupski[1,2,3].* 1) Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Dept of Pediatrics, Baylor College of Medicine, Houston, TX; 3) Texas Children's hospital, Houston, TX.

Many microdeletion/microduplication syndromes are associated with specific abnormal behavioural phenotypes. Potocki-Lupski syndrome (PTLS; MIM #610883) is associated with microduplication in chromosome 17p11.2, and it is characterized by autistic or ASD-like phenotypes, congenital and neurobehavioral abnormalities, and developmental delay. The reciprocal 17p11.2 microdeletion is associated with Smith-Magenis Syndrome (SMS; MIM #182290), a multiple congenital anomaly disorder with neurobehavioral features including aggression and self-injury, as well as mental retardation. We previously generated mouse models for PTLS, *Dp(11)17/+*, and SMS, *Df(11)17/+*, that harbor either a duplication or deletion of a ~2 Mb region syntenic to the PTLS/SMS region. These mouse models recapitulate some of the physical and neurobehavioral phenotypes seen in patients, including abnormal social interactions, motor, and learning defects. This unique mouse model system allows the systematic study of copy number variation (CNV) in relation to specific physical and neurobehavioral phenotypes because *Df(11)17/+*, *Df(11)17/Dp(11)17*, *Dp(11)17/+*, and *Dp(11)17/Dp(11)17*, mice can be analyzed to evaluate the effect of one, two, three, and four copies, respectively, of the SMS/PTLS critical region. In addition, we have developed mouse strains with a point mutation in the major dosage-sensitive gene, RAI1/Rai1, as well as different sized deletions, which allow us to also observe the effect of flanking regions on the CNV manifestation, perhaps through chromatin structure alteration. Utilizing these mouse models, the behavioral phenotypes were studied with a battery of behavioural tests, including the tube, partition, hotplate, 3-chamber, rotarod, conditioned fear, Morris water maze, open field analysis, light-dark, and hotplate tests to determine how CNV of the SMS/PTLS critical region can be linked to behavioural phenotypes. In this study, we confirm previously published results and extend these studies to include new tests and other mouse strains with different-sized deletions. Furthermore, copy number normalization in *Df(11)17/Dp(11)17* mice is examined to determine whether it is able to partially correct the phenotypes examined. Our results indicate that both dosage of RAI1/Rai1 and possibly other modifier genes or elements result in neurobehavioral anomalies, suggesting the presence of gene(s) functioning in the regulation of neurobehavioral pathways in this region.

1950/F

Specific detection of single-stranded DNA in the presences of double-stranded DNA in ChIP-Seq data improves recognition of meiotic hotspots of recombination. *P. Khil[1], F. Smagulova[2], K. Brick[1], I. Gregoretti[1], S. Sharmeen[1], R.-D. Camerini-Otero[1], G. Petukhova[2].* 1) Genetics and Biochemistry Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892; 2) Department of Biochemistry & Molecular Biology, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road Bethesda, Maryland 20814.

The absence of simple and reliable methods to map recombination hotspots on a genome-wide basis presents a major block to further research on the mechanisms of meiotic recombination in mammals. Existing methods, such as sperm genotyping, do not scale well to allow analysis of whole genomes. One powerful approach for the mapping of protein-bound sequences is chromatin immunoprecipitation followed by next-generation sequencing (ChIP-Seq). We have devised an extension to ChIP-Seq that uses molecular features uniquely found in meiotic hotspots to significantly improve the efficiency of the procedure. Following DSB formation, recessed single-stranded DNA ends are formed at the hotspots. These ssDNA strands are covered with Dmc1 and/or Rad51 proteins. Thus, in the first step we use immunoprecipitation to isolate Dmc1- or Rad51-bound sequences. To improve the specificity of hotspot detection we developed an approach to distinguish single-stranded DNA from double-stranded DNA in sequencing data. The rationale for the method is the following: In the process of library preparation for sequencing single-stranded DNA can form hairpins with 5' overhangs which are extended and filled-in by polymerase. The newly added sequence differs in sequence from genomic DNA but is identical to the reverse complement of the sequence from the other end of the fragment. Thus, the presence of a 5-10 nt transferred sequence indicates that the fragment of the DNA was single-stranded rather than double-stranded. A specific single-stranded DNA signature originates from the hotspots whereas background DNA is largely double-stranded. Compared to standard ChIP-sequencing without an ssDNA detection step, both the sensitivity and specificity can be improved more than ten-fold. We applied our method to map hotspots in the mouse and find that if we use this ssDNA signature, we can map more than 10 thousands hotspots in each of two mouse strains. Another important advantage is the ability to gain insights in the molecular structure of the hotspots. For example, we can distinguish hotspots where DSBs are formed at a single location versus several locations clustered together and precisely define the location of initiating DSBs. In addition to mapping meiotic hotspots, this approach can also be extended to other applications where the detection of single-stranded DNA intermediates is necessary.

1951/F

Acute myeloid leukemia 1 over-expression improves muscle pathology in mdx mice. *SH. Lai[1], R. Shi[1], M. Sutherland[2,3], S. Muger[3], R. Marathi[1], N. Kumari[1], YW. Chen[1,2].* 1) Center for Genetic Medicine Research, Children's National Medical Center, Washington , DC; 2) Department of Integrative System Biology and Department of Pediatrics, George Washington University, Washington DC; 3) Center for Neuroscience Research, Children's National Medical Center, Washington DC.

Duchenne Muscular Dystrophy (DMD) is an X-linked muscle disorder caused by mutations in dystrophin gene. Our previous study showed that acute myeloid leukemia 1 (AML1) was up-regulated in non-symptomatic patients with DMD but not in patients with advanced disease. We hypothesized that AML1 was involved in a compensatory mechanism at the early stage of DMD. To study the role of AML1 in the skeletal muscle and DMD, a tet-repressible muscle-specific Aml1 transgenic mouse model (mCK-tTA/TRE-Aml1) was generated. Over-expression of Aml1 in skeletal muscle does not cause an abnormal phenotype. The mice were then crossed with mdx mice which is a widely used animal model of DMD to generate mCK-tTA/TRE-Aml1/mdx mice. Aml1 over-expression was induced at 2 weeks of age and the muscles of the mCK-tTA/TRE-Aml1/mdx mice were collected 4 weeks afterwards. The muscles of mCK-tTA/mdx littermates were used as controls. To evaluate the pathological changes, Hematoxylin and Eosin staining was performed followed by quantifying the different changes (necrotic fibers, myofibers with centralized nuclei and inflammatory foci). Anti-laminin antibody was used to identify the membrane boundaries when measuring the minimal Feret's diameter. The results showed that the mdx muscles over-expressing Aml1 had fewer myofibers with centralized nuclei than their control littermates ($p < 0.05$). The small-calibre regenerating fibers were less abundant and fiber size variability was reduced in the mice over-expressing Aml1 in both gastrocnemius and diaphragm ($p < 0.05$). To investigate the mechanism, we studied proliferation and differentiation of primary myoblasts from the mice over-expressing Aml1. The results indicated that Aml1 promotes both cell proliferation and differentiation in primary myoblasts.

1952/F

Constitutively Nuclear *Foxo3* in Oocytes of Primordial Follicles Promotes Conservation of Ovarian Reserve. *E. Pelosi[1], S. Omari[1], M. Michel[1], A. Forabosco[2], D. Schlessinger[1], C. Ottolenghi[3].* 1) Laboratory of Genetics, NIA/NIH-IRP, Baltimore, MD, USA; 2) Unita' di Genetica Medica, Universita' di Modena, Modena, Italy; 3) Université Paris Descartes, Paris, France.

During female reproductive life, the number and state of ovarian follicles depends on initial numbers of oocytes and follicles and the subsequent balance of activation, maturation and atresia. By age 50 numbers are depleted, and menopause ensues in women, with the cessation of ovarian function. *Foxo3* is a transcription factor that is required to maintain follicles in a quiescent state. FOXO3 is active in the nucleus, but when phosphorylated, it exits the nucleus and becomes inactive. We made a construct in which critical regulatory sites of *Foxo3* were mutated, so that a transgene would remain constitutively active. The transgene was put into mice under the control of a c-kit promoter, in order to drive its expression preferentially in the oocytes of quiescent follicles. We then tested for effects of the transgene with forced nuclear localization on follicle dynamics. In mouse ovaries 3 days post natum, the activation of primordial follicles was delayed and lesser in extent in the presence of the transgene, accounting for a significantly larger pool of primordial follicles at puberty. This was accompanied by an up-regulation of expression of early follicle genes, and a down-regulation of maturation markers. In transgenic peri-menopausal ("old") mouse ovaries we also observed significant down-regulation of aging-associated markers. At the morphological level, relative follicle counts showed a larger number of primordial and still growing follicles in the old transgenic ovaries. We infer that the transgenic ovaries retained some active folliculogenesis. The transgene was then introduced into knockout (*Foxo3-/-*) mice, i.e., where it is the only *Foxo3* allele present. At 14dpn, a stage at which all primordial follicles had disappeared from *Foxo3-/-* ovaries, ovaries from the mice with the transgene still exhibited primordial follicles. Consistent with a rescue of quiescent follicles, ovaries of the *Foxo3-/-* animals harboring the transgene appeared globally younger in their gene expression profiles, assessed by microarray analysis, than did ovaries from age-matched knockouts. Our study supports proposals that *Foxo3* is a guardian of the primordial follicle reserve, and creates a model that could help evaluate and characterize the function of genetic variants putatively involved in the determination of female reproductive lifespan.

1953/F

Genome & Transcriptome Analysis of Human Genomes: Maximising the Benefits of Next-Generation Sequencing using latest Technologies. *K. Stangier, U. Schoeck.* GATC Biotech AG, Konstanz, Germany.

The Illumina HiSeq 2000 provides the industry's highest sequencing output and quality at fastest data generation rate. This can be optimally used in combination with different paired end and mate pair libraries as well as enrichment strategies. **Genome Re-Sequencing** Illumina sequencing data can be generated by using paired end and mate pair data to identify InDels and SNPs simultaneously in whole human genome sequencing, which is especially useful in cancer research. GATCs prorietary tagging system allows barcoding of samples for the combination of any number of samples with any size of targeted region. Depending on size and number of samples, different enrichment technologies can be applied to sequence a specific subset of the human genome. **Transcriptome Sequencing** Enrichment kits for exons can be customised to target all exons including additional specific regions of interest. Using a proprietary tagging protocol it is possible to multiplex samples for sequencing on Illumina sequencing technologies in order to generate expression profiles from several samples in a cost effective way. For both applications, special bioinformatics analysis tools are used. We will show a variety of visualised results generated from different technical approaches and analysis tools. **Conclusion** High quality results will be achieved when optimally combining several library preparations and Next Gen technologies with state-of-the-art bioinformatics.

1954/F

A secure repository for human sequencing data with rich phenotypes. *M.F. Shumway, E. Yaschenko, S. Sherry, R. Agarwala, M. Feolo, L. Hao, M. Kimelman, G. Godinskiy, C. O'Sullivan, A. Sturcke, A. Mnev, J. Ostell.* National Center for Biotechnology Information (NCBI), National Library of Medicine, Bethesda, MD.

NCBI has developed a controlled access repository for detailed genetic information including sequence and alignment data. This system builds on the existing Genotype and Phenotype archive (dbGaP) infrastructure [1] by placing inside it an instance of the Sequence Read Archive (SRA) [2] specially designed for archiving human sequencing datasets under privacy control, usage restrictions, or ethical constraints. Controlled Access SRA will acquire and distribute data to approved users via dbGaP using existing mechanisms. An important difference between dbGaP and open SRA submissions is that study, subject, and sample metadata and phenotypes must be submitted before sequencing data can be accepted. Controlled Access SRA will archive and distribute human clinical and research sequencing data gathered under subject consent. Controlled Access SRA will also house human meta-genome datasets where a level of human contamination may exist. These datasets can be optionally screened to remove human contaminants and exported to open SRA. The presentation will review the experience of Controlled Access SRA in the context of the first phases of sequencing and alignment data from The Cancer Genome Atlas (TCGA) project [3] and the Human Microbiome Project (HMP) [4]. References [1] Mailman MD, Feolo M, Jin Y, Kimura M, Tryka K, Bagoutdinov R, Hao L, Kiang A, Paschall J, Phan L, Popova N, Pretel S, Ziyabari L, Lee M, Shao Y, Wang ZY, Sirotkin K, Ward M, Kholodov M, Zbicz K, Beck J, Kimelman M, Shevelev S, Preuss D, Yaschenko E, Graeff A, Ostell J, Sherry ST. The NCBI dbGaP database of genotypes and phenotypes. Nat Genet. 2007 Oct;39(10):1181-6. [2] Shumway M, Cochrane G, Sugawara H. Archiving Next Generation Sequencing Data. Nucleic Acids Research, Jan 2010. [3] http://cancergenome.nih.gov [4] http://www.nihroadmap.nih.gov/hmp.

1955/F

Accurate imputation and phasing for paired-end read sequencing data with low coverage. *E. Eskin, B. Han.* Dept Computer Sci, Univ California, Los Angeles, Los Angeles, CA.

Many methods have been developed for imputing uncollected genotypes based on the microarray technology, but few methods are available for imputation based on the sequencing technology. To our knowledge, the only imputation method that currently supports sequencing data is MACH. However, MACH uses the simple model that "decouples'' sequence reads into SNP-wise information. This way, if a read or paired-end read covers multiple SNPs, the information that the observed alleles came from the same chromosome, or the partial phasing information, is ignored. If the coverage is low, the partial phasing information can substantially affect the accuracy. The challenge is that it is very difficult to incorporate partial phasing information into the traditional hidden Markov model (HMM). We solve this challenge by introducing a new model, rotating hidden Markov model (RHMM). The key idea is to place the multiple alleles from a read to the one of two chromosomes in the model to keep the partial phasing information, and then allow two states corresponding to the two chromosomes to freely "rotate'' so that no phasing information is imposed between two separate reads. In our simulations using 1x coverage paired-end read data, the imputation accuracy of RHMM is the same as that of HMM, but the phasing accuracy of imputed genotypes dramatically increases from 48% to 87% by an almost 2-fold change. Similarly to the other imputation methods, our method can utilize information from the sample data as well as from the reference data. Our method IMPERS (IMputation for Paired-End Read Sequencing) is publicly available for research community.

1956/F

Resequencing of a patient with inflammatory bowel disease and subsequent evidence-based treatment. *A. Franke[1], I. Thomsen[1], M. Schilhabel[1], P. Rosenstiel[1], S. Schreiber[1,2].* 1) Institute of Clinical Molecular Biology, Christian-Albrechts-University Kiel, Kiel, Germany; 2) Department of General Internal Medicine, University Clinic S.-H., Kiel, Germany.

Resequencing entire individual patient genomes has become technologically and economically feasible. While several projects have so far demonstrated the successful application of genome resequencing in the field of cancer genetics as well as monogenic diseases, much (>50%) of the heritability for complex (polygenic) chronic inflammatory diseases remains to be discovered. Hence, we have started to resequence the entire genome of a patient with inflammatory bowel disease using the SOLiD system. The patient has not yet responded to any available treatment for the disease and has undergone multiple surgeries. Based on the first genetic findings and subsequent studies, an individual treatment was designed that later proved to be successful. The full experiment with the final sequencing data will be presented at the conference.

1957/F

Re-sequencing of the entire HLA region for informative polymorphism detection and haplotype map construction. *K. Hosomichi, T. Shiina, T. Wang, S. Suzuki, H. Inoko, I. Inoue.* Molecular Life Science, Tokai University School of Medicine, 143 Shiokasuya, Isehara, Kanagawa, Japan.

The human leukocyte antigen (HLA) super-locus on the chromosomal position 6p21 includes six classical HLA genes and at least 132 protein coding genes that play important roles for the regulation of the immune system as well as some other fundamental molecular and cellular processes. This small segment of the human genome associates with more than 100 kinds of diseases including common diseases such as diabetes, rheumatoid arthritis, psoriasis, asthma and various other autoimmune disorders. Furthermore, the HLA region strongly associates with some drug hypersensitivities. Primary aim of the HLA re-sequencing is to provide reference sequences of the most common representative HLA haplotypes in the Japanese and other populations. The 3.8-Mb entire HLA region from *MOG* to *KIFC1* were amplified by using 447 long-range PCR (LR-PCR) primer sets. We performed the LR-PCR reactions using genomic DNA of seven Japanese HLA homozygous cell lines - AKB, TOK and T182 (A24-B52-DR15), HOR (A33-B44-DR13), SA (A24-B7-DR1), LKT3 (A24-B54-DR4) and TAB089 (A2-B46-DR8) representing the highest (8.2%), second highest (5.2%), third highest (3.6%), fourth highest (2.3%), and fifth highest (2.2%) haplotype frequency, respectively, within the Japanese population. The PCR amplified products were sequenced by Genome Analyzer (Illumina). Paired end DNA library preparation and sequencing-by-synthesis methodology followed the manufacturer's instructions (Illumina). Reads were mapped to UCSC hg18 (NCBI Build 36) human reference genome and the chr6 alternate haplotype sequences (PGF, COX) in the Wellcome Trust Sanger Institute MHC Haplotype Project. Average depth of the genome coverage on the seven HLA homozygous cell lines (AKB, TOK, T182, HOR, SA, LKT3 and TAB089) was 178x, 122x, 136x, 64x, 301x, 91x and 220x, respectively, and percentage of the genome coverage was 98.2%, 89.7%, 91.1%, 95.6%, 95.6%, 94.6% and 95.9%, respectively. Data derived from each genome were sorted to lists of sequence variants (SNPs and short indels) in basis on score values as a measure of SNP call accuracy. Identification of the variants relating to common disease susceptibility is critically depended on general informative polymorphism and haplotype map information. Our future aim is to produce these critical data and to develop them publicly available as a general resource for the HLA-linked disease studies.

1958/F

Genotyping and resequencing identified *HLA-DPB1* locus as a susceptibility to non-obstructive azoospermia in Japanese patients. *S. Suzuki, K. Hosomichi, T. Wang, T. Cui, S. Mitsunaga, H. Inoko, T. Shiina, I. Inoue.* Molecular Life Science, Tokai University School of Medicine, 143 Shimokasuya,Isehara, Kanagawa, Japan.

Worldwide, approximately 15% of couples attempting pregnancy meet with failure. Male factors are thought to be responsible in 20-50% of all infertility cases. Azoospermia, the absence of sperm in the ejaculate due to defects in its production or delivery is common in male infertility. In this study, we focused on non-obstructive azoospermia (NOA) because the etiologies of obstructive azoospermia are well studied and distinct from those of NOA. Microdeletions of the Y chromosome are thus far the only genetic defects known to affect human spermatogenesis, but most cases of NOA are unsolved. Thus far, we reported significant associations between NOA and *HLA* (human leukocyte antigen) *-DQB1*0604* with odds ratio 3.58 in a relatively small sample size. In the current study, we performed the HLA genotyping (*HLA-A*, *-C*, *-B* and *-DRB1*, *-DQB1*, *-DPB1*) between 355 NOA patients and 544 male controls. As a result, we identified a strong association with *HLA-DPB1* locus locating 409kb upstream of *DQB1*: the strongest association was observed with *DPB1*0401* (P=5.15x10^{-7}) having the highest odds ratio (OR=2.42). We are uncertain whether *DPB1*0401* is a true causality of NOA or merely a surrogate because of the high linkage equilibrium. Therefore, we further resequence the locus with the next generation sequencer (illumina Genome Analyzer IIx) to identify a variation that could be an authentic causality of NOA. We used genomic DNA extracted from six NOA patients who harbor *HLA-DPB1*0401* as a heterozygote. We specifically amplified the loci spanning *HLA-DQB1* to *KIFC1* (750 kb) using a long PCR method. As a result, we detected a total of 828 variations (42 in exons, 314 in introns, 55 in untranslated regions, and 417 intergenic) from six patients. Among those, there were 117 variations (9 in exons, 39 in introns, 7 in UTR, and 62 in intergenic) that could be on the same haplotype with *DPB1*0401* (minor allele frequency > 30%). Six nonsynonymous substitutions of nine SNPs in exons were detected but all of them were known variations. We also detected 16 novel variations, however, they were not located on exon (one in intron, three in UTR, and 12 in intergenic). In conclusion we identified *HLA-DPB1*0401* as a susceptibility allele to NOA that may play a decisive role in the pathogenesis of NOA. Further replication study in different populations for confirmation is needed.

1959/F

ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *K. Wang[1], M. Li[2], H. Hakonarson[1,3].* 1) Center Applied Genomics, Children's Hosp Philadelphia, Philadelphia, PA 19104, USA; 2) Department of Biostatistics and Epidemiology, University of Pennsylvania, Philadelphia, PA 19104, USA; 3) Department of Pediatrics, University of Pennsylvania, Philadelphia, PA 19104, USA.

High-throughput sequencing platforms are generating massive amounts of genetic variation data for diverse genomes , but it remains a challenge to pinpoint a small subset of functionally important variants. To fill these unmet needs, we developed the ANNOVAR tool to annotate single nucleotide variants (SNVs) and insertions/deletions, such as examining their functional consequence on genes, inferring cytogenetic bands, reporting functional importance scores such as SIFT scores, finding variants in conserved regions, or identifying variants reported in the 1000 Genomes Project and dbSNP. ANNOVAR can utilize annotation databases from the UCSC Genome Browser or any annotation dataset conforming to Generic Feature Format version 3 (GFF3). We also illustrate a "variants reduction" protocol on 4.7 million SNPs and indels from a human genome, including two causal mutations for Miller syndrome, a rare recessive disease. Through a stepwise procedure, we excluded variants that are unlikely to be causal, and identified 20 candidate genes including the causal gene. Using a desktop computer, ANNOVAR requires ~4 minutes to perform gene-based annotation and ~15 minutes to perform variants reduction on 4.7 million variants, making it practical to handle hundreds of human genomes in a day. ANNOVAR is freely available at http://www.openbioinformatics.org/annovar/.

1960/F

Exome resequencing in a densely affected multigenerational SLE pedigree. *G. Wiley[1], C.P. Lin[1], I. Adrianto[1], J. Kelly[1], K.L. Moser[1], K.K. Kaufman[1,2,3], J.B. Harley[2,3], C. Gray-McGuire[1], P.M. Gaffney[1].* 1) Oklahoma Medical Research Foundation, Oklahoma City, OK; 2) The University of Oklahoma Health Sciences Center, Oklahoma City, OK; 3) Oklahoma City VA Medical Center, Oklahoma City, OK.

Systemic lupus erythematosus (SLE) is a complex autoimmune disease that has heretofore been the subject of intense genetic scrutiny. While genome-wide association studies in SLE have successfully identified approximately 30 new risk loci the low odds ratios of associated loci leave a substantial portion of the estimated heritability of SLE unexplained. By comparison, the high odds ratios of highly penetrant rare polymorphisms (e.g. *TREX1*, OR=25) suggest the possibility that some of the missing heritability of SLE may exist in the form of rare variants. To begin to explore the role of rare variants in SLE susceptibility we resequenced the exome in a unique, densely affected multigenerational SLE pedigree with 7 affected individuals. A densely affected pedigree was specifically chosen with the assumption that rare variations conferring higher SLE risk are more likely to be represented within such a family as opposed to the general population. The entire exonic region for the individuals within this family were captured through the use of the Agilent SureSelect sequence capture system and sequenced to 15x coverage using an Illumina GAIIx second-generation sequencer. Assembly of sequence to reference, variant calling, and other analysis were carried out using the CLC Genomics Workbench bioinformatics suite. Variants were verified with Illumina Omni1 Quad genotyping assays. Identified variants were screened using effect on protein function as determined by the SIFT algorithm. Over 1700 coding variations were identified as shared between all case subjects within the family with approximately 40% encoding a nonsynonymous amino acid change. Of these amino acid changes approximately 7% are predicted to be damaging to the encoded protein. We have identified several nonsynonymous changes in proteins previously associated with SLE (*TNIP1*) as well as in loci not previously implicated in SLE including *NFKBIL1* and *NOTCH1*.

1961/F

Candidate Gene Identification for Distinct Mendelian Disorders Using Exome Resequencing. *V. Schaibley[1], W. Peng[1], J. Xu[1], J. Bedoyan[2], C. Keegan[1,2], D. Martin[1,2], J. Li[1].* 1) Department of Human Genetics, University of Michigan, Ann Arbor, MI; 2) Department of Pediatrics, University of Michigan, Ann Arbor, MI.

The Online Inheritance in Man database describes the phenotype for thousands of Mendelian disorders. However about 60% of the diseases with established loci do not have known causative genes. If the disease is sufficiently homogeneous and if causative variants are of high enough penetrance, whole-exome sequencing can be an effective strategy for candidate gene identification. Several recent studies have demonstrated the ability of whole-exome sequencing to identify genes for both autosomal dominant and recessive Mendelian disorders. We applied whole-exome capture (Agilent SureSelect) followed by next-generation sequencing (Illumina GAII) to study two Mendelian disorders in which the underlying genetic cause is unknown. The two disorders include a rare Mendelian disorder characterized by congenital sensorineural hearing loss and mental retardation and another where affected individuals exhibit a well-defined constellation of severe birth defects. These disorders not only represent a diverse spectrum of phenotypes, they also show distinct transmission patterns, specifically X-linked recessive and *de novo* autosomal dominant. We generated high quality exome sequence data of >20X coverage for 7 individuals: 1 with the hearing loss and mental retardation syndrome and 6 with the severe birth defect complex. To analyze the novel variants found in each of these diseases we applied a combination of bioinformatic filters, incorporating previous information on haplotype sharing or regions of homozygosity, functional annotation, and candidate gene sharing between multiple unrelated affected individuals. We were able to narrow the number of candidate genes for each disorder from thousands to a small set, highlighting several candidate genes for future in-depth investigation. While each condition brought different challenges in data analysis, interpretation, and functional follow-up, our experience demonstrates the feasibility of using high-throughput whole-exome sequencing to accelerate gene discovery for a wide array of Mendelian disorders.

1962/F

Application of exome resequencing with targeted analysis for autosomal dominant disorders with positional information. *L. Huang[1], L. Racacho[2], L. McDonell[1], S. Douglas[3], D.E. Bulman[2], K.M. Bycott[1].* 1) Children's Hospital of Eastern Ontario Research Institute,Ottawa, ON, Canada; 2) Ottawa Hospital Research Institute, Ottawa, ON, Canada; 3) Faculty of Medicine, University of Toronto, Toronto, ON, Canada.

Next-generation sequencing technologies have dramatically accelerated the sequencing of genes that have been implicated in human disease. Two strategies can be employed for DNA enrichment prior to sequencing: capture of a specific region (contiguous or gene-specific); or capture of the human exome with analysis limited to the region(s) of interest. The latter strategy has the benefit of significant cost savings but the potential disadvantage of less coverage. For example, Agilent's SureSelect Human All Exon Kit covers the genes in the NCBI Consensus CDS Database but not hypothetical genes. To evaluate this further we used exome resequencing to identify variants in two patients from two families with autosomal-dominant disorders that have prior positional information based on linkage analysis. A novel locus for autosomal dominant hereditary spastic paraparesis was mapped to a 2.4 Mb region on chromosome 3 in a large Canadian family. The critical region contains 16 genes, 9 of which are covered by the Agilent's SureSelect Human All Exome Kit. The captured whole exome was sequenced using an Illumina GAII sequencing system with a 72bp read length. Twenty-four of 31 million reads were of good quality and were aligned to the human reference sequence (NextGENe). The average sequence coverage was 7x. Four unreported single nucleotide alterations within the coding sequence of four genes in the critical region were identified. Confirmation of these changes using Sanger sequencing is underway. In a smaller family with autosomal dominant spinocerebellar ataxia, three candidate regions of 3.6, 1.9 and 1.2 Mb in length were identified on chromosomes 10, 11, and 19, respectively. The Agilent's SureSelect human all exome kit covers 23/29, 17/22, and 36/40 genes in the three regions, respectively. Twenty-six of 33 million reads were of good quality and were aligned to the reference sequence. The average sequence coverage was 11x. Fourteen unreported single nucleotide alterations within the coding regions of 12 genes were identified. Confirmation of these changes using Sanger sequencing is underway. Our studies demonstrate that exome sequencing coupled with targeted analysis may be an efficient and cost-effective way to quickly identify mutations associated with human disease.

1963/F

Accurate Variant Detection within Targeted Genomic Regions across Unamplified and Whole Genome Amplified HapMap Families using MicroDroplet-based PCR and SOLiD Sequencing. *J.B. Warner[1], A. ELSharawy[2], J. Brayer[1], M. Forster[2], P. Rosenstiel[2], S. Schreiber[2], A. Franke[2], J. Olson[1], D. Link[1].* 1) RainDance Technologies, Lexington, MA; 2) Institute of Clinical Molecular Biology (ICMB), Christian-Albrechts-University of Kiel, Germany.

Many hypothesis driven studies such as Genome Wide Association Studies or Candidate Gene Studies require the ability to efficiently target specific regions of the genome to detect sequence variations across a population of samples. With the increased sequence capacities offered by today's 'Next Generation' sequencing platforms, investigators are now able to generate massive amounts of sequence data. The ability to target specific regions of the genome allows researchers to screen larger numbers of samples against regions relevant to their research. In this study we will present the use of RainDance's microdroplet-based PCR and ABI SOLiD sequencing to specifically target 383 exons. This targeted approach leverages the sensitivity and specificity of the Polymerase Chain Reaction (PCR) to efficiently capture and represent the sequence context from these regions.

There are several advantages with this approach to targeted resequencing. The PCR based approach allows a high degree of stringency and flexibility to design to the targets of interest. Also, the sequence coverage is uniform across all of the amplicons allowing for efficient use of sequencing capacity. This allows for sample indexing strategies, reducing the sequencing cost per sample.

In this study we evaluated six HapMap samples from two family trios from the 1000 genomes project. One of the trios was also evaluated after whole genome amplification (WGA). All samples were evaluated with several barcoding strategies at different stages of the ABI SOLiD workflow. Genotyping data for all samples were compared back to their reference genotypes and across the different sample treatments to determine the concordance of the variant detection. Family structure was also evaluated to confirm Mendelian patterns of inheritance within each family trio. Each sample was barcoded and sequenced under the following conditions: 1) one sample per octet, 2) samples were pooled pre-emulsion PCR (emPCR) and 3) samples were pooled post-emPCR.

SNP detection was performed for each sample and compared with the reference genotypes for each HapMap sample. The average genotype concordance maintained across all of the samples was 99.5%. We also did not detect any errors in the Mendelian patterns of inheritance of genotypes between the parents and offspring within each trio.

1964/F

Performance Comparison of Three Commercial Softwares for Analysis of Illumina Genome Analyzer Next Generation Resequencing Data. *J. Durtschi[1], P. Ridge[1], S. Dames[1], W.L. Wooderchak[1], P. Bayrak-Toydemir[1,2], K.V. Voelkerding[1,2].* 1) 1ARUP Institute for Clinical & Experimental Pathology, Salt Lake City, UT; 2) Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT.

Background A key bioinformatic requirement for NGS sequencing is accurate analysis software. The current study evaluated the performance of three commercial software packages that have features, flexibility, ease of use, and customer support attractive for clinical application. **Methods** Variant calling ability in exons and processing speed at a range of software settings was compared for DNAStar Seqman NGen, SoftGenetics NextGene, and CLCBio Genetic Workbench. Illumina Genome Analyzer data sets included; 1) 76 base, single end read data from 9 genes of a Long Range-PCR enriched hypertrophic cardiomyopathy (HCM) panel; 2) 76 base, single end read data from the same HMC genes enriched on a Febit capture chip and; 3) 36 base, pair end read data from an Agilent SureSelect X chromosome capture enrichment. Software parameters were adjusted for each software to improve performance. Some targeted regions were Sanger sequenced providing variant confirmation. **Results** The LR-PCR data set generated average coverages of over 1000. The high coverage and enrichment specificity of the LR-PCR data yielded perfect exon variant calling in the three softwares using typical variant parameters of 25 read percentage and minimum 20 coverage (in addition to software specific limits). The Febit data included problematic co-capture leading to 12, 14 and 14 Sanger disputed exon false positives in *ACTC1* from DNAStar, CLCBio and SoftGenetics, respectively. Alignment to the whole human genome, currently relatively easy with CLCBio and SoftGenetics, greatly reduced this problem. Other exon regions in the Febit data yielded 2, 3 and 3 incorrect calls from DNAStar, CLCBio SoftGenetics, respectively in low complexity exon regions of *MYBPC3* and *TPM1*. X chromosome data was Sanger sequenced only in a cross-homologous gene, *ABCD1* indicating one true variant in exon 6 that was identified by all softwares. Each software also identified more than 10 false positive exonic variants in this region. **Conclusions** Our ongoing comparison of these three softwares with clinical target data shows that each performs comparably with respect to variant calls. Each also has specific advantages including processing and display features. The differences in alignment and variant detection methods between softwares cause some marginal differences in accuracy of difficult variant calls, specifically low coverage, low complexity and high homology regions.

1965/F

Establishment of two different targeted re-sequencing workflows and a benchmark of whole-exome approaches. *A. ElSharawy, M. Forster, B. Stade, M. Schilhabel, I. Thomsen, P. Rosenstiel, S. Schreiber, A. Franke.* Institute of Clinical Molecular Biology, Christian-Albrechts-University, Kiel, Germany.

Next generation sequencing (NGS) has revolutionized throughput and cost per base call, but bottlenecks exist at the front-end, especially the targeted sequencing of specific genomic regions in large numbers of samples. Until the whole-genome re-sequencing of large numbers of individuals becomes feasible, targeted re-sequencing of both whole-exomes and a complete set of specific genomic loci present the next logic attractive options. Here we established two different targeted next-generation re-sequencing workflows on the SOLiD NGS system, using microarray-based and PCR-based enrichment technologies. In both protocols, we employed molecular bar-coding and pooling strategies to increase throughput, reduce cost per base, and make the identification of variant carriers more efficient. In the microarray-based workflow, we analyzed the data with different software tools, benchmarking against the known HapMap, dbSNP, and whole genotypes derived from re-sequencing an entire genome. The results showed high enrichment key measures and that SNP discovery depended strongly on chosen tools, suggesting a combination of target region matching, SNP backmapping and a final manual inspection as a convenient approach to attain more confident results. In the PCR-based workflow, we evaluated the performance of different pooling schemes on whole-genome amplified and un-amplified genomic DNA HapMap samples. The results of the latter approach showed efficient parallel detection of both homozygous and heterozygous SNPs in all tested samples. Given the fact that most of the known relevant somatic mutations that deliver the development of cancer in human are populated at protein-coding regions (and given that around 90% of all monogenic diseases derive from mutations in the coding sequence or splice sites), we also evaluated the performance of leading whole-exome re-sequencing approaches on different NGS technology platforms. We evaluated many enrichment measures as well as SNP concordance and overlap between different technologies using the generated re-sequencing data in addition to that of whole genome sequencing and dbSNP. At the conference, we will present a state-of-the-art-summary of our benchmarks and the targeted enrichment field.

1966/F

In-Run QC for Paired-End Sequencing with the Illumina GAIIx. *K.N. Hetrick II, B.D. Craig, M.W. Barnhart, S.M.L. Griffith, B.A. Marosy, K.F. Doheny.* CIDR, Johns Hopkins Univ, Baltimore, MD.

The Center for Inherited Disease Research (CIDR) provides high quality genotyping services and statistical genetics consultation to investigators working to discover genes that contribute to common disease. CIDR is currently evaluating the Illumina® GAIIx sequencer and Agilent© SureSelect™ enrichment technology for use in providing our investigators with a next-generation sequencing service. We initially relied on Illumina's real-time analysis (RTA) reports, CASAVA pipeline post-run reports as well as our own post-run analyses in order to determine sequence data quality, enrichment efficiency, and sample-prep library complexity which, for a paired-end (PE) run, could take 10 to 14 days from the start of the sequencing run. In order to cut this time in half, we have designed an in-run QC analysis workflow that uses only the first read of a PE sequencing run, treating it as a single-end (SE) run. Using Bowtie, this workflow aligns the first read to a reference genome and produces a SAM/BAM output file. The Bowtie output allows us to estimate the percentage of reads mapped to a reference genome, the proportion of map-able bases that aligned to the targeted enrichment regions, sequence depth coverage summary statistics and library complexity for each individual experiment. When compared to our PE final results for 24 samples using a custom Agilent Sure-Select bait pool targeting 3.5 Mb (using BWA as the aligner for the PE run); the mean difference between the percent of mapped reads for Bowtie and BWA was 0.8%, the Bowtie SE analysis flagged a mean of 3.4 times more reads as molecular duplicates per experiment than BWA (BWA mean 10%, bowtie mean 32%), a mean difference of 6.7% of map-able bases aligning to target (BWA mean 65.5%, Bowtie mean 58.8%) and a 1.7% mean difference of targeted bases with 10x or greater coverage. This in-run QC analysis process can save CIDR half the instrument time used by alerting us to catastrophic target enrichment failures so that we can cancel the sequencing run. This process also enables us to flag potentially sub-optimal experiments during the sequencing run allowing us to reprioritize samples that need to be redone in subsequent experiments. This method of in-run QC analysis workflow has been automated in the CIDRSeqSuite (refer to Barnhart, et al.) which can allow us to analyze the experiments in a few hours and determine if we should continue on with the sequencing run.

1967/F

Custom Design of Library Target Selection for Next Generation Sequencing. *B. Marosy[1], N. Pankratz[2], B. Craig[1], K. Hetrick[1], M. Barnhart[1], S. Griffith[1], D. Snyder[1], J. Romm[1], T. Foroud[2], KF. Doheny[1].* 1) CIDR/IGM, JHU-SOM, Baltimore, MD, USA; 2) Dept Medical and Molecular Genetics, IU-SOM, Indianapolis, IN, USA.

The Center for Inherited Disease Research (CIDR) provides high quality genotyping services and statistical genetics consultation to investigators working to discover genes that contribute to common disease. CIDR has begun to evaluate Next Generation Sequencing in conjunction with targeted library selection as a future service. Genome Wide Association and linkage studies that identify a chromosomal region may require additional follow-up sequencing in order to find one or more causal variants that contribute to the genetic trait. The Agilent© SureSelect™ Target Enrichment method was used to capture specific regions of interest and was followed by paired end sequencing using the Illumina® GAIIx. Baits were custom designed to capture genomic features within a previously identified 19Mb linkage peak. Using the UCSC genome browser genes, hESTs, conserved regions, sno/miRNAs and predicted first exon/promoter regions were identified for inclusion in the target design. GALAXY was used to import tracks/bed files from UCSC and provided tools to extract exons, add flanks, merge or subtract regions, and provide base coverage information. In the final design, 150bp flanks were added to each exon and 3kb/2kb flanks were added upstream/downstream to each gene. Secondary regions containing additional known causative genes were also included in the design. Regions containing CIDR's SNP barcode panel were added to confirm sample identity and provide QC to identify potential problems, such as contamination. These regions were then submitted in two passes to the Agilent eArray website, which provided additional parameters such as tiling and repeat masking for bait design. The first pass included 2x justified tiling with a 20bp 'avoid overlap' and masking using Repeat Masker. After the first pass any portion of the targeted regions not covered by baits was then resubmitted in a 2nd pass in an attempt to provide complete coverage across the regions of interest. The 2nd pass included 2x centered tiling with a 40bp 'avoid overlap' and masking using Window Masker. By resubmitting these 'missed' regions, 4% of exonic bases were recovered with 7% of exonic bases not covered in the final design. A total of 57,680 baits were designed covering 3.46Mb. Subsequent sequencing data shows an average capture specificity of 66.5% of all regions and 85% for regions targeted within the 19Mb linkage peak. At 1X and >10X depth, >99% and >98% of the targeted bases are covered, respectively.

1968/F

DNA capture and massively parallel sequencing to identify novel alleles for recessive non-syndromic hearing loss. *H. Shahin[1], T. Walsh[2], M.K. Lee[2], A.M. Thornton[2], A. Abu Rayyan[1], S. Loulus[1], A.S. Nord[2], K.B. Avraham[3], M-C. King[2], M. Kanaan[1].* 1) Dept of Life Sciences, Bethlehem University, Bethlehem, Palestine; 2) Depts of Medicine and Genome Sciences, University of Washington, Seattle. WA; 3) Dept of Human Molecular Genetics and Biochemistry, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

In studies of genetics of deafness, families with inherited hearing loss have been extremely valuable, providing paths to discovery of genes essential to mechanisms of hearing. Until now, such studies have depended on Sanger sequencing of genes in candidate genomic linkage regions. Constraints of PCR-based sequencing have largely limited gene discovery to conventional mutations in known genes in relatively small regions of linkage. The advent of DNA enrichment techniques coupled with next generation sequencing technology has the potential to dramatically accelerate the pace of gene discovery. We have applied in-solution DNA capture and massively parallel sequencing to identify the causative alleles from two large Palestinian families that map to recessive non syndromic deafness loci. We designed cRNA oligonucleotides to capture all RefSeq and CCDS annotated exons from the DFNB83 locus: 45MB on chromosome 9, and the DFNB85 locus: 11MB on chromosome 17. We captured 1823 exons from germline DNA of an affected individual from the DFNB83 family and 1372 exons from an affected individual of the DFNB85 family. Each captured library was sequenced on an Illumina Genome Analyzer IIx to a >100x depth. We filtered DNA variants against publicly available databases and categorized the remaining rare and private variants for predicted severity on gene and protein function. We did not observe any rare truncating alleles in the captured exons. We are currently evaluating conserved missense mutations, intronic variants distant from known exons that may alter splicing and read depth data for copy number variants such as deletions and duplications.

1969/F

Buyer beware: limitations of whole-exome sequencing capture methods and the search for rare, Mendelian variants. *K.B. Jacobs[1,2], M. Yeager[1,2], M.G. Cullen[1,2], X. Zhang[1,2], J. Boland[1,2], J. Bacior[1,2], V. Lonsberry[1,2], C. Matthews[1,2], D. Roberson[1,2], Q. Chen[1,2], L. Burdett[1,2], I. Menashe[2], X.R. Yang[2], L.R. Goldin[2], M.L. McMaster[2], N.E. Caporaso[2], P.R. Taylor[2], M.T Landi[2], J. Sampson[2], N. Chatterjee[2], M.L. Nickerson[3], K. McGee[3], M. Dean[3], J. Khan[4], M.A. Tucker[2], S.J. Chanock[2], A.M. Goldstein[2].* 1) Core Genotyping Facility, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD 21702, USA; 2) Division of Cancer Epidemiology and Genetics, NCI, NIH, Bethesda, MD 20892, USA; 3) Laboratory of Experimental Immunology, CCR, NCI, NIH, Bethesda, MD 20892, USA; 4) Oncogenomics Section, Pediatric Oncology Branch, CCR, NCI, NIH, Bethesda, MD 20892, USA.

New technologies such as 'whole'-exome sequencing capture methods have led to renewed excitement about discovering rare, Mendelian, high-risk susceptibility gene variants in humans. To assess the currently available sequence capture approaches with respect to coverage across the exome, we evaluated the content and the empirical performance of the currently-available 'whole'-exome sequence capture methods [NimbleGen Sequence Capture 2.1M Human Exome Array; Agilent SureSelect Human All Exon Kit] on three sequencing platforms [454 FLX Ti (4 runs); ABI SOLiD (1 quadrant); Illumina GA II (2 lanes)]. The protein coding sequences (CDS) reported in the RefSeq database (build 36.3) served as the gold standard for our assessment. NimbleGen capture probes target 77% of CDS bases and Agilent capture probes target 83% of CDS bases. We observed 21.5 Mbps (65%), 25.0 Mbps (76%) and 23.4 Mbps (71%) of the 33.0 Mbps of CDS with ≥8x sequence depth for Nimblegen/454, Agilent/SOLiD, and Agilent/Illumina, respectively. Since identification of rare gene variants requires high per-gene coverage, we also computed the proportion of genes with >90% of CDS bases covered with ≥8X sequence depth. Only 42%, 55%, and 45% of genes were covered by Nimblegen/454, Agilent/SOLiD, and Agilent/Illumina, respectively. Since coverage across the gene CDS is incomplete, failure to identify a causal mutation should be interpreted cautiously. It is notable that many characterized genes are not included in this first generation whole-exome capture products and that only 40-50% of genes are well covered across all known exons with the amount of sequencing performed. Our results underscore the limitations of current 'whole'-exome capture products and have important implications for designing studies in families and unrelated populations. In spite of the limitations, these products may still be useful to screen for disease variants within exons that are satisfactorily covered. The design of future 'whole'-exome capture products will likely be more expansive and performance will likely improve, however careful attention must be paid to coverage in practice. Funded by NCI Contract No. HHSN261200800001E.

1970/F

Analysis of GBA mutations in a French-Canadian Parkinson's disease cohort. *A. Noreau[1], J.-B. Riviere[1], S. Diab[1], P. Dion[1], S. Chouinard[1], M. Panisset[1], N. Dupre[2], G.A. Rouleau[1,3].* 1) The Centre of Excellence in Neuromics, CHUM Research Center and the Department of Medicine, University of Montreal, Montreal, QC, Canada; 2) Faculty of Medicine, Laval University , Department of Neurological Sciences , CHA - Enfant-Jésus, Quebec City, Qc, Canada; 3) Sainte Justine Hospital, Montreal, Qc, Canada.

Background: Gaucher disease is a rare mendelian disorder characterized by accumulation of the lysosomal enzyme glucocerebrosidase (GBA), mainly cause by mutations in the GBA gene. Pedigree analysis revealed an increase incidence of Parkinson's disease (PD) in relatives of patient's with Gaucher disease. Recently, a multicenter analysis of the Glucocerebrosidase gene (GBA) reported that a higher frequency of missenses mutations could be observed PD patients by comparison to healthy controls individuals. Moreover this result was even more significant when making a separate GBA mutation rate examination of the Jewish descent individuals within the cohort; this subgroup presented a 15 % mutation for two specific missenses (L144P and N370S) which is five times higher than what can be observed in Jewish-healthy controls. In that study, they also present an increased rate of mutations in the non-jewish population, less impressive but significant as 3% in PD patients match up to less than 1% in controls. The main goal of our study was to evaluate the possible involvement of the GBA mutations in a french-canadian PD cohort. **Methods**: We completely resequenced the GBA gene in a cohort of 213 French-Canadian patients with a clear PD diagnosis and 190 healthy-age-match French-Canadian controls. PCR and sequencing primers were designed using the chromosome 1 genomic contig sequence (NT_029419) enabling PCR of all 11 coding exons. Primers were designed enabling preferential amplification of GBA over the GBA pseudogene, located on the same chromosome, 16 kb upstream. **Outcomes**: The resequencing success rate reach more than 99 % and we observe an increased amount of GBA mutations in Parkinson's patients. Heterozygotes mutations was found in 10,8 % of the Parkinson's patients compare to 5,8 % in ethnically-healthy controls. This study provides evidence that GBA is probably involved in PD in the French-Canadian population.

1971/F

Development of new HLA-B*3505 genotyping method using Invader assay. *N. Hosono[1], S. Chantarangsu[1], K. Kiyotani[1], S. Takata[1], Y. Tsuchiya[1], S. Mahasirimongkol[2], W. Chantratita[3], T. Mushiroda[1], N. Kamatani[1], Y. Nakamura[4], M. Kubo[1].* 1) Center for Genomic Medicine, Riken, Japan; 2) Center of International Cooperation, Minstry of Public Health, Thailand; 3) Ramathibodi Hospital, Mahidol University, Thailand; 4) Human genome Center, University of Tokyo, Japan.

OBJECTIVE: Several pharmacogenetic studies have revealed strong genetic associations between specific human leukocyte antigen (HLA) alleles and the susceptibility to drug hypersensitivity. Recently, we reported HLA-B*3505 as a strong genetic biomarker for the prediction of the nevirapine (NVP)-induced skin rash in a Thai population. Due to its high specificity and positive predictive value, HLA-B*3505 genetic test will facilitate pharmacogenetic study to avoid a subset of the NVP-induced skin rash. METHODS: We developed a new HLA-B*3505 genotyping method by a combination of the Universal Invader assay and sequence-specific primer PCR method. We performed the assay using an ABI-7500 system, and total reaction time was 50 minutes. We tested the assay using 324 Thai individuals and the results were compared with the data obtained by the sequence-based typing method. RESULTS: From the sequence alignment of HLA Class I alleles detected in the Thai population, we selected the most discriminative SNP (rs1140412) as a target SNP for the Invader reaction and rs4997052 for a sequence-specific reverse primer site. When we performed the assay, the fluorescence intensities of HLA-B*3505-positive samples rapidly increased. Meanwhile, the intensities of HLA-B*3505-negative samples were apparently low. At the endpoint of the reaction, our assay could easily determine the presence or absence of HLA-B*3505. Our assay detected HLA-B*3505 for 100% concordance with the results obtained by a sequence-based typing method. CONCLUSION: Our assay is simple, rapid, and closed-tube homogeneous format. We believe our method will be a useful tool for pharmacogenetic testing of the NVP-induced skin rash in Thailand.

1972/F

A new sequencing primer and workflow increase 5' resolution and throughput on HLA sequencing. *P. Ma[1], SC. Hung[1], S. Berosik[1], M. Wenz[1], S. Schneider[1], A. Chhibber[3], T. Agostini[2], D. Berchanskiy[2], D. Dinauer[2].* 1) Genetic Systems, Life Technologies, Foster City, CA; 2) Applied Market, Life Technologies, Brown Deer, WI; 3) Dept of Bioengineering and Therapeutic Sciences, UCSF, San Francisco, CA.

High quality and high accuracy are the hallmarks of Sanger re-sequencing projects. We have developed a new sequencing primer and workflow that improves 5' sequence resolution, increases throughput, and reduces hands-on time. The novel sequencing primer chemistry produces high quality bases from base 1 on POP-7™ that previously only could be resolved on the slower POP-6™ polymer. The new primer chemistry and workflow also eliminates the need for a separate PCR clean-up step. These improvements reduce the entire workflow from PCR to finished sequence data to under 5 hours, compared to 8 hours for the standard workflow. We used our enhanced sequencing primer and workflow to investigate Human Leukocyte Antigen (HLA) polymorphisms on twelve DNA samples by using the Invitrogen SeCore® HLA-DRB1 primer set and Group Specific Sequencing Primers. Sequencing reactions generated with the traditional sequencing primer and with the new sequencing primer were electrophoresed on Applied Biosystems 3500xl™ Genetic Analyzer using POP-7™. For each sequencing primer, we compared 5' resolution and basecalling accuracy and quality. On average the traditional primers produced high quality readable bases by base 25 after the sequencing primer while the new primers produced high quality bases by base 5, and by base 1 in many cases. Because of improved resolution, basecalling accuracy was increased. This simplified process without a separate PCR clean-up step reduced the overall workflow time by 40%. For HLA genes, obtaining readable sequence within 5 bases of the primer offers improved polymorphism detection and more efficient use of allele specific sequencing primers for heterozygous ambiguity resolution. In conclusion, the novel primer chemistry and workflow generates data superior in quality relative to other currently used solutions and offers significant time savings as well.

1973/F

Multi-sample pooling and Illumina Genome Analyzer sequencing to determine gene sequence variation for database development. *R.L. Margraf[1], J.D. Durtschi[1], S. Dames[1], D.C. Pattison[1], J.E. Stephens[1], K.V. Voelkerding[1,2].* 1) ARUP Institute for Clinical & Experimental Pathology, Salt Lake City, Utah; 2) Department of Pathology, University of Utah School of Medicine, Salt Lake City, Utah.

Multi-sample pooling and Illumina Genome Analyzer (GA) sequencing is a cost effective way to sequence many samples to determine sequence variation within a population. Determination of sequence variation within a genetic locus is essential for developing clinically relevant databases, which are critical for molecular assay design and clinical test interpretation. In this study, multi-sample pooling combined with Illumina GA sequencing was investigated using the *RET* proto-oncogene as a model. All samples were also Sanger sequenced for *RET* exons 10, 11, and 13-16. Based on a preliminary experiment where a 10 sample pool was sequenced on the Illumina GAII using 36 base reads; we predicted £30 samples (or a minimum 1% variant reads for singletons) could be pooled to reliably detect singleton variants by Illumina GA sequencing without needing additional confirmation testing. A second experiment using a 30 sample pool and 50 sample pool was performed to test the predicted pooling limit and also to test the recent Illumina GAIIx upgrades and 76 base length reads. The automatable SequalPrep™ method was used to normalize PCR products before pooling. For comparison, a single 'control' sample was run in a different flow cell lane. Data was evaluated using an established variant detection method (% variant reads) and a new subtractive correction method utilizing the control sample. In total, 59 variants were detected in the pooled samples, which included all the Sanger identified variants. Both the 30 and 50 pool had 17 known singleton variants due to Sanger sequencing. These 17 singleton variants in the 30 pool had 1.70±0.53% average read frequency (expected 1.67% allele frequency), while these same variants within the 50 pool had 1.13±0.23% average frequency (expected 1%). A total of 12 novel intronic variants (not in NCBI dbSNP) were detected within all the Illumina GA sequenced samples and four of the novel variants were within 200bp of the exons, at potential areas of primer design. These data sets confirmed the predicted maximum 30 pool limit for reliable singleton variant detection (all singleton variants were above 1.3% read frequency), since some singleton variants in the 50 pool would be difficult to distinguish from background sequencing errors if the singleton variants were unknown. These pooling protocols and data analysis can be used for other genes for development of locus-specific databases and to facilitate molecular diagnostic test design.

1974/F

Mutation screening of 95 patients with bilateral sensorineural hearing loss using HybSelect and massively parallel sequencing - experience of a clinical diagnostic laboratory. *A. Santani[1], X. Gai[2], M. D'Arcy[2], M. Grauer[2], A. Caruso[3], H. Wu[3], T. Tischler[1], A. Keller[4], V. Boisguerin[4], L. Francey[5], D. Clark[5], I. Krantz[5], C. Stolle[1].* 1) Dept of Path & Lab Med, The Children's Hospital of Philadelphia, Philadelphia, PA; 2) Bioinformatics Core Facility, The Children's Hospital of Philadelphia, PA; 3) febit Inc, Lexington, MA; 4) febit Biomed gmbh, Heidelberg, Germany; 5) Dept of Genetics, The Children's Hospital of Philadelphia, PA.

Hearing loss is the most common birth defect, affecting 1 of every 500 newborns with bilateral sensorineural hearing loss (BLSHNL). More than 50% of affected individuals have a genetic etiology. Over a decade of research in this field has revealed extensive genetic heterogeneity, with more than 40 genes known to date. The universal newborn hearing screening program, together with notable progress in the elucidation of the genetic basis of hearing loss, have resulted in an increased demand for detecting the molecular basis of hereditary hearing loss through DNA testing. Diagnostic testing for BLSHNL is offered as a panel of tests in different centers around the world including the molecular genetics diagnostic lab at CHOP. However, diagnostic testing can be time consuming and expensive. Targeted capture and resequencing of multiple genes can significantly reduce the time and expense to that comparable for a single gene disorder. Here we report the use of a multiplex approach to simultaneously capture and resequence 95 patients with BLSHNL using the microfluidic Geniom biochips approach. Of the 95 patients, 13 patients had a confirmed molecular diagnosis. We enriched and then sequenced 138.8 kb of the human genome encompassing the coding regions of 35 genes implicated in BLSHNL. DNA samples were captured first with HybSelect technology and then sequenced using the SOLiD platform, in either 1-plex, 4-plex, or 8-plex. We captured on average 92.6% (range:69.2%-98.6%) of the targeted regions at an average coverage depth of 13.5X (range: 2.1X-44.6X). We assessed this method's performance in detecting homozygous and heterozygous variants and found 75% concordance with previously reported data. Of the 1545 non- synonymous SNPs or indels, ~196 variants were unique. To date, potentially disease causing mutations were identified and confirmed by Sanger sequencing in 20 previously undiagnosed patients. 4 nonsense mutations and 19 missense variants were identified. 19 mutations were previously reported in the Human Genome Mutation Database & 4 were novel variants. Although the multiplex capture was highly specific, we found that a key issue was non-uniform capture that will require additional optimization to be suited to the performance requirements of a clinical molecular laboratory. Nonetheless, our results demonstrate the utility of this multiplexed capture approach for efficient and cost effective screening of multiple genes implicated in disease pathways.

1975/F

Phenotype driven next generation sequencing of X-linked intellectual disability genes. *E. Chin[1], C. Alexander[1], J. Sun[1], A. Tanner[1], L. Tribble[2], M. Friez[2], M. Hegde[1].* 1) Dept Human Gen, Emory University, Atlanta, GA; 2) Greenwood Genetic Center, Greenwood, SC.

X-linked intellectual disability (XLID) is a common, clinically complex and genetically heterogeneous collection of conditions arising from mutations in genes on the X chromosome. It affects approximately 1/1000 males with females affected to a much lesser degree. Research has identified >100 different XLID genes, affecting a wide range of cellular processes. Many X-linked genes remain undiscovered, especially for non-syndromic forms of XLID. Approximately 11%; of genes located on the X chromosome are implicated in XLID; most, however, contribute individually to only <0.1%; of the total landscape. In recent years, progress has been made in delineating the clinical features associated with some forms of XLID, yet it remains virtually impossible to accurately predict the causative gene in non-syndromic cases that predominate in this group of disorders. For most genes very few clinical cases have been reported so the precise clinical phenotype is not clear. We have chosen 93 genes with a clearly established association with XLID to be a part of a next generation resequencing panel. We have validated the use of highly multiplexed PCR by simultaneously resequencing all 93 XLID genes using 24 positive controls. Highly multiplexed PCR using a customized RainDance Technology primer library designed to capture the genes of interest was used to enrich the target genes. We describe here for the first time evaluation of the highly multiplexed PCR technology in a clinical laboratory setting using XLID as a model. A web-based tool is available for users to input clinical information which allows a more refined selection of genes in smaller panels of 30 or 60 genes. This web-based tool will also allow further characterization of the phenotype associated with mutations in these genes. The combination of highly multiplexed PCR and next generation sequencing allows high resolution analysis capable of detecting coding and non-coding point mutations as well as deletions and duplications. The Collaboration Education and Test Translation (CETT) Program has provided support for this project which also includes development of educational materials to assist clinicians in utilizing testing for this large set of genes.

1976/F

Leber's hereditary optic neuropathy associated with Multiple Sclerosis like picture in a man. *R. Cittadella[1], V. Andreoli[1], P. Valentino[2], F. Trecroci[1], M. Caracciolo[1], O. Gallo[1], G. Di Palma[1], A. La Russa[1].* 1) Inst Neurological Sci, CNR, Cosenza, Italy; 2) Institute of Neurology, University "Magna Graecia", Catanzaro, Italy.

Purpose: Leber's hereditary optic neuropathy (LHON) is the most common mitochondrial disease, and it is characterized by degeneration of the retinal ganglion cells with demyelination and atrophy of the optic nerve. The primary LHON mutations 3460, 11778, and 14484 are found only in LHON pedigrees and putatively play a genetic role in its pathogenesis. Multiple sclerosis (MS) is a multifocal demyelinating central nervous system disease in which genetic susceptibility factors and non-genetic factors are postulated to be involved, most likely all with a small contribution to the overall risk. Here we report a male patient with LHON homoplasmic T14484C and heteroplasmic G15257A mutations who developed an MS-like picture. Methods: The patient is a 35-year-old young man who developed a sudden episode of paresthesia and weakness of the legs and feet bilaterally. There was no family history of neurological disease or loss of vision. Neurological examination revealed a gait disturbance with hypostenia and hypoesthesia of lower limbs bilaterally. Somatosensory evoked potentials with stimulation of the posterior tibial nerve at the ankle were absent bilaterally. Pattern visual evoked potentials revealed a delayed response bilaterally. Brain MRI revealed sparse areas of demyelination along the periventricular white matter without gadolinium enhancement. Spinal cord MRI showed diffuse demyelinating lesions extending from D6 to D11 with gadolinium enhancement. Spinal fluid examination showed oligoclonal bands.Total leukocyte DNA was extracted using standard protocols. DNA was screened for the LHON nt-3460, nt-11778, nt-14484, and nt-15257 mitochondrial DNA (mtDNA) base changes using polymerase chain reaction and restriction enzyme fragment length polymorphisms analysis. Mutations were confirmed by directed sequencing. Summary of results: Analysis of mtDNA revealed a homoplasmic T14484C change and heteroplasmic for the G15257A, which are common mutations causing LHON. Conclusion: Here we report one additional male patient displaying LHON and MS like picture (Harding's syndrome). Similarly to previous reports of LHON and MS in men, distinguishing MRI features were the major involvement of the spinal cord, while only sparse demyelinating lesions were seen on brain MRI. On this basis, screening for the Leber's mutations in male MS patients who display such features might be considered, as this has important prognostic and genetic implications.

1977/F

Single nucleotide polymorphism in the MMP-9 gene is associated with susceptibility. *A. La Russa[1], E. De Marco[1], P. Valentino[2], V. Andreoli[1], F. Trecroci[1], M. Caracciolo[1], G. Di Palma[1], R. Cittadella[1].* 1) Institute of Neurological Sciences,National Resear, Cosenza, Cosenza, Italy; 2) Institute of Neurology - Magna Graecia - Catanzaro, Italy.

Purpose: Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS). It is characterized by the presence of demyelinated plaques or multifocal inflammatory lesions caused by autoreactive immune cells migrating through the damaged blood-brain barrier (BBB) into the CNS during the active stage of the disease. MMPs, and especially MMP-9, seem to play a role in the influx of inflammatory cells into the CNS and in the breakdown of the BBB and have been demonstrated to cleave human myelin basic protein in vitro. MMP-9 is higher in the cerebrospinal fluid and serum of patients with MS, compared to healthy controls and represents a useful marker for the evaluation of the clinical type, disability and severity of the disease. The transcriptional activity of the MMP-9 gene is influenced by two polymorphisms identified in the promoter region. These are a (CA)n microsatellite polymorphism from position -90 and a single nucleotide polymorphism at position -1562. In this study we wished to investigate the role of the MMP-9 gene in patients MS Methods: The functional -1562C/T and -90 (CA)n repeat polymorphisms were analyzed in 243 patients with MS and 173 healthy sex and age matched controls. All patients and controls were Caucasian and were born in Italy. All gave written informed consent prior to participation in the genetic studies. Genomic DNA was extracted from peripheral blood leukocytes of patients and controls using a standard procedure. The C/T polymorphism at position -1562 of the MMP-9 gene promoter was genotyped by PCR-restriction fragment length polymorphism analysis. To determine the numbers of the CA repeats in the MMP-9 promoter region, PCR products were generated using already published primers. Capillary electrophoresis was used to separate the amplicons. GENESCAN™ software was used to determine allele size. Summary of Results: A significant increase of the -1562T allele carriers was found in patients with MS compared to controls. Moreover, haplotype analysis showed that the haplotype formed by the -1562T allele and the L allele ((CA)£20) was overrepresented in patients with MS versus controls. Conclusion: Our study suggests that the MMP-9 gene is implicated in the susceptibility to MS. This result, together with that obtained in a distinct ethnic population with MS reporting association with disease progression, supports the hypothesis of an important role played by this gene in MS.

1978/F

An integrated analysis identifies several factors influencing the expression of CDKN2A and CDKN2B in glioblastoma. *J. Feng, W. Liu, S. Kim, J. Kim, J. Sun, Z. Zhang, Y. Zhu, J. Sun, J. Xu.* Center for Cancer Genomics, Wake Forest University School of Medicine, Winston-Salem, NC.

Glioblastoma multiforme (GBM) is the most prevalent primary brain tumor and is among the deadliest of all human cancers. CDKN2A and CDKN2B are two tumor suppressor genes encoded at 9p21.3 that have established roles in GBM. Germline polymorphisms at 9p21.3 and somatic variations such as deletions, mutations and DNA methylations at CDKN2A/CDKN2B are associated with GBM susceptibility, development and prognosis. Despite these reports, these events are only isolatedly documented, and their functional connections and relative contributions to the disease phenotype remain yet to be characterized. In order to obtain a comprehensive view of these genomic and cellular events related to GBM, we have performed an integrated analysis by leveraging the full spectrum of GBM data available from the Cancer Genome Atlas (TCGA) Research Network. Our univariate analysis indicated that the expression of CDKN2A and CDKN2B was strongly affected by copy number variations (CNVs, p=2.01 × 10-44 and 5.91 ×10-37, respectively). DNA methylation may also have an influence, with that on one CpG site (cg10210238) significantly associated with CDKN2B expression levels (p=0.029), and that on another (cg17449661) marginally associated with CDKN2A expression (p=0.079). However no correlations were found between the germline or somatic polymorphisms with their mRNA levels. Through multivariate analysis, we further found that CNVs accounted for a significant proportion (25.6% and 29.5%, for CDKN2A and CDKN2B respectively) of the total variations of the expression levels of CDKN2A/CDKN2B, whereas DNA methylation variations made a small but significant contribution (3.67% and 6.06%, respectively). Taken together, these analyses indicate that CDKN2A/CDKN2B expression is predominantly influenced by copy number variations, and to a less extent by DNA methylation at specific CpG sites. The fact that the majority of the variances (69.4% for CDKN2A and 61.6% for CDKN2B) remain still unexplained suggests that there are yet other factors that strongly affect the expression of CDKN2A and CDKN2B but await further identification.

1979/F

Utility of autopsy-derived tissue for gene expression studies. *S. Gupta, G.M Hilton, M.K Halushka, D.E Arking.* Johns Hopkins University School of Medicine, Baltimore, MD.

Gene expression studies are currently used to identify differentially expressed transcripts between cases and controls, and to identify functional genetic variants (eQTLs). While many of these studies are performed in blood or lymphoblastoid cell lines, the majority of eQTLs are tissue specific, and thus, gene expression studies will need to be conducted in the relevant tissue for a disease. One major technical concern about using autopsy-derived tissue is how representative it is of physiologic conditions because of the effect of postmortem interval on tissue degradation. This study evaluates the variability of RNA message in autopsy-derived tissues. Heart tissues were obtained from a cardiac transplantation and a rapid autopsy. Tissues were allowed to autolyze in a simulation of standard autopsy conditions. Hearts were initially maintained at 37°C, and then cooled at 1°C / hour for 12 hours, after which they were cooled at 2-3°C/hr (to 4°C). This simulated a body cooling at room temperature for 12 hours, before transportation and storage to a cold room in the morgue. Twenty-one tissue samples were harvested from each heart at time points 0,6,12,18 and 24 hours. These heart samples, along with 2 HeLa RNA controls were run on the Affymetrix 1.0 ST Exon arrays. Exon arrays have 1.4 million probesets that map to over a million exon clusters. We analyzed expression data from ~200,000 core probe sets that were supported by putative mRNA from RefSeq. The gene intensities were quantile normalized and summarized by using Robust Multi-Array Analysis. The probes were filtered to retain those flagged as present (DABG<0.05), at at least one time point. Approximately, 163,000 probes mapped to ~14,000 transcripts. Comparing the 0 hour explant and autopsy tissues to the later harvest time points, we observed that only <0.5% of genes were differentially expressed (FC > 2 and p<0.05), which was similar to results observed comparing technical replicates (HeLa 1 vs HeLa 2). Our data also demonstrated biologically relevant expression differences between the two hearts. For example, CTGF, a secreted protein that is strongly induced in human and experimental heart failure, was 5-fold higher in the transplant heart (p=0.011). These results demonstrate that RNA from autopsy-derived tissue, even after 24 hours of autolysis, can be used to identify biologically relevant expression pattern differences, thus serving as a practical source for gene expression experiments.

1980/F

***ABCA13*: the naming of a gene.** *R. Seal, S. Gordon, M. Lush, M. Wright, E. Bruford*. HUGO Gene Nomenclature Committee (HGNC), European Bioinformatics Institute, Hinxton, Cambridgeshire, United Kingdom.

The HUGO Gene Nomenclature Committee (HGNC) aims to approve a gene symbol and name for every human gene. Standardisation of gene symbols is important as it allows researchers to refer to the same gene without ambiguity and facilitates data retrieval. The primary rule of the HGNC is that every approved gene symbol must be unique. Gene symbols should also be acceptable to researchers to ensure their widespread use, and should be based on structure, function or homology wherever possible. The HGNC encourages the development of a common root symbol for members of a gene family, with a hierarchical numbering system to distinguish the individual members, as this is an efficient way to name large numbers of related genes and makes each family member instantly recognisable. We provide individual web pages on our site for many established gene families and have over one hundred specialist advisors that help us to accurately maintain these families. Here we describe the naming of one particular gene family member, *ABCA13* (full name: ATP-binding cassette, sub-family A (ABC1), member 13). We explain how and why the gene was named and how the symbol has subsequently been used. The first step was the development of the ABC (ATP-binding cassette) gene superfamily and sub-family nomenclature scheme, following in depth discussions between the HGNC and the ABC research community. This was followed by the identification and naming of *ABCA13* as part of this family, its appearance in the biomedical literature and databases, its adoption for the mouse and rat orthologs as *Abca13*, and its subsequent breakthrough into the international media. For further information on human gene nomenclature, please email us at hgnc@genenames.org, or visit http://www.genenames.org/. The work of the HGNC is supported by the NHGRI and the Wellcome Trust.

1981/F

Long segment Hirshprung's disease and bifid epiglottis in a patient with a denovo mutation in the GL13 gene. *H.H. SHUHAIBER, K. Surapaneni, E. GUZMAN, K. ANYANE-YEBOA*. Columbia University Medical Centre, New York, N.Y.

Pallister-Hall syndrome (PHS) is a pleiotropic disorder first described in 1980 and characterized by hypothalamic hamartoma, central or postaxial polydactyly, hypopituitarism, and variable visceral anomalies1.In 2005, mutations in the zinc-finger transcription factor gene (GLI3) on the chromosomal locus 7p13 were found to be the causative factor in 95% of patients with clinically suspected PHS. We describe a neonate who was the product of an uncomplicated pregnancy and normal spontaneous vaginal delivery at 34 4/7 weeks to an otherwise healthy 19 year old G1P1 with an initial birthweight of 3105 grams. On newborn exam, the patient was found to have bilateral polydactyly, microphallus and hypospadius. On day of life three, the patient was found to have bilious emesis .Resected bowel pathology was consistent with a long-segment Hirschprung's disease. Further evaluation showed patient's karyotype to be 46XY, with low growth hormone and testosterone. MRI imaging demonstrated a large nonenhancing prepontine mass causing superior displacement of the optic chiasm and mildly deforming the ventral pons. Molecular DNA analysis showed that patient was heterozygous in the GLI3 gene for a framshift mutation defined as c.2400delA and predicted to result in premature protein truncation p.Ala801-ProfsStop8. The presence of a hypothalamic hamartoma is the primary clinical feature of PHS that helps in differentiating it from other genetic polydactyly syndromes with overlapping features. Additional major findings in PHS aside from central polydactyly include a bifid epiglottis, GI anomalies (Hirschprung's disease and imperforate anus), renal abnormalities, panhypopituitarism, and dysplastic nails. Two studies have reported finding a bifid epiglottis in 50-58% of patients diagnosed with PHS (Azzam and Ondrey). The association between polydactyly and bifid epiglottis in PHS patients may possibly be secondary to the developmental observation that the hand and foot plates, digital rays, and epiglottis develop between 32 and 46 days of gestation. It has been shown that haploinsufficiency of GLI3 causes GCPS while protein truncation mutations in GLI3 are associated with PHS, which may partly reflect the absence of bifid epiglottis in GCPS patients. The characteristic finding of a bifid epiglottis and a hypothalamic hamartoma seem to be key differentiating features of PHS from other genetic polydactyly syndromes.

1982/F

Sequence interpretation challenges for mutation detection for congenital muscular dystrophy - a complex multigene panel. *C.A. Valencia, K. Gokhale, C. Alexander, E. Chin, S. Bhide, M. Yau, M. Hegde*. Department of Human Genetics, Emory University School of Medicine, Decatur, GA.

Congenital muscular dystrophies (CMDs) are a group of genetically and clinically heterogeneous hereditary myopathies characterized by congenital hypotonia and muscle weakness, contractures, and delayed motor development. Given the recent improvement of molecular technologies, the classification of CMDs has significantly changed from phenotype driven towards a more molecular based categorization. So far up to twelve genes are known to cause various forms of CMD, including *COL6A1*, *COL6A2*, *COL6A3*, *ITGA7*, *FKTN*, *FKRP*, *POMGNT1*, *POMT1*, *POMT2*, *SEPN1*, *LARGE* and *LAMA2*. Single gene sequencing is effective when a single missing protein is identified by a muscle biopsy and loss of that protein fits the phenotype. However, a comprehensive gene sequencing panel is necessary when ambiguous results arise or when muscle biopsies are difficult to obtain. To overcome these difficulties, clinical laboratories are now pursuing novel strategies to perform large scale mutation detection such as cost-effective targeting of a specific fraction of the genome. We have developed a next generation panel for CMD using highly multiplexed PCR using RainDance and SoLiD. We have used previously identified eleven positive controls in different CMD associated genes which consist of point mutations and indels. Analysis performed using Softgenetics, NextGene software, permitted the successful detection of all mutations and polymorphisms. The CMD panel genes are highly complex and extremely polymorphic which create challenges for interpretation and classification of variants. As genomics is rapidly moving towards clinical whole genome sequencing, the CMD next generation sequencing serves as a good model in understanding the interpretation challenges we will be facing.

1983/F

A preliminary comparison of the distal gut microbiome in HIV-infected subjects before and after HAART. *L.M. Bull[1], B. Youmans[2], Y. Shang[2], S.K. Highlander[1,2], J.F. Petrosino[1,2], K. Worley[1], Q. Xiang[1], R.A. Gibbs[1]*. 1) Human Genome Sequencing Ctr, Baylor College Med, Houston, TX; 2) Molecular Virology and Microbiology, Baylor College of Med, Houston, TX.

The importance of the gastrointestinal tract's commensal bacteria in the maintenance of the mucosal barriers has become evident. In acute HIV infection the primary site of T cell depletion is from the (GI), and HIV viral replication plays a fundamental role in perpetuating microbial translocation, suggesting that the microbiome may have significant impact on HIV disease progression. To examine the relationship between HIV infection and the distal colon's microbiome, we conducted a pilot study to evaluate the difference in microbial community structure and diversity in treatment naïve HIV infected patients (CD4 cell count <250 cells/mm^3), before and after HAART. All subjects were matched by sex and race to HIV negative controls. 16S rDNA from fecal samples was amplified and sequenced using standard Sanger and 454 FLX Titanium, and the taxonomic groups were compared. We found that in all samples, the predominant bacterial classes were the Clostridia (Phylum Firmicutes) and Bacteroidetes (Phylum Bacteroidetes). There was greater variation in the number of minor groups found in the controls which were not identified in the HIV-infected cases. We also found that in all case groups there was an average reduction of the Bacteroidetes to half of the community membership representation that was observed in the controls. Most significantly, there was a clear shift in phylum membership between the cases and the controls that trended with severity of the immune depletion. While all cases showed changes at the phylum level compared to the controls, the most advanced disease (CD4 =118cells/mm^3) case showed the greatest reduction in Bacteroidetes and a greatest increase in Firmicutes. The shift in the higher bacterial taxa either completely or partially reversed after 6-12 months on HAART. Principal component analysis using bacterial families differentiated between the cases before and after HAART for participants that were compliant on medication. Our pilot study suggests that the shift in the GI microbial structure maybe correlated with HIV viral load in a dose-dependent manner, and that upon partial restoration of immune system on HAART, the higher taxa composition is significantly different and likely to cluster with the HIV negative controls. This is the first study to examine the relationship between HIV infection and the human distal colon's microbiome, and the first to report a possible association between HIV viral load and key microbial community membership.

1984/F

Genetic Variants in the promoter region of the gene encoding C-Reactive Protein (CRP) are associated with serum CRP levels in African Americans. *A. Doumatey, G. Chen, A. Adeyemo, H. Huang, J. Zhou, C. Rotimi.* Center for Research on Genomics and Global Health, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD.

Purpose: C-reactive protein is an acute phase reactant protein produced primarily by the liver and associated with diabetes, hypertension, and cardiovascular diseases. Its level is also influenced by other factors such as obesity, smoking. Multiple studies have reported significant association between serum CRP levels and polymorphisms within the CRP gene; however these associations explain only a small proportion of the reported heritability. Thus, it was hypothesized that genetic effects on CRP are certainly present outside its locus. Genome-wide approach has been used in other populations mainly in Europeans to identify other loci that may control serum CRP level. The aim of this study was to identify genetic variants that influence serum CRP in African Americans and to replicate the findings of previously published GWAS in other populations. Methods: A total of 836 unrelated African Americans recruited through the Howard University Family study (HUFS) were genotyped using the Affymetrix 6.0 fixed SNPs array. After quality controls, the analyses focus on 2,366,856 genotyped and imputed SNPs. CRP levels were measured using a clinical auto analyzer (COBAS Integra 400 plus, Roche Diagnostics). The associations were assessed under the additive model with adjustment for proportion of African ancestry, age, gender and BMI. Results: We found significant associations with serum CRP on chromosome 1 at CRP locus (rs3093058, p= 5.20E-10; rs9628671, p= 9.09E-10) and on chromosome 5 near MAN2A1 locus (rs25913, p= 2.58E-06; rs31610, p= 5.61E-06 and rs25915, p= 6.02E-06) Conclusion: In this study, we observed associations at CRP locus with serum levels of CRP as reported in populations of European ancestry. Importantly, we observed a novel locus that may be influencing CRP levels in African Americans.

1985/F

Integrating Personal Genome Testing with SNP array into standard Genetic Practice - a model from an Executive Health Clinic in Canada. *J. Davies, L. Velsher, J. Aw.* Medcan Clinic, Toronto, Ontario, Canada.

The Medcan Clinic is a preventive healthcare clinic focused on identifying health risks before signs and symptoms occur. Medcan provides an array of in-house services, such as diagnostic imaging, genetics, optometry, fitness and nutritional counselling, to customize patients' medical care and optimize healthy outcomes. Since 2005 clients could request a Genetic Risk Assessment, which involves an in-person interview with a masters-trained genetic counsellor and assessment of the family history to identify single gene and/or multifactorial disorders of concern. If there is concern, further genetic testing and/or a consultation with a clinical geneticist is arranged. Since July 2009, the clinic has expanded this service to offer a Personal Genome Testing option. In addition to the Genetics Risk Assessment, the client provides a saliva sample for analysis using the Navigenics platform. Prior to submitting the DNA sample, the client is fully counselled regarding the purpose, limitations, and possible risks of the assessment, including the possibility for insurance discrimination. The results of the array are first conveyed to the client in a personal interview with the genetic counsellor, who reviews the key features of the report. The client is then given a printed copy of the report as well as access to the report on line. Copies of the report are not kept in the patient's file, and are not sent to the patient's physician unless specifically requested by the patient. The client may request follow-up visits with the counsellor if there are any concerns. The array used provides information regarding 27 clinically significant conditions, for which some action is available to reduce lifetime risk. The associations used for the array have been validated in peer- reviewed studies involving independent populations. The combination of pedigree analysis and SNP array screens for both the less common possibility of a Mendelian disorder (e.g. BRCA mutation) and the more common association of SNPs with adult onset conditions. This program combines the benefits of the information available from the SNP array with the value of a family health history review and detailed explanations by a trained genetics professional. In this way, the technology provided in a 'Direct to Consumer' test is made available within the framework of genetics 'best practice'.

1986/F

Personal Genomic Testing - Initial Results of a Genetic Service Combining Family Health History and SNP Array. *L. Velsher, J. Davies, J. Aw.* Dept Gen, Medcan Clinic, Toronto, ON, Canada.

The Medcan Clinic is a preventive healthcare clinic focused on identifying health risks before signs and symptoms occur. Medcan provides an array of in-house services, such as diagnostic imaging, genetics, optometry, fitness and nutritional counselling, to customize patients' medical care and optimize healthy outcomes. Since July 2009, clients can request Personal Genome Testing, which includes a detailed review of their family history and a SNP array using the Navigenics platform. So far, we have provided the service to 284 clients. 242 (85%) of these requested the full genome testing after reviewing the option on the website or speaking to the genetic counsellor or trained sales person prior to their visit. The other 15% were originally seen for a Genetics Risk Assessment, which involves only the pedigree analysis, and decided then to proceed with the genomic SNP test. 4% of the individuals who chose Personal Genome Testing were adopted. Only 3% were referred by their doctors for the service. During the same time period, 73 clients who were seen for the pedigree assessment declined the SNP array. We are compiling a data base of our cases, combining family history and SNP array results for each condition tested by the array. One trend we are seeing is that an increased risk for common disorders based on family history correlates with increased risk by SNP array about 50% of the time. However, the reverse is not true. In most cases with a higher than average risk the family history is negative. We are also conducting a survey of clients, to assess their satisfaction with the test, whether they report changes in their health care or behaviour, and whether clients are experiencing changes in anxiety levels because of the test. Anecdotal trends so far indicate a low level of anxiety and a high degree of satisfaction among those who have had the full Personal Genome Testing. This may be due to the detailed information given by the genetic counsellor before and after the actual test is done. It may also be a function of the select population in the clinic, where most of the clients are highly educated, motivated individuals who are early adopters of new technology and are pro-active about their health.

1987/F

Functional genomic screening to identify novel pathways involved in HIV pathogenesis. *D. Dykxhoorn[1,2], M. Dominska[1], S. Liu[1].* 1) John P. Hussman Institute for Human Genomics, Dr. John T. Macdonald Foundation Department of Human Genetics, University of Miami Miller School of Medicine, Miami, FL; 2) Department of Microbiology and Immunology, University of Miami Miller School of Medicine, Miami, FL.

The human immunodeficiency virus (HIV)/AIDS pandemic remains a global health concern affecting millions of individuals world-wide resulting in tremendous pain and suffering. Over a protracted clinical course, HIV attacks the host's immune system leaving the infected individual susceptible to a wide variety of opportunistic infections. HIV susceptibility and disease progression show a substantial level of inter-individual variability. This heterogeneity is influenced by a number of important factors, including the immune status of the infected individual, genetic, epigenetic, viral and environmental factors. The identification of genes that impact susceptibility and resistance to HIV infection is key to unravelling the complexities of HIV-host interactions. Using an unbiased, genome wide RNA interference (RNAi)-based genetic screen, we identified over 250 host factors (HIV-dependency factors (HDFs)) which are required for HIV-1 replication but whose silencing had no overt cytotoxic effects within the context of the screen. Further analysis of these factors have implicated novel roles for factors involved in retrograde Golgi-to-ER trafficking, in viral fusion, and the karyopherin Transportin 3 (TNPO3) in viral integration. Bioinformatic approaches coupled with functional genomic, biochemical and cell biological approaches have been used to identify interactions between HIV and the host cell machinery that are required for early events in the viral life cycle. The characteriztion of these factors will provide novel therapeutic targets for the development of antiretroviral agents that could halt the transmission and spread of this deadly virus.

1988/F

A large-scale neurodevelopmental genomics study with in-depth phenotype-genotype assessment. *H. Qiu[1], M.E. Calkins[2], F.D. Mentch[1], J. Richard[2], D.J. Abrams[1], R.W. Grundmeier[3], R. Chiavacci[1], J. Loughead[2], P.M. Sleiman[1], L. Hermannsson[1], R.C. Gur[2], R.E. Gur[2], H. Hakonarson[1].* 1) Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, PA; 2) Department of Psychiatry, School of Medicine, University of Pennsylvania, Philadelphia, PA; 3) Center for Biomedical Informatics, Children's Hospital of Philadelphia, Philadelphia, PA.

Mental disorders are complex, with underlying genetic, epigenetic and environmental factors that are far from being well understood. Symptoms of mental disorders often emerge during childhood and adolescence and undergo changes modulated by processes related to brain maturation and experience. Application of genomic methods to mental illnesses is still hindered by the paucity of information beyond diagnosis pertinent to mental illness. In a recent large-scale genomics study focusing on Neurodevelopmental Genomics and Trajectories of Complex Phenotypes (NGTCP), we aim to fill the information gap by conducting clinical and neurobehavioral phenotypic characterization of a large cohort of 10,000 prospectively accrued community sample, age 8 to 21 years, who were already genotyped using high-density SNP arrays.

Participants undergo a clinical assessment composed of personal and family psychopathology screening interviews, with their electronic health records (EHR) also linked as supplementary information. Computerized neurocognitive battery (CNB) tests are administered to collect measures in the following domains: 1) Abstraction and mental flexibility, 2) Attention, 3) Working Memory, 4) Verbal Memory, 5) Face Memory, 6) Spatial Memory, 7) Language and Reasoning, 8) Spatial Processing, 9) Emotion Processing, and 10) Sensory-motor Processing Speed. In addition, neuroimaging is conducted in 1,000 participants randomly selected using stratified random sampling. The neuroimaging studies include Structual Imaging (sMRI), Diffusion Tensor Imaging (DTI), Functional Imaging (fMRI) and Resting ASL perfusion MRI. For these 1,000 imaged subjects, DNA methylation profiling is performed and EBV cell lines are established for functional-biological studies such as expression profiling and deep sequencing.

This integrated resource of comprehensive phenotypic and genomic parameters will be presented and is anticipated to lay sound foundations for advanced genomic studies to elucidate the neurodevelopmental trajectories of complex mental disorders in the future.

1989/F

Race, Gender, Adiposity and Leukocyte Telomere Length in Healthy White and Black Adolescents. *H. Zhu[1], X. Wang[1], B. Gutin[1], D. Keeton[1], J. Thomas[1], I. Stallmann-Jorgensen[1], V. Bundy[1], H. Snieder[2], P. Harst van der[3], Y. Dong[1].* 1) Department of Pediatrics, Medical College of Georgia, Augusta, GA; 2) Department of Epidemiology, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands; 3) Department of Cardiology, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands.

Objectives: Telomere length, an emerging marker of biological aging, has been investigated in adult populations. However, little is known about the effect of race, gender, adiposity and the adipokines on telomere length in adolescent populations. **Study design:** Leukocyte telomere length (T/S ratio) was assessed cross-sectionally in 667 white and black boys and girls aged 14-18 years (48% blacks, 51% girls) using a quantitative PCR method, which normalized telomere extension product (T) to the amount of extension product of a single copy gene (S). Predictor variables included body mass index, waist circumference, percentage body fat, visceral adipose tissue, leptin and adiponectin. Multiple linear regression analyses were used to determine the effect of race, gender and adiposity on telomere length. **Results:** Significant race and gender difference in telomere length were identified, with black adolescents having longer telomeres than white adolescents (age and gender adjusted T/S ratio ± SE: 1.32 ± 0.01 vs. 1.27 ± 0.01, p=0.009), girls having longer telomeres than boys (age and race adjusted T/S ratio ± SE: 1.31 ± 0.01 vs. 1.27 ± 0.01, p=0.01). None of the adiposity measures or adipokines explained a significant proportion of the variance in telomere length. **Conclusions:** This is the first study conducted in a biracial adolescent cohort showing that (1) race and gender differences in telomere length have already emerged during adolescence; but (2) adiposity and adipokines are not associated with telomere length at this age.

1990/F

MTHFR 677C>T polymorphism in Iranian migranous patients. *M. Ansari[1], S. Saeidi[1], M. Moghadasi[2], A. Ebrahimi[3], F. Abbasi[1], F. Rezaei[4], MS. Fallah[3,4].* 1) Clinical Biochemistry, Tehran University of Medical Sciences, Tehran, Tehran, Iran; 2) Dep't of Neurology, Iran University of Medical Sciences, Tehran, Iran; 3) Kawsar Human Genetics Research Center, Kawsar Genomics & Biotech Center, Tehran, Iran; 4) Aramesh Pain Clinic, Tehran, Iran.

Migraine is a prevalent and debilitating disease affecting a large proportion of the population. MTHFR is suggested to play a role in migraine susceptibility. There are conflicting data on the association between the MTHFR 677C>T polymorphisms and migraine disease. This may have some correlation with the response to the vitamin therapy. This study performed to find the association between the MTHFR 677C>T polymorphism and migraine in Iranian patients. Migrainous patient according to the international headache society criteria entered in the study. Healthy people without any positive history of periodic headache and no family history of migrainous head in the family recruited as control group. MTHFR 677C>T polymorphisms were investigated in cases and controls using PCR-RFLP method. Thirty-nine migrainous patient (9 male and 30 female) and 58 healthy controls (20 male and 38 female) entered in the study. The CC genotype was most frequent in both group (19 (48.7%) and 31 (53.4%) in case and control group respectively) followed by CT genotype in 16 (41.0%) cases and 25 (43.1%) controls. Genotype frequency didn't show any significant difference between groups (chi square test). In this study we didn't find any correlation between MTHFR 677TT genotype and migraine. More studies with more sample is needed in Iranian population.

1991/F

ENCODE Whole-Genome Data at UCSC: Fall 2010 Snapshot. *K.R. Rosenbloom, T. Dreszer, B. Raney, C. Sloan, V. Malladi, M. Cline, G. Barber, L. Meyers, K. Roskin, B. Suh, A. Hinrichs, A. Zweig, P. Fujita, K. Learned, B. Rhead, V. Swing, A. Coelho, R. Kuhn, D. Karolchik, D. Hausler, W.J. Kent, ENCODE Consortium.* Center for Biomolecular Science and Engineering, UC Santa Cruz, Santa Cruz, CA.

The Encyclopedia of DNA Elements (ENCODE) Project is an international scientific collaboration charged with creating a comprehensive catalog of functional elements in the human genome. In September 2007, the pilot project focused on 1% of the genome, was scaled up to whole-genome scale. Two years into production, the participating experimental groups have submitted a wealth of high-throughput epigenomic and transcriptome data to the ENCODE Data Coordination Center at UC Santa Cruz. At the mid-course milestone (January 2010 data freeze), results from 860 experiments in 112 cell lines and tissues had been submitted to the DCC for public release in the Genome Browser. A total of 23 experimental assays were represented, including ChIP-seq of 98 transcription factors and 10 histone modifications, RNA-seq in 8 cellular compartments, DNase and other measurements of open chromatin, chromatin interaction maps, and high-quality gene annotations from the Gencode project. It is anticipated that the number of experiments will have doubled by November 2010. The ENCODE DCC at UCSC provides both raw data (sequence reads and quality scores) and processed data files (alignments, density graphs and peak calls) from the UCSC Download server, data mining via the UCSC Table Browser, and general access to ENCODE information at the UCSC ENCODE Portal (http://genome.ucsc.edu/ENCODE). This year's developments for ENCODE include migration to the GRCh37/hg19 human genome assembly, completion of the first round of integrative analyses, and the addition of 6 new production groups, expanding the project scope to include new experiment types (proteogenomics, epitope-tagged TFBS assays) and inter-species orthology of regulatory elements from ChIP-seq and RNA-seq in mouse cell lines and tissues.

1992/T

Patient-entered, web-based, EMR-compatible, comprehensive cancer family history collection tool: clinical workflow, demographics, and patient satisfaction from pilot study. *M. Doerr[1], B. Leach[1], X. Men[2], J. Moline[1], K. Stanuch[1], E. Edelman[3], C. Eng[1].* 1) Genomic Medicine Institute, Cleveland Clinic, Cleveland, OH; 2) Information Technology Division, Cleveland Clinic, Cleveland, OH; 3) National Coalition for Health Professional Education in Genetics, Lutherville, MD.

MyFamilyHealthHistory (MyFHH) is a patient-entered, web-based, EMR-compatible, comprehensive cancer-focused family health history collection tool developed by the Genomic Medicine Institute (GMI) in collaboration with the Information Technology Division, Cleveland Clinic. Clinical Workflow: A specific appointment type automatically triggers a MyFHH invitation email to the patient. The patient logs in via secure web-portal and follows prompts to complete a 3-4 generation pedigree. If not completed, a genetic counselor assistant (GCA) contacts the patient with reminder phone call(s). GCA can also walk the patient through the questionnaire if the patient lacks web access or needs additional support. Demographics: As of June 2010, MyFHH has been piloted with 56 patients (45 women, 11 men) scheduled for cancer genetics appointments at GMI. 5% of participants self-identified as African-American (n=3), the remainder as White (53); 25% (14) reported Jewish ancestry. The average age of participants was 47 years (median = 50, range = 21-68). Consistent with Clinic norms, most (31, 55%) were white women age 40 or older. 45% (25) of patients completed the questionnaire in advance of their appointment without further prompting; 39% (22) completed it after a reminder phone call; 11% (6) required some GCA support (e.g., web-based completion with a GCA helping by phone); and 5% (3) had the questionnaire completed by their genetic counselor (GC). There were no discernable trends in age, race, or gender of those not needing support vs those needing GC/GCA support. Patient Satisfaction: 48 (86%) of the pilot group completed a satisfaction survey following the questionnaire. 81% (39) agreed/strongly agreed that MyFHH was easy to use. 60% (29) of respondents spoke with family members while completing the questionnaire (a “save and come back” feature facilitates information-gathering). 85% (41) felt that the time it took to complete the questionnaire was “just right” (data entry takes 30-60 minutes); 7 respondents (15%) felt it took “a little too long.” Conclusion: MyFHH enables secure, EMR-compatible, comprehensive cancer family history collection prior to genetic clinic visits. 84% of patients were able to complete MyFHH without GC/GCA support; trends were not observed by age, race, or gender. Patient satisfaction with MyFHH is high. Use of MyFHH could potentially streamline counseling sessions and/or allow for the implementation of alternative service delivery models.

1993/T

A genomic and personalized medicine application for population and public health: The Genomically Enabled Elecronic Medical Record - GenE EMR. *W. Cohn[1], S. Jones[1], M. Ropka[1], R. Gaare-Bernheim[1], J. Harrison[1], J. Lyman[1], S. Powell[2], W. Knaus[1].* 1) Public Health Sciences, Univ Virginia, Charlottesville, VA; 2) Internal Medicine, University of Virginia, Charlottesville VA.

The potential public health benefits available from genomics and personalized medicine have yet to be realized. We previously developed Health Heritage, a Web-based family health history application, to provide an easy-to-use tool for consumers to efficiently collect and update their family health history. Combined with evidence-based decision support, Health Heritage can provide recommendations for prevention and risk reduction for 89 conditions in 5 disease areas, based on hereditary and familial risk factors. Our current project, titled “Building a Genome-Enabled Electronic Medical Record (GenE EMR)”, focuses specifically on cancer risk. The project will enhance Health Heritage in three primary ways. First: it will include additional personal and genomic risk factors into the decision support to provide recommendations that are even more personalized. Second: Health Heritage will be integrated into multiple electronic medical records (EMRs) and personal health records, thus increasing ease-of-use and accuracy by both patients and physicians. Third: we will develop ways to, at patient request, securely electronically share information with family members to ease relatives’ burden of compiling and updating health histories. Additionally, we are exploring the economic, ethical, legal and social implications of GenEMR and its features. Although the initial focus is on cancer during this development phase, work with other diseases is planned.

1994/T

Validation of Electronic Health Records in a large scale pediatric genomics study. *F.D. Mentch[1], D.J. Abrams[1], H. Qui[1], S. Ostapenko[2], J.J.M. Connolly[1], A.W. Moy[1], C.E. Kim[1], R.M. Chiavacci[1], L. Hermannsson[1], K. Wang[1], P.M. Sleiman[1], Y. Guo[1], B. Keating[1], S.F.A. Grant[1,3,4], R.W. Grundmeier[2], H. Hakonarson[1,3,4].* 1) Center for Applied Genomics, Children’s Hospital of Philadelphia, Philadelphia, PA; 2) Center for Biomedical Informatics, Children’s Hospital of Philadelphia, Philadelphia, PA; 3) Division of Human Genetics, Children’s Hospital of Philadelphia, Philadelphia, PA; 4) Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, PA.

Electronic Health Records (EHRs) are a rich source of phenotype information for large scale genomics studies. The format and focus of the data, however, can make it challenging to automate validation of purported subject phenotypes on a large cohort with a high degree of confidence. We report initial results of comparing patient interviews, automated EHR phenotype extraction and manual EHR phenotype extraction for an on-going large scale pediatric genomics study conducted at the Children’s Hospital of Philadelpha, including over 35,000 children. Taking the combination of manual abstraction plus self-reporting as the baseline, we find that patient self-reporting in the interview process returns about 50% of the phenotypes of interest; manual abstraction from the EHR database returns 87%; and the automated abstraction process from the EHR database returns 69%. The combination of patient self-reporting and automated EHR extraction return 93%. We propose that automated extraction is a reliable method for validating and enhancing self-reported data and we will test this approach with larger numbers of subjects as the study progresses.

1995/T

Enabling genetically guided patient care through clinical decision support: a systematic review of the literature. *K. Kawamoto.* Department of Community and Family Medicine and Institute for Genome Sciences & Policy, Duke University, Durham, NC.

Computer-based clinical decision support (CDS) has been identified as a critical enabler of genetically guided patient care by various groups, including the U.S. Secretary of Health and Human Services and the Secretary’s Advisory Committee on Genetics, Health, and Society. In order to inform future research in this area, MEDLINE was systematically searched from 1990 through May 2010 using a search strategy adapted from previous systematic reviews of CDS (Kawamoto K et al., BMJ, 2005), genetic health services (Scheuner MT et al., JAMA, 2008), and family history (Wilson BJ et al., Ann Intern Med, 2009). Of 1,892 references screened, 53 manuscripts met the study inclusion criteria. Of the 53 included manuscripts, 36 (68%) were primary research articles, and 17 (32%) were reviews or opinion papers. Two-thirds of the primary research studies focused on CDS for familial cancer syndromes, as did all eight of the identified randomized controlled trials. Of particular note, a large randomized controlled trial conducted across 45 clinics in the U.K. found that the use of a CDS system by primary care clinicians significantly improved the appropriateness of the clinicians’ genetic counseling referrals for familial breast and colorectal cancer (Emery J et al., Br J Cancer, 2007). Beyond hereditary cancer syndromes, investigators have evaluated the use of CDS to support genetically guided care in several other areas, including HIV management, prenatal testing, warfarin dosing, cardiovascular disease risk management, and drug dosing informed by patients’ genotypes for drug metabolizing enzymes. With regard to long-term trends, research in this field is accelerating, with 92% of the manuscripts published on or after 2000. Also, while only 25% of the 36 manuscripts published before 2008 focused on clinical areas outside of hereditary cancer syndromes, 76% of the 17 manuscripts published on or after 2008 focused on additional clinical areas such as pharmacogenetics. Moreover, recent research efforts have focused significantly on the need for a coordinated, cross-institutional approach to CDS that is based on common health information technology standards and is capable of widespread deployment. Future studies will need to address gaps in the current research landscape, including the need for more scalable approaches to genetically guided CDS and the need for rigorous, prospective evaluations of CDS systems in clinical areas beyond hereditary cancer syndromes.

1996/T

Veterans' Response to the Use of Family Health History Online Tool. *N. Arar.* Dept Medicine, Div Nephrology, Univ Texas Health Sci Ctr, San Antonio, TX.

Objective: To assess veterans' satisfaction in using the online Surgeon General family health history (SG FHH) tool, and intentions regarding the utilization of FHH information. Method: A total of 35 veterans visiting the primary care outpatient clinic in San Antonio, TX were invited to complete the online self-administered SG- FHH tool and the study's surveys. Subjects were provided with a printed copy of their pedigree then asked to participate in a short semi-structured interview to assess their intentions regarding the utilization of their FHH information. The modified ACSI survey assessed drivers of satisfaction (content, functionality, look and feel, navigation, and performance) in using the SG-FHH online tool. The semi-structured interviews focused on facilitators and barriers to using FHH information. Semi-structured interviews were content analyzed using qualitative methods. Results: The majority of participants (71%) was male, 78% were over 50 years of age, and described themselves as non-Hispanic White. About 56% indicated that they had completed some technical school. All participants found the SG- FHH online tool very useful. The total time spent in completing the FHH forms was 25 minutes. About 67% of the participants said that they have easy access to a computer or the internet and demonstrated their ability to complete all FHH forms. Most subjects (88%) viewed the functionality, look and feel, navigation, and performance of the NG-FHH tool favorably. Participants also indicated that the tool has met their expectations. Content analysis of the semi-structured interviews showed several barriers to veterans' use of FHH information. These include (1) lack of patients' knowledge regarding the importance of FHH information, (2) concerns about privacy and confidentiality related. Our findings suggest that strategies to improve veterans' knowledge regarding the importance of FHH information and address their concerns about privacy may enhance successful implementation of FHH information into VHA clinical practice. Impact: Promoting the use of online FHH tool [e.g., SG-FHH tool] will have important implications to the primary care providers and their patients. It will also improve screening for common complex diseases and enable providers to focus their resources on developing critically important health behaviors for populations at high familial risk.

1997/T

Health Needs Assessment as a Pre-requisite for Evidence-based Service Development in Developing Countries. *I. Nippert[1], U. Kristoffersson[2], J. Schmidtke[3], A. Kent[4], R. Raouf[5], C. Barreiro[6], A. Christianson[7].* 1) Women's Hlth Res, Univ Muenster, Muenster, Germany; 2) Dept Clin Gen, Univ Hosp Lund, Lund, Sweden; 3) Inst Hum Gen, MHH, Hannover, Germany; 4) GIG, London, UK; 5) Children with special needs Dep, Ministry of Health, Cairo, Egypt; 6) Hospital de Pediatria SAMIC, Buenos Aires, Argentina; 7) Div Hum Gen, Univ Witwatersrand, Johannesburg, South Africa.

Introduction: Many developing countries have yet to confront the issues of developing policies for the provision of medical genetic services and medical genetic testing. Given their resource limitations they need practical approaches that have been proven to work in real settings. Approaches are required that will result in the development of appropriate needs-based services, that are sensitive to specific country contexts, health service patterns, available resources and capacities as well as to legislative factors and cultural and societal norms. *Methods:* An international multidisciplinary working group developed a model approach for capacity building for the translation of genetic knowledge into practice and prevention in developing countries (CAPABILITY, funded by the EC's 6th Framework programme, LSSG-CT-2006-037275). The model is based upon a systematic health needs assessment (HNA) for medical genetic services and was validated via a joint demonstration project in Argentina, Egypt and South Africa. *Outcome:* A validated capacity building approach sensitive to specific health care contexts and service pattern in developing countries including the magnitude of assessed needs, available resources and capacities, service gaps as well as ethical, socio-cultural and legal implications. *Conclusion:* Significant challenges face the development of medical genetic services in middle- and low-income countries. HNA is a systematic approach for planning and providing cogent health services in a setting of finite resources, epidemiological transition, technological advance and changing public expectations. HNA can assist developing countries determine and prioritise their medical genetic health needs and establish well organised and structured services, both clinical and laboratory, appropriate to their needs and resources.

1998/T

An exploratory study of public and professional perceptions on the use of family history and genomic profiling to assess complex disease risk. *B. Wilson[1], D. Castle[2], M. Weir[1,2], D. Jillions[1], K. Morin[2], J. Little[1], D. Avard[3], M. Cappelli[4], J. Carroll[5], T. Caulfield[6], P. Chakraborty[7], H. Etchegary[8], L. Lemyre[9], F. Miller[10], B. Potter[1], G. Wells[11].* 1) Epidemiology & Community Medicine, University of Ottawa, 451 Smyth Rd. Ottawa, ON K1H 8M5 Canada; 2) Department of Philosophy, University of Ottawa, 234-70 Laurier Ave. E. Ottawa, ON K1N 6N5 Canada; 3) Centre of Genomics and Policy Faculty of Medicine, Dept. of Human Genetics McGill University 740 Dr. Penfield Avenue, Room 5210 Montreal QC H3A 1A4 Canada; 4) Children's Hospital of Eastern Ontario 401 Smyth Road, Room R1120 Ottawa, ON K1H 8L1 Canada; 5) Granovsky Gluskin Department of Family Medicine Mount Sinai Hospital 60 Murray Street, 4th Floor, Box 25 Toronto, ON M5T 3L9 Canada; 6) Health Law Institute University of Alberta Edmonton, AB T6G 2H5 Canada; 7) Children's Hospital of Eastern Ontario Ontario Newborn Screening Program 401 Smyth Road Ottawa, ON K1H 8L1 Canada; 8) Eastern Health,Clinical Epidemiology, Faculty of Medicine, Memorial University Room H1761, Level 1 Health Sciences Centre 300 Prince Phillip Drive St. John's NL A1B 3V6 Canada; 9) School of Psychology Institute of Population Health 1 Stewart St, Room 312 Ottawa, ON K1N 6N5 Canada; 10) Department of Health Policy, Management and Evaluation Faculty of Medicine, University of Toronto 155 College Street, 4th Floor Toronto, ON M5T 3M6 Canada; 11) University of Ottawa Heart Institute H1-1, 40 Ruskin Street Ottawa, ON K1Y 4W7 Canada.

Background Genomic profiling for susceptibility to complex disorders offers the goal of personalizing disease prevention interventions to individual patient risk, e.g. by defining the right age to start a preventive regimen and/or selecting specific interventions. Two genomic profiling approaches are DNA based and family history (FH) based. The former is still a largely hypothetical possibility whereas the latter is part of standard medical practice. Even if genomic profiling by either method can be shown to produce acceptable clinical validity (ability to correctly predict risk), the clinical or public health utility will be influenced by its rate of adoption by health care professionals, and its acceptability to target populations. We report here the results of a pilot study on (hypothetical) DNA-based genomic profiling for colorectal cancer, discuss the lessons learnt, and describe how the issues are being addressed in current research. Pilot study methods and findings We conducted focus groups and semi-structured interviews with community groups representing the target populations for screening, and relevant professional provider groups. We identified the following salient themes: the attraction of a 'clean' alternative to fecal occult blood testing; attitudes towards knowing one's genetic make-up; professionals' framing of DNA based genomic profiling as 'genetic testing'; and professionals' perceptions regarding health service capacity to integrate new technologies into current screening programs. Methodological issues and current research In the pilot study, we noted the difficulty of imparting information on the nature of a hypothetical technology while also assessing participants' reactions. After a broad literature review, we identified over 100 approaches to public engagement but a dearth of published empirical validations. For our main study, we selected an approach designed for technologies which are new or unfamiliar. The technique involves the carefully staged, incremental presentation of information on the topic of interest (including ethical issues), and the use of open and closed questions to gather reactions as progressively more details are provided. We will present preliminary findings from the main study, and report critical details on the way in which the approach was specifically implemented and initial insights into its performance as a way of evaluating unfamiliar technologies.

1999/T

Evaluation of Clinical Diagnostic Potential of Next Generation Sequencing using Hypertrophic Cardiomyopathy as a model. *K. Kaur[1], J. Broxholme[2], R. Copley[2], J. Thistleton[3], S. Reid[3], L. Ormondroyd[4], A. Seller[3], E. Blair[5], I. Ragoussis[2], J. Taylor[1], H. Watkins[4].* 1) Oxford Biomedical Research Centre, Oxford, United Kingdom; 2) Wellcome Trust Centre for Human Genetics, Oxford, United Kingdom; 3) Oxford NHS Regional Molecular Genetics Laboratory, Oxford, United Kingdom; 4) Oxford University Department of Cardiovascular Medicine, Oxford, United Kingdom; 5) Oxford NHS Clinical Genetics Department, Oxford, United Kingdom.

The routine diagnosis of genetically heterogeneous Mendelian conditions is limited by current technologies. Conventional Sanger sequencing is too expensive for parallel screening of multiple genes and mutation detection methods are laborious for this purpose. As a result many conditions go untested or tests do not encompass all known genes. Next generation sequencing has the potential to revolutionise the field of clinical genetic diagnostics, by providing massively parallel sequencing at comparable costs to current technologies. We have developed a workflow for routine diagnosis of hypertrophic cardiomyopathy using the GS-FLX next generation sequencing platform. The sensitivity and specificity of this platform for genes currently tested in the NHS (MYBPC3, MYH7, TNNT2 and TNNI3) has been confirmed. Using a long range PCR approach the number of genes tested has been expanded to 15 and the mitochondrial genome has been included. A bioinformatics pipeline has been developed to ensure that the data can be analysed in a routine diagnostic laboratory without specialist bioinformatics knowledge. Our results on a cohort of more than 150 probands demonstrate the feasibility of using next generation sequencing in a clinical diagnostic environment. The process of translating this to the NHS and the impact of testing on diagnostic accuracy, yield, turn-around times and cost-effectiveness will be discussed. This approach has considerable implications for routine testing of other heterogeneous Mendelian conditions and monogenic forms of complex diseases.

2000/T

Financial Analysis of Genetic Testing for Legius Syndrome in the Neurofibromatosis Type 1 Population. *T.M. Muram-Zborovski[1,2], D.A. Stevenson[3], D.H. Viskochil[3], J.C. Carey[3], R. Mao[1,2], B. Jackson[1,2].* 1) Department of Pathology, University of Utah, Salt Lake City, UT; 2) ARUP Laboratories, Salt Lake City, UT; 3) Department of Pediatrics, University of Utah, Salt Lake City, UT.

Legius syndrome (LS) is a single gene disorder, which clinically overlaps with the pigmentary features of neurofibromatosis type 1 (NF1). LS individuals may meet NIH diagnostic criteria for NF1, creating diagnostic difficulty. Both LS and NF1 individuals may also have macrocephaly and/or learning disabilities. Distinctive manifestations of NF1 appear with age, lending a definitive diagnosis of NF1. These features include neurofibromas, Lisch nodules, optic gliomas, and distinctive bone manifestations (long bone dysplasia, sphenoid wing dysplasia, dystrophic scoliosis). The diagnostic difficulty remains with those individuals with pigmentary findings who do not develop additional manifestations, with or without macrocephaly and learning disabilities. A diagnosis of LS compared to NF1 may provide both peace of mind, because they lack a tumor phenotype, and cost savings due to less aggressive medical monitoring. However, these savings are balanced against the genetic testing costs. Our aim was to identify the most cost-effective age for genetic testing for LS in an NF1 clinic population. Delaying genetic testing beyond a specific age could lower global testing costs, since fewer patients would require testing as the development of distinct NF1 features would eliminate the possibility of Legius syndrome. Prior to this age cutoff however, all individuals would need to be managed as if they had NF1. Using TreeAge Pro 2009 software, we generated a model of the NF1 population of routine NF1 management costs at a typical NF Clinic to determine a population cost-per-individual between the ages of 1.5 and 18 years, using the assumption based on published data that 2% of these individuals meeting NIH diagnostic criteria for NF1 have Legius syndrome. Our model includes annual genetics and ophthalmology examinations and a 10% probability per year for an MRI scan. Over time, the majority of individuals with NF1 will develop age-related manifestations, declaring themselves diagnostically as having NF1. Therefore, as the model adjusts for age, there are fewer individuals without a definitive diagnosis who require genetic testing. We analyzed the cost effectiveness of testing at 2-year age intervals from 4 until 10 years-of-age. Within the NF1 population, for those individuals who do not develop additional manifestations of NF1, it is not only cost-effective but cost-saving to perform genetic testing for LS, provided that one delays until the child is six years of age.

2001/T

A practical measure of workload for molecular genetic diagnostic laboratories. *S. Stenhouse[1], R. Butler[2], A. Curtis[3], J. Deller[4], K. Kelly[5], R. Mountford[6], G. Norbury[7], Clinical Molecular Genetics Society and UK Genetic Testing Network.* 1) Institute of Medical Genetics, Yorkhill Hosp, Glasgow, United Kingdom; 2) Molecular Genetics, Institute of Medical Genetics, Cardiff and Vale NHS Trust, Heath Park, Cardiff. United Kingdom; 3) Northern Genetics Service, Institute of Human Genetics, Central Parkway, Newcastle upon Tyne, United Kingdom; 4) UK Genetic Testing Network, Southside, 105 Victoria Street, London, United Kingdom; 5) Molecular Genetics, Department of Medical Genetics, Polwarth Building, University of Aberdeen, Foresterhill, Aberdeen, United Kingdom; 6) Merseyside and Cheshire Regional Molecular Genetics Laboratory, Genetics Department, Liverpool Women's NHS Foundation Trust, Crown St, Liverpool, United Kingdom; 7) DNA Laboratory, Genetics Centre, 5th Floor Guy's Tower, Guy's Hospital, London, United Kingdom.

There has long been a need for a robust measure of workload for molecular genetic testing. This is required to predict staffing requirements and provide a logical method for pricing genetic tests. A working group from the CMGS and UKGTN was assigned to investigate this and produce a model for pilot studies.

The initial premise was that the measure should be transparent and flexible to allow comparisons between laboratories and to take account of developing technologies. The unit chosen for the measure was the final diagnostic report which is an easily counted unit. As reports can be relatively simple or very complex a weighting system was required. The unit chosen to weight the reports was 'an amplicon'(or equivalent) which is again an easily counted measure. The weights were then assigned to a band from A to G. The weight for any disorder can be decided in a straightforward manner by counting the amplicons tested and assigning it to a band. The basic unit, Band A, is sample reception and DNA extraction with a weight of one. Band G has a weight of 40.

This system was piloted in 6 UK laboratories and the total annual number of units divided into the total annual budget to provide a cost per unit and a guide price for tests. The laboratories varied in size and testing repertoire to ensure a broad base for the trial. Similarly the total number of units can be divided into the number of staff involved in the testing to derive an approximate figure for staff productivity allowing appropriate service planning.

The mechanics of the measure will be described in detail.

2002/T

Integrating parallel sequencing into diagnostic pathways. *G.R. Taylor, R.F. Charlton, P. Roberts, D.T. Bonthron, C.E. Chu.* Genetics, Leeds Inst Mol Med & Leeds Teaching Hospitals, Leeds, United Kingdom.

We have applied clonal sequencing in a diagnostic setting for the detection of mutations and copy number alterations and describe its effect on the performance and cost of a the service. Base-calling was less accurate than by Sanger sequencing, but complete concordance was seen in 110 Sanger sequenced cases and those sequenced using an Illumina GAII provided that the depth of clonal sequencing was greater than 50. This value implies base calling accuracies below the Phred-like scores commonly used and increases the cost of diagnostic testing. Nevertheless the overall cost of BRCA1 and BRCA2 gene testing was reduced by over 50% and this figure will improve as the workflow is refined. We explored he choice of wide (many genes, few patients) versus deep (few genes, many patients) sequencing in a diagnostic setting using gene sets for cardiomyopathy testing and hereditary cancers. Preliminary findings in terms of costs and patient benefits will be presented. We and others have shown that clonal sequencing can be used to provide copy number information. A comparison of the cost and performance of array CGH with copy number counting using whole genome sequencing shows that in many instances, sequencing is a more economical alternative.

2003/T

The NIH Undiagnosed Diseases Program: New Insights on the Application of Clinical Research Techniques to Unknown Diseases. *D. Adams, C. Tifft, T. Markello, C. Toro, G. Golas, L. Wolfe, W. Gahl.* Undiagnosed Diseases Program, NIH, Bethesda, MD.

The NIH Undiagnosed Diseases Program (UDP) is a pilot program designed to address the needs of persons with debilitating medical conditions for which no diagnosis has been found despite an extensive workup. The goals of the UDP include finding accurate diagnoses, and discovering new diseases that provide insight into human health. In the first two years, over 3000 inquiries have been received, 1192 medical records have been reviewed, 227 people have been accepted and 84 have been referred to other ongoing studies within the NIH. To date, 160 accepted individuals have been brought to the NIH for in-depth, one-week inpatient medical evaluation. Approximately 15% of individuals have received a specific diagnosis and a larger number have generated strong candidates for research follow-up. Most applicants have a multisystem phenotype; many have a preponderance of neurological symptoms. Diagnoses have been made by the UDP team before, during and after admission to the NIH Clinical Center. Diagnoses based on clinical evaluation and clinically-available testing include amyloidosis, Smith-Magenis syndrome, familial ALS and hereditary spastic paraplegia. Diagnoses based on research testing include SCA 28, CDG IIb, and a novel arterial calcification syndrome. Research biochemical screening tools include such techniques as organelle immunocytochemistry (Invitrogen, Carlsbad, CA) and biochemical profiling of cellular respiration (Seahorse Bioscience, North Billerica, MA). Research molecular analyses include SNP array screening for indels, contiguous homozygosity, uniparental disomy, consanguinity, and crossover-delineated linked regions. Whole exome data analysis, involving eight families, is ongoing and has yielded an experimentally-verified, disease-causing mutation in one family. Interim analysis of data derived from studied individuals is being used to refine our diagnostic approach. For example, few patients appear to have uncommon presentations of known conditions, and extensive clinical-hypothesis-driven Sanger sequencing has yielded few useful results. Consequently, we have moved toward earlier use of whole exome sequencing and have defined a set of guidelines for determining which family members' should provide DNA samples to maximize the use of genetic models to filter DNA variants. Future development of the NIH UDP pilot will include expansion of in-house research and collaboration to follow-up the abundant basic research leads arising from UDP cases.

2004/T

Family history in risk assessment for common complex diseases: a critical review of practice guidelines and modeling study. *Q. Hasanaj, B. Wilson, J. Little, Z. Montazeri.* Epidemiology and Community Medicine, University of Ottawa, Ottawa, Canada.

Background: Family history (FH) is a risk factor for many common complex diseases, and provides insights into the effects of shared disease susceptibilities arising from genomic susceptibility, shared environments, and common behaviors. Although it is not a modifiable risk factor, in principle, inclusion of FH in disease risk assessment algorithms might improve the accuracy of disease risk stratification; this, in turn, may lead to better matching of disease prevention interventions to individual risks. Practice guidelines often mention FH in the context of risk assessment. However, in isolation, FH is not highly predictive for complex disease risk, so it is important to examine how it alters the predictive accuracy of other accepted risk factors. **Objectives:** (1) To review how 'positive FH' is defined in disease-specific guidelines, (2) To review how preventive recommendations are revised for people with a positive FH and (3) To calculate the incremental improvement in individual risk prediction which is gained by incorporating FH with other forms of clinical information recommended in guidelines. **Methods:** We performed an environmental scan of chronic disease guidelines currently in use in North America using standard systematic review methods. We extracted data on the definitions of 'positive FH' which were used (if any) and the way in which preventive recommendations were altered according to presence of positive FH. We also evaluated the guidelines using the AGREE instrument. We then used data from a large colorectal cancer case control study to determine the predictive ability of FH and other classical risk factors (e.g. age, sex, selected dietary patterns, etc) individually and in combination. We developed logistic regression models and calculated the area under the receiver operating curve (AUC). **Results:** There was no consistency of definitions of 'positive FH' between guidelines for the same condition and there was a wide range in how preventive recommendations are modified in response. The logistic regression modelling and AUC calculations are currently underway.

2005/T

Simultaneous detection of 26 amino acids in human plasma using 6-aminoquinoly-N-hydroxysuccinimidyl carbamate (AQC) as derivatizing agent. *S. Attri, G. Sharma, P. Kumar, S. Singhi, P. Singhi, I. Panigrihi, S. Sharda.* Pediatrics, Postgraduate Inst Medl Education & Research, Chandigarh, India.

The AQC has been used for the derivatization of primary and secondary amino acids to yield fluorescent derivatives with high sensitivity; however there is need to improve and modify the existing Waters' AccQ.Tag method that can analyze maximum number of amino acids in a single run without compromising the sensitivity. All the reagents used were from Sigma, USA except the AccQ.Fluor reagent kit from Waters Assoc (Milford USA). Separations were carried out using Lichrospher 100 RP-18e (5μm) 250x4.0 mm column connected to 100CN 4.0x4.0 mm guard column on a quaternary HPLC system from Perkin Elmer using modified gradient elution instead of AccQ.Tag column from Waters Assoc. The working eluents were acetate buffer (pH 5.8 and 6.8), acetonitrile and water. A fluorescent detector was used to quantify all the amino acids with excitation at 250nm and emission at 395 nm. The photodiode array detector was used at 248 nm to obtain a better quantification of tryptophan. In the present method all the 26 amino acids were assayed with high sensitivity, accuracy and good reproducibility on very small amounts of plasma samples within 50 min whereas only 18 amino acids were detected using original AccQ.Tag method and 24 amino acids by quaternary eluent systems in hydrolysate samples. High resolution was achieved for a number of important amino acids including glutamine, glycine, proline, homocysteine, tyrosine, ornithine, phenylalanine, lysine and leucine. Linearity of the peak areas for different concentrations, ranging from 2.5-200 picomoles, of individual amino acids was determined. A good linearity ($r2 \geq 0.98$) was achieved in the standard mixture for each amino acid. Recovery of amino acids incorporated at the time of derivatization ranged from 95 to 106%. We have established the normative data of amino acids in plasma for 50 children, the profile being comparable to the western data reported in literature. The present method was highly sensitive, accurate, reproducible and cost-effective for amino acid analysis in metabolic laboratory in pediatric setup.

2006/T

Routine use of CANTAB system for detection of neuropsychological deficits in patients with PKU. *M. Bik-Multanowski, J.J. Pietrzyk.* Chair Pediatrics, Jagiellonian Univ, Krakow, Poland.

Adolescents and adult patients with phenylketonuria (PKU) demonstrate compliance problems often leading to discontinuation of their dietary treatment. In such non-compliant patients neuropsychological abnormalities are often reported. Prefrontal cortex-dependent deficits are considered to be early symptoms of brain dysfunction in these patients. However, no standardized tests for reliable, early detection of such deficits were available until recently. The aim of the study was to assess the usefulness of a battery of standardized, computerized neuropsychological tests application for detection of prefrontal cortex dysfunction in patients with PKU. Methods: A group of 47 patients aged >16 years were tested by means of CANTABeclipse system (Cambridge Cognition Ltd), which includes normative database for assessment of results of the tests used. Neuropsychological tests assessing prefrontal cortex-dependent functions such as attention (Rapid Visual Information Processing - RVP), working memory (Spatial Span - SSP and Spatial Working Memory - SWM), impulsive behavior control (Stop Signal Task - SST) as well as reaction time (Simple Reaction Time - SRT and Choice Reaction Time - CRT) were applied. Results: Differences were observed between a group of 20 patients on well-controlled diet and a group of 27 patients with poor dietary control with regard to the results of the tests. The overall frequency of all results of the individual tests below accepted range (<2SD) in patients with poor dietary control was significantly higher than in the well controlled patients ($p=0.022$; Fisher's exact test). Similarly, working memory deficits were found in the group of patients lacking dietary control (the number of errors observed in SWM was higher; $p=0.006$ in Student's t-test) as well as worse impulsive behavior control was observed (the number of errors in SST was higher; $p=0.021$ in Students t-test). Conclusion: CANTABeclipse system allows for reliable detection of neuropsychological deficits in non-compliant adolescents and adults with PKU. Study was sponsored by government research grant NN402329233.

2007/T

Neurocognitive Findings in Individuals with Phenylketonuria and Treatment with Sapropterin Dihydrochloride (BH4). *D.K. Grange[1], D.A. White[2], S.E. Christ[3].* 1) Div Genetics and Genomic Medicine, Dept. Pediatrics, Washington University, St Louis, MO; 2) Dept. Psychology, Washington University, St Louis, MO; 3) Dept. Psychological Sciences, University of Missouri, Columbia, NO.

Background/Objective: Phenylketonuria (PKU) is a disorder in which phenylalanine (Phe) metabolism is disrupted. The disorder is associated with dopamine disregulation and white matter abnormalities in the brain. Impairments in cognition (particularly executive abilities) are common, even in patients treated early and continuously with dietary Phe restriction. Sapropterin dihydrochloride (BH4) is a pharmaceutical agent that lowers Phe in BH4 responders. We are evaluating changes in brain and cognition that occur following BH4 treatment. Method: Brain and cognition are evaluated in PKU patients at baseline before BH4 treatment (20mg/kg/day) using MRI/DTI (diffusion tensor imaging) and neuropsychological tests focused on executive abilities. For BH4 responders, follow-up evaluation is conducted after 6 months of BH4 treatment. Data collection is ongoing. At this time, participant ages range from 7 to 35 years (M=18; SD=8). Evaluation at baseline has been conducted with 19 PKU patients and 12 controls, and at follow-up with 5 PKU patients and 5 controls. Results/Conclusions: Baseline findings to date indicate that executive performance is significantly poorer for PKU patients than controls across a range of tasks assessing abilities such as inhibitory control (go/no-go, $p=.04$; stimulus-response compatibility, $p=.03$), strategic processing (verbal fluency, $p=.007$; word list learning, $p=.001$), and working memory (2-back, $p=.001$). These results reflect specific and pervasive impairments in executive abilities prior to treatment with BH4. Follow-up findings provide evidence of improvement in executive abilities following treatment with BH4. Baseline findings from newly enrolled patients will be presented, as well as specific findings from the follow-up neuropsychological assessments and MRI/DTI data.

2008/T

A novel splice mutation in CYP27A1 associated with cerebrotendinous xanthomatosis (and pulverulent cataract). *S. Joyce[1], R. Bourkiza[2], E. Meyer[1], A. Reddy[2], H. Patel[2], M. Chan[2], E.R. Maher[1].* 1) Medical & Molecular Genetics, University of Birmingham, Birmingham, United Kingdom; 2) Barts and the London NHS Trust, London, United Kingdom.

Cerebrotendinous xanthomatosis (CTX) is a lipid storage metabolism disorder, inherited in an autosomal recessive manner. CTX is characterized by neurological findings (such as cerebellar ataxia and dementia), premature atherosclerosis, tendon xanthomas and cataracts. It is caused by mutations in CYP27A1, leading to sterol 27-hydroxylase deficiency and abnormal cholestanol tissue deposition. A consanguineous Bangladeshi family with three children with pulverulent cataract and global developmental delay were ascertained. Since learning difficulties could also be observed in two of the other siblings who showed no evidence of cataract, the underlying diagnosis and inheritance pattern was unclear. Genetic linkage studies, employing an autozygosity mapping approach, were undertaken to ascertain the underlying genetic cause. The results showed linkage to the chromosomal region 2q35-q36 which contained the CYP27A1 gene. Direct sequencing of CYP27A1 revealed a homozygous splice mutation in all family members with cataracts. This finding confirms the validity of this approach when identifying genes responsible for disorders, even when the aetiology of the condition and diagnosis are uncertain. It highlights the importance of evaluating if CTX is an appropriate diagnosis when the proband has cataracts, especially since the condition can be treated if identified before irreversible brain damage.

2009/T

Assessment of a high-throughput DNA melting analysis assay for rapid screening of gene variants in the Ornithine Transcarbamylase Gene. *K. Sumner[1], L. Hubley[1], S. Dobrowolski[1], G. Pont-Kingdon[1], R. Margraf[1], H. Best[1,2], E. Lyon[1,2].* 1) ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT; 2) Dept of Pathology, University of Utah School of Medicine, Salt Lake City, UT.

Abstract: Ornithine transcarbamylase (OTC) deficiency is the most common inherited defect of the urea cycle and is caused by mutations in the OTC gene (Xp21.1). Over 450 disease-causing mutations have been reported, most of which are family specific, with the vast majority occurring within the exons and intron/exon boundaries of the OTC gene. All 10 exons in the OTC gene are 154bp or smaller making this an ideal target for exon scanning. **Methods:** Exons and intron/exon boundaries were amplified in the LightCycler 480 Real-Time PCR System (Roche Applied Science, Indianapolis, IN) using the LightScanner Master Mix with LCGreen Plus+ Melting Dye (Idaho Technology Inc., Salt Lake City, UT) and then subsequently melted on the same instrument or they were amplified on the GeneAmp PCR System 9700 (Applied Biosystems, Carlsbad, CA) and melted on the LightScanner System (Idaho Technology, Inc., Salt Lake City, UT). Melting curves were then analyzed for differences in melting signatures. As a control, a redundant genotyping melt was also performed to characterize three common OTC polymorphisms. Melting profiles were obtained for 11 deidentified, normal female samples that were submitted to ARUP Laboratories for unrelated testing. In addition, the OTC gene was sequenced in each of these patients to confirm melting curve results. **Results:** One sample was excluded due to poor amplification. Ninety-nine out of 100 exons analyzed were concordant with their sequencing results. Seven exons from 3 samples had deviant melting curves, 8 exons from the same 3 samples contained sequence variations. One sample with a homozygous polymorphism had a melt similar to wild-type samples when using the LightCycler 480 but was distinguishable from the wild-type samples using the genotyping melt assay when the sample was amplified on the GeneAmp PCR System 9700 and melted on the LightScanner System. **Conclusions:** Ninety-nine out of the 100 exons analyzed were concordant with their sequencing results giving the assay an overall accuracy of 99% (excluding all exons from the poorly amplified sample) when amplified on the LightCycler 480 using the LightScanner Master Mix with LCGreen Plus+ dye and then subsequently melted on the same instrument. In summary, DNA Melting analysis is a rapid and inexpensive method to screen for OTC deficiency.

2010/T

A mutation potentially altering ubiquinone binding of ETF dehydrogenase manifests as mild glutaric aciduria type II with severe complex II-III deficiency in liver and skeletal muscle. *J. Vockley[1,2,3], M. He[4,5], N. Payne[1], K.M. Gibson[1,2,4,6], L.A. Wolfe[1,7].* 1) Pediatrics, Children's Hosp of Pittsburgh of UPMC, Pittsburgh, PA; 2) Department of Pediatrics, University of Pittsburgh, School of Medicine, Pittsburgh, PA; 3) Department of Human Genetics, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA; 4) Department of Pathology, Children's Hospital of UPMC, Pittsburgh, PA; 5) Department of Human Genetics, Emory University, School of Medicine, Decatur, GA; 6) Department of Biological Sciences, Michigan Technology University, Houghton, MI; 7) Undiagnosed Diseases Program, National Human Genome Research Institute, Bethesda, MD.

Purpose: Describe clinical and biochemical features of a male with recurrent hospitalizations for recurrent vomiting associated with mild hyperammonemia and elevated CK. Case Review: A 22-year-old male developed severe hypoglycemia (20 mg/dl) and lethargy during an acute illness at 4 months and subsequently grew and developed normally. At age 4 years he developed recurrent vomiting associated with mild hyperammonemia and dehydration requiring frequent hospitalizations for IV fluids. Glutaric aciduria Type II was suspected based upon biochemical findings and managed with cornstarch, carnitine and riboflavin supplements. He did not experience metabolic crises or hospitalizations between ages 4-12 years. He was noted to experience recurrent vomiting, mild hyperammonemia without hypoglycemia, and generalized weakness, primarily associated with acute illnesses and growth spurts. At approximately age 18 years, he developed exercise intolerance and proximal muscle weakness leading to further evaluation. Methods Used: Retrospective records review, prospective evaluation and clinical biochemical testing identified multiple acyl-CoA dehydrogenase and complex II/III deficiencies in both skeletal muscle and liver. Plasma acylcarnitine analyses at this time were unremarkable, while urine organic acid analysis revealed only moderate and variable levels of hexanoylglcyine (HG). Subsequent molecular characterization of the ETFDH gene revealed novel heterozygous mutations, p.G274X:c.820G>T (exon 7) and p.P534L: c.1601C>T (exon 12), the latter within the iron sulfur-cluster and predicted to affect ubiquinone reductase activity of ETFDH as well as the docking of ETF to ETFDH. These mutations were confirmed in parental DNA samples. Results: Our patient, and his unique alleles, support the concept of a structural interaction between ETFDH and other enzyme partners, and predicts that the conformational change upon ETF binding to ETFDH may play a key role in linking ETFDH to II/III super-complex formation interrelating metabolic pathways.

2011/T

Pilot study to evaluate the effects of Sapropterin on adult individuals with Phenylketonuria with measurable maladaptive behaviors. *S. Yano[1], K. Moseley[1], J. Ottina[1], C. Azen[2], R. Koch[1].* 1) Pediatrics/Gen Div, 1G24, LAC+USC Medical Center, University of Southern California, Los Angeles, CA; 2) General Clinical Research Center, University of Southern California/Keck School of Medicine, Childrens Hospital Los Angeles, CA.

Background: We report 12 month data on a pilot study to evaluate changes in behavior while on sapropterin (Kuvan®), a drug that is used for the treatment of PKU. Kuvan® functions like BH4, a co-factor for phenylalanine (phe), tyrosine, and tryptophan hydroxylases. Kuvan® may affect tyrosine and tryptophan hydroxylases in the brain and affect behavior without a reduction in blood phe levels. Objectives: To evaluate effects of Kuvan® on maladaptive behavior in patients with PKU. Material and methods: Ten subjects (>18 years) with maladaptive behavior were enrolled in a 12-month study. Kuvan® was given at 20mg/kg/day. Baseline and quarterly measures of plasma amino acids, as well as baseline, six-month and 12-month evaluation of the Vineland II Adaptive Behavior Scales (VABS-II) and a PKU Behavior Check List were obtained. Results: Comparison of 12-month data to baseline showed no change in blood phe levels (p=0.33), but increased blood tyrosine levels (p=0.05) and decreased blood phe/tyrosine ratio (p= 0.067). The VABS-II showed no change in communication, daily living skills, socialization, or motor skills, but significant improvement for internal behavior including anxiety, nervousness, and unexplained sadness (p=0.018). On the PKU Behavior Check List, subjects showed significant improvement in the sum of scores over the 15 negative behaviors (p<0.0001). Conclusion: PKU subjects who did not respond to Kuvan in blood phe level, showed significant improvement in maladaptive behavior, may suggest effects of Kuvan in the CNS. Long term evaluation of CNS effects of Kuvan is warranted.

2012/T

Is that mitochondrial has any role in Freidreich Ataxia? *M. Naseroleslami[1], M. Heidari[3], K. Parivar[1], S. Sanjarian[1], M. Houshmand[2,4].* 1) Science and Research branch,Islamic Azad University, tehran, Iran; 2) National Institute of Genetic Engineering and Biotechnology, Tehran,Iran; 3) Faculty of Shahid Sadooghi University,Yazd, Iran; 4) Special Medical Center, tehran, Iran.

Introduction: Friedreich's ataxia (FRDA) is an inherited recessive disorder characterized by progressive neurological Disability. The expansion GAA Repeat cause a sever decrease in the expression of frataxin. A deficiency in the protein frataxin causes this disease and the number of triple repeat GAA in the patients is more than 33 repeats. Frataxin interfere with the mitochondrial oxidative process and enhance iron accumulation .decreased oxidation activity a strong negative correlation with the number of GAA Repeats. In 97% of FRDA patients there is expansion of unstable GAA trinucleotide repeats in intron 1 and 2%-3% patients are heterozygote with point mutations. Materials and Methods: Spectrophotometry, temperature gradient gel electrophoresis (TTGE) and PCR-sequencing methods were used to analysis of DNA sample from 25 FA patients. For study role of mitochondrial and role of oxidative stress in FRDA patients. Results: Biochemical study showed that complex I activitiy and intracellular ATP were significantly reduced in patients compared to controls and we found strong, correlation between complex I activity and intracellular ATP content in FRDA patients, (n=12) and control subjects (n=25). (r = 0.93; P<0.002), This study suggested that a biochemical defect in complex I activity and ATP production, which may be due to iron accumulation in mitochondria, can be involved in age of onset of FRDA. We observed 8.6 and 9 Kb deletions in 9 from12 patient (75%) by multiplex PCR and Southern blot analysis. Our results showed also that NADH dehydrogenase (ND) genes mutations in FRDA samples were higher than normal controls (P < 0.001) and we found statistically significant inverse correlation (r = -0.8) between number of mutation in ND genes and age of onset in FRDA patients. In study by TTGE, for the first time, we detected 26 mtDNA mutations; of which 5 (19.2%) was novel and 21 (80.8%) have been reported in other diseases. Investigation of exons 3 of FRDA in 50 patients with a clinical diagnosis of Friedreich ataxia showed SNP (C 832729 T) in heterozygote and homozygote state. No pathogenic mutation was found in our patient by now but investigation of other exons and also other region of FRDA genes continue in our lab. Discussion: 1) Mitochondria play important role in FA. 2) Mutation analysis needs for patients who had shown expansion in one allel.

2013/T

Evaluation of oral feeding of N-acetylmannosamine-related sugars as therapeutics for a knock-in mouse model of *Hereditary Inclusion Body Myopathy* (HIBM). *C. Ciccone[1], A. Astiz-Martinez[1], L. Vincent[1], M. Lin[1], T. Yardeni[1,2], S. Kakani[1], P. Zerfas[3], W.A. Gahl[1], M. Huizing[1].* 1) Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD; 2) Graduate Partner Program, Sackler School of Medicine, Tel Aviv University, Israel; 3) Division of Veterinary Resources, Office of Research Services, National Institutes of Health, Bethesda, MD.

Hereditary Inclusion Body Myopathy (HIBM) is a recessive adult-onset neuromuscular disorder, characterized by progressive muscle weakness caused by mutations in UDP-GlcNAc 2-epimerase/ManNAc kinase (*GNE*), the key enzyme in sialic acid biosynthesis. We created a *Gne* knock-in mouse model with the human Persian-Jewish M712T founder mutation. Mutant mice died before postnatal day 3 (P3) of unexpected glomerular disease involving effacement of podocytes and hyposialylation of podocalyxin, a glomerular sialoglycan. Administration of the sialic acid precursor N-acetylmannosamine (ManNAc) partially rescued the renal phenotype and allowed survival beyond P3 in 46% of mutant mice. We evaluated the effects of a variety of ManNAc-related sugars on glomerular disease in our mouse model. Sialic acid, mannose, GlcNAc, galactose, or mannosamine were administered in the drinking water to pregnant and nursing mice (1 g/kg/day). Survival of mutant pups beyond P3 was monitored and renal phenotypes were assessed by histology, western blotting, and electron microscopy (EM). Oral treatment of sialic acid, GlcNAc, mannose, GlcNAc and galactose did not improve survival beyond P3, nor did these sugars restore the hyposialylation in mutant mice. Surprisingly, oral administration of mannosamine proved more effective than ManNAc for survival of mutants beyond P3, achieving 56% survival. Western blotting of mannosamine-fed mutants showed improved sialylation of podocalyxin, and improved podocyte ultrastructure on EM. Further research is needed to elucidate whether mannosamine is converted into ManNAc, which feeds into the sialic acid synthesis pathway, or whether mannosamine rescues the phenotype through another mode of action (e.g., chaperoning GNE, assisting folding). This finding may assist in further elucidating the pathology of the glomerular disease in our mice and the muscle phenotype in HIBM patients. Most importantly, mannosamine should be considered as therapy for patients with HIBM, and may also be beneficial for treatment of glomerular diseases with podocytopathy due to hyposialylation, including certain forms of minimal change nephropathy and focal segmental glomerulosclerosis.

2014/T

Further characterization of Congenital Disorder of Glycosylation IIb in siblings. *G.A Golas[1], L.A Wolfe[1], M. He[2], B. Xia[2], W. Zhang[2], X. Song[3], R. Cummings[3], D.R. Adams[1], S. Yang[1], A. Gropman[1,4], C.J. Tifft[1].* 1) Undiagnosed Diseases Program, NHGRI Bethesda, MD; 2) Department of Human Genetics, Emory University, School of Medicine, Decatur, GA; 3) Department of Biochemistry, Emory University, School of Medicine, Decatur, GA; 4) Department of Neurology, Children's National Medical Center, Washington, D.C.

Purpose: Describe clinical and biochemical features of two siblings presenting to the Undiagnosed Diseases Program (UDP). Case review: Two siblings born to non-consanguineous, Northern European parents with negative family history and maternal hypothyroidism and gestational diabetes were evaluated. The older sibling was delivered at 40 weeks gestation by emergency C-section. Birth measurements were normal. Postnatal course was complicated by hypotonia with poor latch and suck requiring bottle feeding. By 8 months of age, he had global developmental delay. Comprehensive molecular and biochemical evaluations were unrevealing. Evaluation at age 11 by the UDP, revealed mild dysmorphia, moderate hypotonia, joint laxity, normal reflexes, and no organomegaly. Weight was 90th centile and height 10th centile. He was non-verbal and able to sit independently and ambulate with assistance. His sister was delivered at 40 weeks gestation by repeat C-section. Birth measurements were normal. She developed generalized seizures within the first 24 hours. The CK was 592 U/L and ammonia 79 umol/dL. Brain ultrasounds and CT were normal. When evaluated at age 6 by the UDP, she was mildly dysmorphic with nystagmus, severe hypotonia, joint laxity, adducted thumbs bilaterally, normal reflexes, and no organomegaly. Height and weight were both below the 3rd centile, at the 50% centile for a 3-=BD year old. She was non-verbal and unable to sit independently. Methods used: Prospective evaluation and clinical biochemical testing. Evaluations revealed generalized cerebral atrophy, delayed myelination and low NAA by brain MRI/MRS in both sibs, cortical visual impairment with optic nerve atrophy. CSF studies identified cerebral folate deficiency in the younger sibling. Urine oligosaccharides on both sibs demonstrated a tetrasaccharide band. Further investigation, identified it as Hex4 with 3 glucose and 1 mannose. The mannose is at the reducing end. DNA studies on the identified three mutations in the GCS1 gene and single mutations in the mother and unaffected sibling. This gene encodes alpha-glucosidase Ia, a protein described in the Congenital Disorder of Glycosylation type IIB in a single case report in 2000. Results: This case underscores the difficulties of diagnosing complex multi-system disease especially when affected siblings have slightly different phenotypes. It also expands the phenotype of CDGIIb.

2015/T

Somatic mosaicism in Menkes disease suggests choroid plexus-mediated copper transport to the developing brain. *P. Johnson[1], A. Donsante[1], L. Jansen[2], S. Kaler[1].* 1) Molecular Medicine Program, NICHD/NIH, Bethesda, MD; 2) Division of Pediatric Neurology, Seattle Children's Hospital Research Institute, University of Washington, Seattle, WA.

The primary mechanism of copper transport to the brain is unknown, although this process is impaired in Menkes disease, an X-linked disorder caused by mutations in a copper transporter, ATP7A. Potential central nervous system entry routes for copper include brain capillary endothelial cells that originate from mesodermal angioblasts and form the blood-brain barrier, and choroid plexuses, which derive from ectoderm, and form the blood-cerebrospinal fluid barrier. We exploited a rare (and first reported) example of somatic mosaicism for an ATP7A mutation to shed light copper transport into the developing brain. In a 20 month old Menkes disease patient evaluated before copper treatment, blood copper and catecholamine concentrations were normal, whereas levels in cerebrospinal fluid were abnormal and consistent with his severe Menkes phenotype. We documented disparate levels of mosaicism for an ATP7A missense mutation, P1001L, in tissues derived from different embryonic origins; allele quantitation showed P1001L in approximately 27% and 88% of DNA samples from blood cells (mesoderm-derived) and cultured fibroblasts (ectoderm-derived), respectively. These findings imply that the P1001L mutation in the patient preceded formation of the 3 primary embryonic lineages at gastrulation, with the ectoderm layer ultimately harboring a higher percentage of mutation-bearing cells than mesoderm or endoderm. Since choroid plexus epithelia are derived from neuroectoderm, and brain capillary endothelial cells from mesodermal angioblasts, the clinical, biochemical, and molecular (Table) findings

Source	% Normal allele (PCR/ Densitometry)	% Normal allele (Q-RT-PCR)
Blood	77.1 +/- 5.48	68.9 +/- 4.16
Fibroblasts	12.24 +/- 5.24	12.34 +/- 4.71

in this infant support a critical role for the choroid plexus epithelia in copper entry to the developing brain.

2016/T

Social Neuropeptides Oxytocin and Vasopressin are Dysregulated in Williams Syndrome. *J.R. Korenberg[1], L. Dai[1], H. Hossein Pournajafi-Nazarloo[2], U. Bellugi[3], C.S. Carter[2].* 1) Center for Integrated Neuroscience and Human Behavior, University of Utah, Salt Lake City, UT 84108, USA; 2) Brain-Body Center, University of Illinois at Chicago, Chicago, IL 60612, USA; 3) Laboratory for Cognitive Neuroscience, Salk Institute, La Jolla, CA 92037, USA.

The social neuropeptides, oxytocin (OT) and Arg-vasopressin (AVP) are involved in the control of social and reproductive behaviors broadly in mammals but whether they play a role in the internal circuitry regulating human behavior is unknown. Williams Syndrome (WS) is a neurobehavioral disorder that is caused by a ~1.5 Mb deletion of 7q11.23 and is uniquely associated with a gregarious personality, a strong drive to approach strangers and an attraction to music. WS provides an unprecedented opportunity to link genetics, neurobiology, and behavior. We hypothesized that alterations in OT and AVP might in part underlie the increased social behavior and response to music in WS. To test this, we established levels of OT and AVP at two baseline points and at eight points after stimulation with music (a self-defined positive stimulus), followed by a mild stressor (cold pressor). Samples collected beginning 30 mins after placement of an indwelling catheter and analyzed using enzyme immunoassays. The data from 13 subjects with WS and 8 age, gender and ethnicity matched controls revealed altered regulation of OT and AVP in subjects with WS. WS subjects showed 5-fold higher mean basal oxytocin (p is less than 0.001) and 3-fold higher vasopressin (p=0.15) levels vs. controls. Longitudinal changes in OT (expressed as % Δ vs. baseline in the peak response during and immediately following the stimulus) exhibited both greater variability (p=0.025 with music; p=0.007 with cold) and greater average increases in WS subjects vs. controls (30% vs. 9% geometric mean increase, p = 0.21, with music; 21% increase vs. 2% decrease, p= 0.01, with cold). Longitudinal changes in AVP exhibited similar but weaker trends, which did not reach statistical significance, for greater variability and greater average increases in WS subjects vs. controls. The results indicate that a subgroup of WS subjects exhibited amplified peak releases of OT, and possibly AVP, in response to music and cold compared to controls. This is the first direct evidence in humans, showing that endogenous circuitry involving OT and AVP might regulate the response to social-emotional stimuli. Furthermore, the genetic circuits that regulate OT and AVP are largely unknown and the data implicate one or a cluster of genes in the WS region. WS provides a unique genetic model for understanding the neurobiology of human sociality and emotion.

2017/T

Biochemical predictors of outcome in methylmalonic aciduria. *H. Vernon[1], A. Hamosh[1], L. Kratz[2], D. Valle[1].* 1) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Biochemical Genetics Laboratory, Kennedy Krieger Institute, Baltimore, MD.

We performed a retrospective analysis of clinical and laboratory data from 11 patients with methylmalonic aciduria (MMA) seen at Johns Hopkins Hospital over the past 16 years: 5 with mut (0), 3 with B12 responsive MMA, 2 with cobalamin B defect, and 1 with mut (-). There were a total of 219 admissions for these patients: 189 in patients with mut (0), 21 in patients with B12 responsive MMA, 8 in patients with Cbl B defect, and 1 in the patient with mut (-). Of the 8 patients who developed renal failure 5 have mut (0), 2 have cobalamin B defect, and 1 has B12 responsive MMA. Additionally, there were 29 acute adverse events: in the mut (0) group there were 6 episodes of basal ganglia (BG) infarction and 20 episodes of pancreatitis; in the B12 responsive group there were 2 episodes of BG infarction; and in the Cbl-B defect group there was 1 episode of BG infarction. To better understand the pathophysiology of MMA, we analyzed plasma and urine methylmalonate levels, blood urea nitrogen, creatinine, acid/base status, ammonia, amylase, lipase and complete blood counts in each patient over time. In 6 patients, there was sufficient data available to compare inpatient and outpatient methylmalonate levels (a total of 41 hospitalizations). Of the 8 patients with renal failure, 4 showed a lifetime trend of increasing plasma and urine methylmalonate levels. Despite this relationship, acutely increased levels of methylmalonate did not correlate with intercurrent hospitalizations, because after the initial diagnostic admission in each of these patients there was no significant difference between inpatient and outpatient levels of methylmalonate. The number of methylmalonate determinations was not sufficient to allow for a correlation to the acute adverse events. However, since there was no difference between outpatient and inpatient methylmalonate levels, we speculate that intercurrent illness leading to hospitalization may reflect changes in tissue sensitivity to relatively constant concentrations levels of accumulated metabolites. This model predicts that, in addition to reducing metabolite accumulation, therapeutic efforts should be directed at correcting tissue sensitivity.

2018/T

An unusual transferrin glycoform in a patient with developmental delay, failure to thrive, anemia, and dysmorphic features suggests a new type of congenital disorder of glycosylation. *M.F. Walsh[1,2], K.M. Raymond[3,4], H.F. Freeze[5,6], P. HE[5], G.H.B. Maegawa[1,2].* 1) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins Hospital, Baltimore, MD; 2) Department of Pediatrics, Johns Hopkins Hospital, Baltimore, MD; 3) Biochemical Genetics Laboratory, Mayo Clinic, Rochester, MN; 4) Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN; 5) Sanford-Burnham Medical Research Institute, La Jolla, CA; 6) Sanford Children's Health Research Center, La Jolla, CA.

Congenital Disorders of Glycosylation (CDG) are a heterogeneous group of disorders of abnormal N-linked oligosaccharides caused by deficiency of specific enzymes in N-linked oligosaccharide synthetic pathway, resulting in a broad clinical spectrum (Sparks, 2009). CDGs were previously termed carbohydrate deficient syndromes (Hagberg, 1993). Nearly all proteins that travel through the endoplasmic reticulum - Golgi conduit are N-glycosylated (Freeze, 2006). Some characteristics include variable degrees of developmental delay, hypotonia, failure to thrive, diarrhea, and electrolyte imbalances (Denecke, 2005). Case Report: We describe a 16-month-old male with failure to thrive, developmental delay, mild dysmorphic features, anemia and watery stools. Child was born full-term via vacuum assisted vaginal delivery and was documented IUGR. Salient features at birth included hypospadias requiring a two staged surgery and testosterone therapy. At 16-months, his weight was 9.35kg (<3%), his height was 78 cm (20-30%), and his head circumference was 46cm (25-50%). On P/E, frontal bossing, hypertelorism, genitourinary anomalies, a sacral dimple, torticollis, and pes plan valgus were noticeable. Failure to thrive investigations included normal testing for: celiac disease, cystic fibrosis, and thyroid disease. IGF levels were borderline low prompting an MRI, which, was normal. Folate, vitamin B12, iron levels and hemoccult testing were normal in assessment of the patient's normocytic anemia. Inherited metabolic investigations were inconclusive. Standard CDG testing performed by Immuno-Affinity Chromatography / Mass Spectrometry revealed an unusual transferrin glycoform. Normally transferrin has 2 N-linked sugar chains and mass of 79551 Da. Our patient also has a prominent, unprecedented transferrin variant of 81701Da, consistent with complete 3 N-glycan chains and loss of 1 Fe. This finding suggests a novel congenital disorder of glycosylation. Conclusion: This patient's diagnosis of a congenital disorder of glycosylation is based on the presence of a novel hyper-glycosylated variant of transferrin. Parental testing is underway to evaluate if the glycosylation pattern is inherited or de novo; mapping is currently in progress to further evaluate the significance of the novel glycoform. Since the transferrin glycosylation pattern has never been previously reported, this may uncover another type of CDG, correlating with the clinical phenotype herein described.

2019/T

Systemic Biomarker Profiling of Metabolic and Dysplastic Skeletal Diseases Using Multiplex Serum Protein Analyses. *D. Wenkert[1], M.P. Whyte[1], D.C. Dwyer[2], D.L. Lacey[2], M. Stolina[2].* 1) Shriners Hosp Children, St. Louis, MO; 2) Amgen, Incorporated, Thousand Oaks, CA.

Understanding of the etiology and pathogenesis of metabolic and dysplastic skeletal diseases has come from both genetic and biochemical investigations. Here, we describe our approach to systemic biomarker profiling using multiplex serum protein analyses in healthy controls and to study age- and disease-mediated changes in patients as well as family members with these disorders. The Research Center at Shriners Hospital for Children, St. Louis, Missouri, serves as a national referral center for inpatient diagnosis, treatment, and investigation of pediatric dysplastic, nutritional, and metabolic bone disorders where collection of fasting blood is routine. At Amgen (Thousand Oaks, California), Luminex-based, micro-bead, multiplex kits are used to assay serum osteopontin, osteocalcin, ligand of receptor activator NF-κB (RANKL), osteoprotegerin (OPG), fibroblast growth factor 23 (FGF23), matrix metalloproteinases (MMP 1, 3, 7, 8, and 13), and cytokines. ELISA kits are used for serum prostaglandin E_2 (PGE_2), bone-specific alkaline phosphatase (BAP), tartrate-resistant acid phosphatase (TRACP 5B), and collagen formation/degradation markers. Serum levels of sclerostin (SOST) and dickkopf (DKK1) were evaluated by custom ELISAs (developed at Amgen, Inc). All sera were obtained after donors fasted for at least 4 hours. To establish reference ranges, we studied 9 healthy children and 18 healthy adults (ages 6-60 yrs). MMPs (1, 3, 7, 8, and 13) correlated positively with age (R^2 = 0.26 - 0.64, $p<0.05$), whereas osteocalcin, BAP, c-terminal propeptide of collagen type I (CICP), and TRACP 5B correlated negatively (R^2 = 0.23 - 0.71, $p<0.05$). The patients represented 30 established and 14 unique diagnoses. Affected family members (dominant disorders) and obligate carriers (recessive disorders) were also studied, comprising a total of 110 individuals. Our analyses revealed that serum concentrations of FGF23 were significantly increased in X-linked hypophosphatemia (XLH), TRACP 5B in osteopetrosis, and RANKL in juvenile Paget's disease, whereas decreases in BAP were documented for hypophosphatasia, validating our array strategy. Hence, our multiplex serum protein analyses could reveal unique markers for specific bone disorders, and enhance understanding for a wide range of skeletal diseases.

2020/T

Mucoployssaccharidosis type IIIB and GalNAc Transferase Double Knockout Mice. *E.E.A. Mohammed[1], E.M. Whitley[2], E.M. Snella[1], M.M. Rutz-Mendicino[1], F.D. Echevarria[1], N.M. Ellinwood[1,3].* 1) Animal Science, Iowa State University, Ames, IA; 2) Veterinary Pathology, Iowa State University, Ames, IA; 3) Veterinary Clinical Sciences, Iowa State University, Ames, IA.

Mucopolysaccharidosis type IIIB (MPS IIIB) is a neuropathic lysosomal storage disorder (LSD) resulting from an inherited deficiency of *N*-acetyl-α-D-glucosaminidase (Naglu) activity, an enzyme required to degrade the glycosaminoglycan heparan sulphate (HS). A deficiency in Naglu activity leads to the lysosomal accumulation of HS as a primary storage substrate, and the gangliosides GM2 and GM3 as secondary storage products. To test the effect on neuropathogenesis of ganglioside accumulation, we bred mice deficient in both Naglu and GalNAcT activity. The latter is the enzyme required for the synthesis of GM2 and other complex gangliosides. Contrary to our expectation and to double knockout (DKO) studies where GalNAcT was knocked out in combination with other LSDs, our DKO mice showed a drastically shortened lifespan (20 ± 3.4 weeks, versus 45 ± 6.9 wks (MPS IIIB), and 45 ± 11.03 wks (GalNAcT)). To confirm that HS storage was the primary element resulting in the accelerated disease in our DKO mice, and not a locus tightly linked to the Naglu gene, we replicated our study with MPS IIIA mice, and found a virtually identical result (26 ± 4.9 weeks, versus 53 ± 2.1 wks). All DKO mice showed motor signs of hind limb ataxia and hyper-extension, which were not seen in the single KO or normal mice. At approximately 5 months of age the MPS IIIB DKO showed a unique pattern of vacuolization and nerve fiber degeneration in the corpus callosum as well as the relatively early intracytoplasmic vacuolation of many neurons and glia, the latter also seen in the MPS IIIB mice. We evaluated motor performance on a rocking Roto-rod beginning at 2 months of age. At five months of age the MPS IIIB DKOs performed far worse (p £ 0.0006) than all other genotypes (139 ± 25 sec (DKO), versus 252 ± 18 sec (MPS IIIB), 270 ± 19 sec (GalNAcT), and 293 ± 14 sec (norm). In conclusion we identified an accelerated form of MPS IIIB within a DKO model system which showed white matter changes, with attendant performance deficits and a drastically shortened lifespan. This was in stark contrast to our expectations of a salutary response to the elimination of GM2. Despite this, the accelerated pathology and clinical signs represent an improved system to study the MPS IIIB response to therapies, and may be important to investigate neuropathogenesis as well as the role of complex gangliosides in normal CNS function. Funded by; CIAG of ISU, the LSDRC, and The Sanfilippo Children's Research Foundation.

2021/T

A successful tool to predict antibodies against therapeutic proteins: Experience from infantile Pompe disease. *S.G. Banugaria[1], S.N. Prater[1], Y-C. Hsueh[2], Y-K. Ng[3], J.A. Kobori[4], R.S. Finkel[5], R.L. Ladda[6], A.S. Rosenberg[7], Y-T. Chen[2], M-J. Hwang[2], P.S. Kishnani[1].* 1) Department of Pediatrics, Division of Medical Genetics, Duke University Medical Center, Durham, NC, USA; 2) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 3) Department of Pediatrics, University of Hong Kong, Hong Kong, China; 4) Genetics Department, Permanente Medical Group, San Jose, CA, USA; 5) Division of Neurology, Children's Hospital of Philadelphia, Philadelphia, PA, USA; 6) Department of Pediatrics, Penn State University, Hershey Medical Center, Hershey, PA, USA; 7) Division of Therapeutic Proteins, Office of Biotechnology Products, Center for Drug Evaluation and Research, United States Food and Drug Administration, Bethesda, MD, USA.

With the advent of enzyme replacement therapy (ERT) with alglucosidase alfa (Myozyme®) for Pompe disease, the clinical course of the disease has changed. We have previously described the poor outcome in cross reactive immunologic material (CRIM)-negative patients secondary to high sustained antibody titers (HSAT) to ERT. More recently, we have also shown that a subset of CRIM positive patients develop HSAT and, in essence, behaved similar to CRIM negative patients. There is emerging data on success of tolerance induction therapies in abrogating the effect of antibodies if started early or ideally in naïve setting. However, once HSAT are formed, tolerization therapy has uniformly failed to lower antibody titers. While CRIM negative status can be easily identified by western blot and mutation analysis, the current challenge is the early identification of patients at-risk for developing HSAT amongst CRIM positive patients. We evaluated a cohort of 31 CRIM positive infantile Pompe disease patients: 11 high-titer CRIM-positive (HTCP) patients and 20 low-titer CRIM-positive (LTCP) patients. Our hypothesis was that HTCP and LTCP patients differed in the immunogenic potential of their underlying epitopes. A 3D model of acid alfa glucosidase (GAA) was derived through homology modeling using ESyPred3D based on the X-ray structure of MGAM (human intestinal maltase-glucoamylase), with which GAA shares 42.8% amino acid sequence identity. Mutational data in the context of the 3D model of GAA was then subjected to epitope prediction by ElliPro, a structure-based B-cell epitope prediction tool. Epitope prediction was made based on a protrusion index (PI), which is a measure of the extent that an amino acid residue protrudes from a protein 3D structure. LTCP mutations tended to be located in the core of GAA protein with a PI<0.5 while HTCP mutations were more externally located with a PI>0.5. Accuracy of ElliPro for these single mutation sites was 80% and 85.7% for HTCP and LTCP mutations, respectively. The approach taken here utilizing patient mutation data provides a useful strategy to identify Pompe patients at risk of developing HSAT. This tool thus allows for recognition of patients that need immune modulation prior to ERT and represents a novel approach in the field of therapeutic proteins. Methods similar to the one presented here could also represent a useful strategy in predicting antibodies in other disorders treated with therapeutic proteins.

2022/T

Gyk cGPD Double Knockout Mice Have a Longer Lifespan Than Gyk Knockout Mice. *N. MacLennan[1], A. Presson[2], M. Bedernik[1], R. Crawford[1], E. McCabe[1].* 1) Dept Pediatrics, Univ California, Los Angeles, Los Angeles, CA; 2) Dept Bio statistics, Univ California, Los Angeles, Los Angeles, CA.

The first two steps of glycerol metabolism involve glycerol kinase (GK/Gyk) glycerol phoshpate dehydrogenase (GPD). Single knockout (KO) mice of each enzyme produce two very different phenotypes. Gyk KO mice model the phenotype of human GK deficiency (GKD) except that the mice die at dol 3-4. Cytoplasmic or cGPD KO mice have no apparent adverse effects on lifespan or reproductive capability compared to wild type (WT). These mice suggest that alternate pathways of energy metabolism are available to cGPD KO but not to Gyk KO mice. Our goal was to further understand the molecular pathogenesis of GKD by generating a double KO mouse line for Gyk and cGPD. We mated the single KO lines for Gyk (G) and cGPD (C) to generate the following KO lines all having a C57BL6/J (Gyk KO strain) and BalbC (cGPD strain) mixed background strain: double WT = CG++, double KO = CG--, cGPD WT and Gyk KO = CG+-, and cGPD KO and Gyk WT = CG-+. We then performed microarray expression analysis on day of life (dol) 1 mouse pup livers and compared their gene expression levels. CG-- mice have a lifespan of 4-5 days (n=10 KO pups, 5 litters) versus CG-+ and CG++ mice that live a normal murine lifespan (about 2 years). CG+- (Gyk KO) mice die at dol 3-4. There was minimal overlap of canonical pathways among the single and the double KO mice. Four significant ($p<0.05$) canonical pathways total were altered in the CG-- mice including glycerolipid metabolism, lysine biosynthesis and glycerolphospholipid metabolism. None of these pathways in the double KO were significantly altered in the single KO data. CG+- and CG-+ mice had 14/16 and 1/3 unique pathways, respectively. Both CG+- and CG-+ revealed only 2 overlapping significantly altered canonical pathways. In contrast to CG--, CG+- and CG-+ mouse gene function profiles included cell death and cell cycle. The majority of mouse gene function profiles in the CG+- and CG-+ mouse cell death networks were related to apoptosis and survival. While both cGPD and Gyk single KO mice were enriched for cell death/cell cycle genes, this enrichment profile was not prominent in the double KO. We speculate that contributions of gene alterations comprising the unique canonical pathways and functional enrichment profiles of the single cGPD and Gyk KO mice may complement each other to partially ameliorate negative effects from the Gyk KO and account for an increase in lifespan of the double cGPD Gyk KO mouse.

2023/T

Bone Mineral Density in Patients with Homocystinuria. *J.L. Brodsky[1], K. D'Aco[1], C. Coughlin[1], C. Ficicioglu[1], R. Pyeritz[2], M.A. Levine[1].* 1) Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, PA; 2) Medical Genetics, The University of Pennsylvania, Philadelphia, PA.

BACKGROUND: Homocystinuria is an autosomal recessive disorder characterized by homocysteinemia, homocystinemia,hypermethioninemia, and homocystinuria. Defective cystathionine synthetase leads to an inability to synthesize cystathionine from homocysteine and serine in the methionine pathway. Patients with this disorder develop subluxation of the ocular lens, mental retardation, vascular disease, and osteoporosis. While several studies have reported on bone density deficits using radiographs, there has never been a study published using dual x-ray absorptiometry (DXA) to describe bone mineral density (BMD)in this population. OBJECTIVE: To examine the relationship between clinical factors and BMD in patients with homocystinuria. DESIGN/METHODS: Retrospective chart review of all patients with homocystinuria who underwent DXA from July 2002 through January 2010 during routine surveillance at The Children's Hospital of Philadelphia Clinic for Metabolic Disease and The University of Pennsylvania Center for Connective Tissue Disorders. Data collected from the medical record include patient characteristics, disease course and severity, and DXA results. At the time of DXA, data collected include serum homocystine,homocysteine,methionine,calcium,and vitamin D when available, height, BMI% and age at DXA assessment. Descriptive statistics were generated and Spearman correlation analyses performed. RESULTS: DXA results of the lumbar spine (L1-L4) were available for review in 14 patients (6M/8F; age at initial DXA 3.5-49.2 yrs, mean 17.2yrs) with a total of 25 DXA encounters. Serum homocysteine(97.2 μmol/L), homocystine (10.4 nmol/mL) and methionine (324.7 nmol/mL) were elevated, and patients were non-responsive to vitamin B6. Lumbar spine (LS) Z-score was -1.5 SD for the group, with 36% below -2 SDs. Serum 25(OH)D was 27.1±3.6 ng/mL. LS Z-scores were negatively correlated with serum homocystine($p=0.0026$), homocysteine($p=0.0075$), and methionine levels ($p<0.001$) and age of diagnosis($p=0.004$). CONCLUSIONS: Patients with homocystinuria have significantly reduced LS BMD compared to age- and sex-matched controls. BMD is negatively associated with age of diagnosis and serum levels of methionine, homocystine, and homocysteine. These data refine the skeletal phenotype of homocystinuria, and highlight the association between the metabolic defect and low BMD in this population. We propose that bone density should be assessed in all subjects with homocystinuria.

2024/T

Disease Severity in Children and Adults with Hypophosphatasia (HPP). *A. Skrinar[1], J. Smith[1], C. Patel[2], H. Landy[1].* 1) Clinical Research, Enobia Pharma, Cambridge, MA; 2) University of Texas Medical School at Houston.

Hypophosphatasia (HPP) is a rare inherited form of rickets and osteomalacia caused by inactivating mutations in the gene encoding tissue-nonspecific alkaline phosphatase (TNSALP). Birth incidence of severe patients is about 1:100,000. The biochemical hallmarks of this inborn error of metabolism are low circulating levels of ALP with elevated serum or urine levels of TNSALP substrates. The disease manifests as a broad clinical spectrum. There is no approved treatment although clinical trials of a bone-targeted enzyme replacement therapy are underway. Methods: An online survey was developed to assess disease burden in children and adults with HPP. Translations were available in English, French and German. Respondents were recruited through patient advocacy groups and small donations were made for completed surveys. Data from 90 respondents or their caregivers who reported age at symptom onset are presented. Respondents were stratified into 3 subgroups based on age of symptom onset. Results: Approximately 2/3 of the respondents were adults and 1/3 were children at the time of survey completion. Of the 90 respondents, 46 (51%) reported symptom onset in the first year of life, 27 (30%) in childhood (1-12 years) and 17 (19%) in adolescence/adulthood (over 12 years). The most common first symptoms were premature tooth loss (48%), cranial deformity (26%) and gross motor delay (17%) for those presenting in infancy, premature tooth loss (56%), fracture (26%) and bone pain (19%) for those presenting in childhood and fracture (47%) and bone pain (29%) for those presenting in adolescence/adulthood. Bony deformity and developmental delay were more common in those presenting in infancy, $p<.05$. Poorly healing fractures and wheelchair/walking aid use were more common in those presenting in adolescence/adulthood, $p<.05$. There was no significant difference in the median number of fractures (4-5) or the need for surgical fracture repair among the 3 subgroups. No more than 10% of respondents reported an improvement in their disease course over the past 5 years. Pain and limited mobility were among the top rated symptoms that interfered most with daily function. Mean SF-36 PCS, PF and BP scores were below U.S. general population norms indicating significantly diminished function and increased pain. Conclusion: Findings suggest that HPP is associated with debilitating symptoms, functional disability, pain and impaired quality of life regardless of age at symptom onset.

2025/T

An Attenuated form of Morquio Disease seen in Northern Ireland. *F.J. Stewart[1], K. Tylee[2], A. Cooper[2], J.E. Wraith[2].* 1) Dept Medical Genetics, Belfast City Hosp, Belfast, United Kingdom; 2) Willink Biochemical Genetics Unit, St. Mary's Hospital Manchester UK.

Morquio disease (mucopolysaccharidosis type IV) is a lysosomal storage disorder causing predominantly skeletal manifestations. It is caused by a deficiency of galactose-6-sulphatase and is inherited in an autosomal recessive manner. In the classical form of Morquio disease there is extreme short stature with average height being between 90 and 120 cm. There are marked skeletal deformities and many affected individuals require surgery to stabilise their cervical spine. We have identified 10 individuals in Northern Ireland who have an attenuated form of the disease - four sets of siblings and two single cases. All were found to have glycosaminoglycans (GAG's) in their urine. All were found to have reduced levels of galactose-6-sulphatase consisitent with a diagnosis of MPS type IV Morquio disease. Ages ranged from 27 years to 38 years. Height ranged from 142 cm to 160cm (5th to 50th centile). This form of the disease was initially considered relatively mild. However it is now clear that affected individuals have major problems with their joints and 6/10 patients have had at least one major joint replaced with two having had 3 joints replaced. Two further patients are undergoing orthopaedic assessment with a view to having total hip replacements. Our patients have also shown evidence of osteoporosis with decreased bone density being seen in all cases tested so far. Mutations in the GALNS gene have been characterised in all cases and include p.I113F, p.T312S and p.A241A. These patients were all initially considered to have spondyloepiphyseal dysplasia before the correct diagnosis of MPS IV was made. We believe the diagnosis of MPS IV may not be considered in patients who do not show the classical features including extreme short stature. We believe this diagnosis should be considered in young people presenting with epiphyseal dysplasia and also in young adults presenting with joint problems requiring joint replacement surgery at an early age. A urine sample should be screened for GAG's in the first instance followed up by confirmatory enzyme studies if the presence of keratan sulphate is confirmed. Clinical findings in 12 patients with MPS IV A Nelson J, Broadhead D, Mossman J. Clin Genet. 1988 Feb;33(2):111-20.

2026/T

Whole Exome Sequencing identifies AFG3L2 homozygous mutations resulting in a novel autosomal-recessive progressive myoclonic epilepsy-ataxia-neuropathy syndrome. *T.M. Pierson[1,2], D.A. Adams[1, 3], F. Bonn[4], P.F. Cheruki[5], J.K. Teer[6], N.F. Hansen[5], P. Cruz[5], N.I.S.C. Comparitive Sequencing Program[7], J.C. Mullikin[6,7], R.W. Blakesley[6], G. Golas[1,3], J. Kwan[8], T. Markello[1,3], C. Blackstone[2,9], A. Sandler[10], K. Fuentes Fajardo[1], C. Tifft[1,3], E. Rugarli[11], W.A. Gahl[1,3], T. Langer[12,13], C. Toro[1].* 1) NIH Undiagnosed Diseases Program, NIH Office of Rare Diseases and NHGRI, NIH, Bethesda, MD; 2) Neurogenetics Branch, NINDS, NIH, Bethesda, MD; 3) Office of the Clinical Director, NHGRI, NIH, Bethesda, MD; 4) Institute for Genetics, University of Cologne, Cologne, Germany; 5) Genome Technology Branch, NHGRI, NIH, Bethesda MD; 6) Genetic Disease Research Branch, NHGRI, NIH, Bethesda MD; 7) National Intramural Sequencing Center, NIH, Bethesda MD; 8) EMG Section, NINDS, NIH, Bethesda, MD, USA; 9) Cellular Neurology Unit, NINDS, NIH, Bethesda, MD; 10) Division of Surgery, Children's National Medical Center, Washington, DC; 11) Biocenter, University of Cologne, Cologne, Germany; 12) Institute for Genetics, Centre for Molecular Medicine (CMMC), Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Germany; 13) Max-Planck-Institute for Biology of Aging, Cologne, Germany.

In neurogenetics, a single gene may be involved in several distinct disorders. Examples of this phenomenon include genes for signaling molecules, cellular membrane trafficking, and energy metabolism. These genes, initially identified with one disease, were "rediscovered" in the context of another. Whole exome sequencing (WES) is used to search for new pathogenic mutations, but it also identifies variants in known genes that lead to variant phenotypes. We used WES to identify new homozygous mutations in the AFG3L2 gene resulting in a novel progressive myoclonic epilepsy-ataxia-neuropathy syndrome. Two brothers presented in late infancy with progressive ataxia, dysarthria, spasticity, and myoclonic epilepsy. Associated features included ptosis, dystonia, and action myoclonus. Cognition was intact. Testing revealed cerebellar atrophy and polyneuropathies. Muscle biopsy uncovered mitochondrial respiratory dysfunction and abnormal structure. mtDNA was mildly depleted. The parents (first cousins) were normal neurologically, except the mother had asymptomatic mild cerebellar atrophy on MRI imaging. The AFG3L2 gene causes autosomal-dominant spinocerebellar ataxia, type 28 (SCA 28), which is phenotypically similar to other progressive late-onset SCAs with dysarthria, eye movement abnormalities, and ataxia. AFG3L2 is a nuclear encoded mitochondrial protein that forms oligomeric m-AAA protease complexes, which play a major role in mitochondria ribosomal assembly and proteome quality control. AFG3L2 forms homo-oligomeric m-AAA complexes, it also forms hetero-oligomeric m-AAA complexes with paraplegin, the protein mutated in autosomal recessive spastic paraplegia, type 7 (SPG7). Paraplegin is unable to form homo-oligomers and requires AFG3L2 to function; lack of activity results in lower extremity spasticity. Our patients' mutation, resulting in a missense Y616C substitution, reduces AFG3L2 enzymatic activity in yeast expression studies, without a dominant negative effect. This mutation also inhibits the functional interaction between AFG3L2 and paraplegin in yeast expression studies. These results indicate the brothers' complex phenotype is likely the combination of abnormal activity of AFG3L2 in cerebellar cells and paraplegin in motor neurons. In summary, WES was used to identify novel homozygous mutations resulting in a new disorder primarily consisting of a combination of the SCA28/SPG7 phenotypes in association with other mitochondrial signs and symptoms.

2027/T

A novel Anderson-Fabry disease mutation in a Greek female patient presented with early-onset dystonia: An unknown Anderson-Fabry cerebrovascular phenotype? *A. Psychogios.* American Genetics Center, Nicosia, Cyprus.

We report on a novel Anderson-Fabry disease mutation identified in a Greek 28-year-old female with negative family history presented in adolescence with dystonic muscle contractions causing posturing of her feet, legs, and arms. Other clinical features included normal cognition, scanning speech, hand muscle atrophy, periumbilical and right leg angiokeratomata, hypohidrosis and severe constipation. Serial renal, eye and heart examination and imaging was normal. Previous TOR1A gene testing for early-onset primary dystonia (DYT1) was negative. Brain MRI revealed white matter changes and whole body MRA identified a moderate right femoral artery stenosis. Serial leukocyte testing for alpha-galactosidase activity was abnormal, < 3.0 % a-Gal A activity. Urine ceramide trihexoside was increased which supports the deficient alpha-galactosidase activity. GLA gene sequencing from peripheral blood leukocytes identified a heterozygous 5' UTR -30G>A alteration of uncertain clinical significance. This alteration has been recently reported to be associated with increased transcription resulting in higher than normal plasma alpha-galactosidase activity in females (Oliveira et al., 2008) and may account for some of the difficulty found in determining carrier status in females. These results support the presence of another alteration such as a deletion or duplication which may be responsible for the diagnosis of Anderson-Fabry disease in this patient. The frequency of cerebrovascular complications in carrier females has been reported as high as 28% for TIAs at a mean age of 52 years and the frequency of cerebrovascular accidents 7% at a mean age of 42 years (Mac Dermot KD et al., 2001). Experts recommend that enzyme replacement therapy (ERT) be initiated as early as possible in all males with Anderson-Fabry disease, including children and those with ESRD undergoing dialysis and renal transplantation, and in females with significant disease. In conclusion, dystonia could represent an unknown Anderson-Fabry disease manifestation and further studies are needed to clarify whether there is such a connection in females with significant cerebrovsacular disease of unknown etiology.

2028/T

Parkinsonism in Patients with type 1 Gaucher disease in the ICGG Gaucher Registry: Prevalence and Clinical Characteristics. *N. Weinreb[1], M. Balwani[2], J. Bronstein[3], E. Kolodny[4], A. Gwosdow[5], J. Taylor[5], J.A. Cole[5], S. Sathe[4], A. Zimran[6], B. Rosenbloom[7].* 1) Univ Res Foundation, Hollywood, FL; 2) Mount Sinai School of Medicine, New Yotk, NY; 3) University of California, Los Angeles, CA; 4) New York University School of Medicine, New York, NY; 5) Genzyme Corporation, Cambridge, MA; 6) Shaare-Zedek Medical Center, Jerusalem, Israel; 7) Tower Hematology Oncology, Beverly Hills, CA.

PURPOSE: Investigate the prevalence of Parkinsonism among patients with Gaucher disease type 1 (GD1) and evaluate the clinical, genotypic, and phenotypic characteristics. BACKGROUND: An association between Parkinsonism and mutations in the glucocerebrosidase (GBA) gene has been reported. GBA mutations resulting in GBA deficiency cause Gaucher disease (GD). Parkinsonism may occur with a greater than expected prevalence among GD patients. METHODS: GD patients with Parkinsonism were identified through reports to the International Collaborative Gaucher Group (ICGG) Gaucher Registry as of April 2010. A control group was constituted by all GD1 patients enrolled in the ICGG Gaucher Registry with no reports of Parkinsonism. A matched case-control analysis was conducted, whereby patients with and without Parkinsonism were matched by sex and year of birth. Up to 10 control patients were randomly selected and matched to each case patient. Demographics, genotypes and GD-related characteristics were provided for both groups. RESULTS: The matched study cohort comprised of 63 patients with Parkinsonism and 608 patients without Parkinsonism. Demographic and clinical characteristics were similar in both groups; the most prevalent genotype was N370S/N370S (approximately 40%). Patients with Parkinsonism were distinguished by 1) later median age at GD1 diagnosis (42yr) compared to controls (29yr), and 2) median age of reported Parkinsonism onset was 56yr compared to 60yr in the general population (Lancet 2009;373:2055-2066). The prevalence of Parkinsonism in all GD1 patients over 18yr in the ICGG Gaucher Registry was 1.5% (CI 1.1, 1.9). CONCLUSIONS: The clinical phenotype of GD1 patients with Parkinsonism was similar to patients with GD1 alone except the median age of GD diagnosis was older in GD1 patients with Parkinsonism. Older age at Gaucher diagnosis may be attributable to a less severe clinical GD1 phenotype. There is no evidence that severity of the common GD1 clinical manifestations was predictive for onset of Parkinsonism.

2029/T

Alpha-synuclein expression and localization in cultured neurons from glucocerebrosidase-deficient mouse models. *W. Westbroek[1], W. Xiao[1], S.W. Klontz[1], Y.N. Blech-Hermoni[1], M.R. Cookson[2], E. Sidransky[1].* 1) Medical Genetics Branch, NHGRI, NIH, Bethesda, MD; 2) Laboratory of Neurogenetics, NIA, NIH, Bethesda, MD.

Gaucher disease is an autosomal recessive lysosomal storage disorder caused by mutations in the glucocerebrosidase gene (GBA). In patients with Gaucher disease, deficiency of the enzyme glucocerebrosidase (GCase) leads to accumulation of the glycolipid glucosylceramide in reticulo-endothelial cells in the spleen, liver, bones, and, in neuronopathic forms, the brain. A subset of patients with Gaucher disease develop Parkinson disease, an adult-onset neurodegenerative disease characterized by motor dysfunction due to loss of dopaminergic neurons in the substantia nigra and alpha-synuclein protein aggregation into Lewy body structures in the brain. Recent studies have shown that subjects with Parkinson disease are over five times more likely to carry GBA mutations than controls, but the molecular mechanisms by which the two diseases are related remain unknown. We utilized mouse models of Gaucher disease including both the null allele knock-out and point mutation models to investigate the effect of GCase deficiency on alpha-synuclein expression and cellular localization. Primary embryonic hippocampal cells were cultured from E-18 mice homozygous and heterozygous for the null-allele, as well as embryonic mice with other common mutant GBA alleles. We performed immunocytochemistry on distinct primary neuronal cultures with several anti-alpha-synuclein antibodies, followed by laser scanning confocal microscopy. We found that neuronal cells with deficient or absent GCase activity had increased expression and altered localization of alpha-synuclein.

2030/T

Mechanism of neurodegeneration in ATP7A-related distal motor neuropathy is distinct from Menkes disease and occipital horn syndrome. *L. Yi, S. Kaler.* Molecular Medicine Program, NICHD/NIH, Bethesda, MD.

ATP7A is a copper-transporting ATPase that helps regulate and control cellular copper homeostasis. Defects in ATP7A lead to Menkes disease, or its allelic variants occipital horn syndrome, and isolated distal motor neuropathy, a newly discovered condition. Whereas Menkes disease and OHS share certain clinical and biochemical abnormalities, recently identified subjects with ATP7A-related distal motor neuropathy have normal serum copper, normal copper enzyme activities, normal renal tubular function, no central nervous system symptoms, and no connective tissue abnormalities. Conversely, 3 Menkes disease patients and 2 OHS patients whom we studied recently showed no clinical or neurophysiological evidence for motor neuron dysfunction, including the original OHS patient we reported, now 32, an age by which distal motor neuropathy could be expected to be manifest. Together, these clinical findings imply that the mechanism of disease in the new allelic variant is distinctly different than in Menkes/OHS. To characterize the unique missense mutations which cause ATP7A-related distal motor neuropathy, we expressed several (including P1386S and T994I) in a yeast copper transport knockout, ccc2Δ, as well as in Hek293 cells, in a fibroblast cell line from a Menkes patient with a large ATP7A deletion, and in NSC-34 motor neurons. For mammalian cell transfections, we tagged wild type and mutant ATP7A alleles with Venus-fluorescent protein. The mutant alleles complemented ccc2Δ in a range from 60-100% of normal. Expression in Hek293 cells suggested delayed trafficking of mutant ATP7As in response to copper loading, as noted previously in fibroblasts from affected patients. In addition, we observed baseline accumulation of mutant ATP7As at the plasma membrane. This phenomenon was clearer in transfected neuronal (NSC-34) cells in which mutant ATP7A also showed more diffuse intracellular localization than wild type. These results suggest that aberrant ATP7A trafficking, which may be particularly relevant when transport along lengthy axons is required, may sabotage motor neuron function and underlie this phenotype. Protein misfolding with oligomerization or aggregate formation, chronic deficiency of one or more copper enzyme(s), oxidative injury, and altered synaptic activity of ATP7A remain alternative potential disease mechanisms. Delineation of the problem in this form of distal motor neuropathy will help elucidate the normal role of ATP7A in motor neurons.

2031/T

Molecular analysis of patients with unclassified disorders of lysosome-related organelles. *J.A. Curry, A.R. Cullinane, R. Hess, C. Carmona-Rivera, D. Adams, W.A. Gahl, M. Huizing.* Medical Genetics Branch, National Human Genome Institute, NIH, Bethesda, MD.

Altered biogenesis of lysosome-related organelles (LROs) such as melanosomes, platelet delta and alpha granules, lamellar bodies, and lytic granules has been implicated in human pathologies. We investigated 46 patients at the NIH Clinical Center with rare disorders of LRO biogenesis with Hermansky-Pudlak syndrome (HPS)-like phenotypes. These patients presented with hypopigmentation, a bleeding diathesis, and occasionally other symptoms such as immunodeficiency, granulomatous colitis, neurological symptoms or pulmonary fibrosis. Of this cohort, 14 patients had absent platelet dense bodies (a hallmark for HPS) but carried no mutations in the HPS1-HPS6 genes. The remaining 30 patients had reduced or normal numbers of platelet dense bodies, but no mutations in genes associated with oculocutaneous albinism (OCA1-4). For molecular analysis of candidate genes in this cohort, we first considered genes corresponding to mouse models of 'pigment dilution and storage pool deficiency' for which there is not yet a corresponding human subtype. We began by direct sequencing of genes encoding eight subunits of the biogenesis of lysosome-related organelles complex-1 (BLOC-1), including the HPS-7 and HPS-8 genes, not previously tested in this cohort. Additional murine candidate genes include RABGGTA, Rab38, and VPS33. In addition, genes affected in the Drosophila melanogaster 'granule group' of eye color mutants are excellent candidates; these include VPS18, VPS41, rab32 and AP3 subunits. Other molecules, such as coat proteins, SNAREs, syntaxins, rabs, cytoskeletal proteins, and membrane lipids may also be candidates for causing LRO disorders and will eventually be considered. Apart from direct DNA-sequencing, other techniques such as real-time PCR, immunofluorescence microscopy and western blotting will be employed on patients' cells. Although we detected no defects in the genes screened so far, this candidate gene approach appears feasible for identifying novel LRO-related gene defects. Any novel identified gene defect may not only result in prognostic and therapeutic implications for the patients, but also present an opportunity to further elucidate the mechanisms of biogenesis of lysosome-related organelles.

2032/T

Newly identified cardiovascular complications of Menkes disease and occipital horn syndrome. *J. Hicks[1], T. Shawker[2], S. Kaler[1].* 1) Molecular Medicine Program, NICHD/NIH, Bethesda, MD; 2) Diagnostic Imaging, NIH Clinical Center, Bethesda, MD.

Menkes disease is an infantile neurodegenerative disorder caused by mutations in ATP7A, an X-chromosomal gene that encodes a copper-transporting P-type ATPase. Deficiency of the ATP7A gene product results in abnormal cellular copper transport and reduced activities of numerous copper-dependent enzymes. The classical clinical phenotype associated with mutation at the ATP7A locus includes growth retardation, peculiar hair, and focal cerebral and cerebellar degeneration. Some clinical features of Menkes disease are associated with reduced activities of specific copper enzymes. For example, deficiency of lysyl oxidase, a copper enzyme that normally plays a critical role in formation and repair of extracellular matrix via oxidation of lysine residues in elastin and collagen, has been implicated in the generalized vascular tortuosity evident in patients with Menkes disease. In the absence of normal lysyl oxidase activity, formation of covalent cross-linkages that provide tensile strength to blood vessel walls is markedly reduced. Diminished tensile strength predisposes to formation of aneurysms in the walls of high-flow blood vessels, and aneurysms have been reported in some major internal arteries in patients with Menkes disease. Aortic aneurysms are a prominent feature in one mouse model of Menkes disease, Mo-blotchy. Male mice from the Mo-Tohm strain die prenatally due to embryonic vascular abnormalities. The yolk sac of these mice display hemorrhage and arrested development of blood vessels, irregular attachment between mesoderm and endothelial cells, and disrupted collagen linkage. Mo-Tohm/+ females display fragmented elastin in the descending aorta during the neonatal period. We report on three Menkes disease patients in whom complex congenital heart disease (pulmonic stenosis, transposition of the great vessels, and tetralogy of Fallot) was noted. These patients, all identified within a three-year time frame in the United States, highlight the potential role of ATP7A in normal cardiovascular development. In addition, we have documented progressive internal jugular vein dilation in 9/14 Menkes disease infants followed by serial neck ultrasounds, presuambly a consequence of lysyl oxidase deficiency, and of uncertain clinical importance.

2033/T

Identification and Molecular Characterization of Spectrum of Mutations in Galactosemia genes from Galactosemia Patients: A Study from Tertiary Care Center in North India. *R. Singh[1], G. Kaur[2], B.R. Thapa[3], R. Prasad[1].* 1) Biochemistry, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, Chndigarh, India; 2) Department of Physiology, Government Medical College and Hospital, Chandigarh, India; 3) Division of Pediatric Gastroenterology, Postgraduate Institute of Medical Education and Research, Chandigarh, India.

Introduction: Galactosemia is an autosomal recessive metabolic disorder caused by the deficiency of enzymes involved in galactose metabolism resulting in complications like cataracts, hepatocellular damage and developmental delay. Nonetheless, no report is available on mutations in galactosemia genes from our population. Objective: 1) To determine blood GALT activity in infants with cholestasis and to establish a spectrum of mutations in GALT gene 2) To determine blood GALK activity in infants with congenital cataract and to establish a spectrum mutations in GALK gene Methods: 390 infants (2 days-11 months) with cholestasis admitted in Pediatric Gastroenterology over 3.5 years were evaluated for galactosemia. Basic investigations included hemogram, liver function tests, blood culture, urine culture, urine for non-glucose reducing substances, eye evaluation, abdominal ultrasound, TORCH serology, mebrofenin scan and peroperative cholangiogram were done when indicated. Screening for GALT deficiency was done using Perkin-Elmer neonatal GALT kit. The levels of galactose-1-phosphate were also measured. Apart from this, 115 patients with congenital cataract were screened for the galactokinase (GALK) deficiency. Mutation analysis for most common Q188R and N314D mutations in GALT gene was performed by Restriction Fragment Length Polymorphism (RFLP). Single Stranded Conformational Polymorphism (SSCP) analysis and subsequently DNA sequencing were done for identification and characterization of unknown and novel mutations in GALT and GALK genes. Results: 55 (14.1%) infants were found to have reduced GALT activity with male: female: 37:18, jaundice in 54 (98%), hepatomegaly in 50 (91%), splenomegaly in 32 (58%), coagulopathy in 23 (42%), encephalopathy in 9 (16%), septicemia in 10 (18%) and cataracts in 12 (22%) were observed. Increased galactose-1 phosphate levels were fraternized with reduced activity of GALT. A total of 13 mutations and 3 polymorphisms were detected. 8 were novel mutations. N314D mutation was found in 30 patients. Q188R mutation was detected in three patients. Reduced blood galactokinase activity was found in 8 (7%) patients with congenital cataracts. 4 novel mutations were found in GALK gene. Conclusion: N314D mutation was found to be the most common mutation in our population. 8 and 4 novel mutations were also detected in GALT and GALK genes respectively.

2034/T

Comprehensive Biochemical and Molecular Analysis of Congenital Disorders of Glycosylation. *M. He[1], B. Xia[1], X. Li[1], B. Ng[2], H. Freeze[2], R. Cummings[3], W. Zhang[1], D. Matern[4], K. Raymond[4], T. Wood[5], M. Hegde[1].* 1) Human Genetics, Emory University, Georgia, GA; 2) Burham Instituion for Medical Genetics, La Jolla, CA; 3) Biochemistry, Emory University, Georgia, GA; 4) Biochemical Genetics Laboratory, Mayo Medical College, Rochester, MN; 5) Greenwood Genetics Center, Greenwood, SC.

Congenital Disorders of Glycosylation (CDG) are caused by gene defects in biosynthetic glycosylation pathways. To date, 14 different genetic defects in N-glycosylation, 11 in O-glycosylation and 11 in multiple glycosylation are known. Current diagnostic testing are mostly through serum transferrin analysis. However, with 2-3% of the active human genome thought to be involved in the synthesis and recognition of glycoconjugates, screening CDGs by one single transferrin protein is insufficient and a number of different types and patients remain undiagnosed. To improve the diagnosis and therefore an earlier treatment of CDGs, we have developed and launched comprehensive biochemical and molecular clinical CDG screening panels. For biochemical screening of CDG in serum or plasma, we coupled carbohydrate deficient transferrin test with serum N-glycan and O-glycan structural mapping by MALDI-TOF/TOF. This strategy has been applied successfully to the characterization of glycan profiles in 30 micro liter of the serum/plasma from patients suffering from CDG type II, combined CDG type I and type II or deficiencies in COG subunits. In addition to the biochemical CDG screen in blood, we also have implemented a free glycan/oligosaccharide screening in urine by MALDI-TOF/TOF method. Urinary free glycan/oligo screening not only could be used to diagnose the CDGs that are difficult to detect in the blood, such as CDGIIb, but also it specifically detects glycoprotein storage diseases such as the I cell disease, GM1 or GM2 gangliosidosis, galactosialidosis, Pompe, aspartylglucosaminuria etc. The sensitivity and specificity of this method has far exceeded what traditional thin layer chromatography could achieve. For comprehensive molecular CDG test panels, we have developed and launched the comprehensive 24 gene N-glycosylation disorder whole gene sequencing panel, CDG type I, 14 gene panel and multiple glycosylation, 11 gene panel using fluidigm and raindance library construction system coupled with the next generation sequencing and CGH array to detect single and multi exon deletions and duplications. We have also developed a CDG research library containing a list of putative genes which may be associated with CDG. This library will facilitate identification of novel genes associated with CDGs. Our comprehensive biochemical and molecular analysis of CDG provides new algorithms of the diagnosis of CDG.

2035/T

Genetic variation and an increasingly personalized approach to medicine: the Lysosomal Storage Disorder Registries. *E. James[1], F. Stewart[2], S. Prasad[1].* 1) Genzyme Corporation, Cambridge, MA; 2) Belfast City Hospital, Belfast, UK.

Sequencing of the human genome has heralded a new era in medicine. A better understanding of the relationship between genotype and phenotype, as well as the impact of genotype on disease progression and treatment will afford clinicians new opportunities to consider increasingly personalized approaches to disease management. Knowing the sequence of a gene, however, does not establish its function. Consequently, numerous initiatives have been established to further the understanding of the relationship between genetic variation and human health. Increasingly, disease registries are being used to collect observational, clinical effectiveness data in real-world settings. Registries are particularly important in rare genetic (or 'orphan') disorders, where identifying large numbers of patients to enroll in clinical trials is difficult. For example, disease registries now exist for various lysosomal storage disorders (LSDs), including Fabry disease, Gaucher disease, mucopolysaccharidosis Type I, and Pompe disease. Since the establishment of these registries, the understanding of the natural histories of the diseases, phenotypic heterogeneity, and genotype-phenotype correlations has grown, although much is still to be learned. Also, participants from clinical trials are increasingly being entered into registries, enabling follow-up of long-term treatment effectiveness and safety, thus demonstrating how registry data complement and supplement clinical trial data. As of May 7, 2010, the Pompe Disease Registry, established in 2004, contains data for 847 patients. Genotype data are available for a large number of enrolled patients. As the understanding of the genetics of Pompe disease increases, the Registry should help elucidate regional differences in the disease and symptomology in different subgroups. The methodology of how registries operate and how they can contribute to our understanding of disease continue to evolve with increasing advancements in technology and willingness to engage in such projects. This presentation looks at examples from LSD registries on understanding genetic variation and comments on operational aspects of registries to facilitate this. With genetic diseases such as the LSDs, registries are helping establish relationships between genetic mutations and disease, thereby paving the way for an increasingly personalized approach to the treatment of these rare and serious disorders.

2036/T

The heterogeneity of Pompe disease: early data from the Pompe Registry. *S. Prasad[1], B. Byrne[2], L. Case[3], E. Cupler[4], A. Genge[5], P. Kishnani[3] on behalf of the Pompe Registry Boards of Advisors.* 1) Genzyme Corporation, Cambridge, MA; 2) University of Florida, Gainesville, FL, USA; 3) Duke University Medical Center, Durham, NC, USA; 4) Oregon Health & Sciences University, Portland, OR, USA; 5) Montreal Neurological Institute, Montreal, Quebec, Canada.

Background: Pompe disease, a rare autosomal recessive disease resulting from a deficiency of acid α-glucosidase (GAA), is characterized by progressive myopathy. It is classified as infantile (IO; onset £12 months of age) or late (LO; onset >12 months of age) onset, although a range of disease severity and rate of progression is found. Diagnosis is confirmed by identification of absent or deficient GAA enzyme activity or presence of disease-causing GAA gene mutations in both alleles of the GAA gene. More than 289 different sequence mutations in the gene encoding GAA have been identified; 197 appear to be pathogenic. Disease registries are important in establishing information on pathogenesis, genotypic and phenotypic heterogeneity, symptomatology, and natural history of rare diseases. The Pompe Registry was established in 2004. Data from the Registry have begun to demonstrate the phenotypic heterogeneity of the disease in a relatively large cohort of patients. Methods: A query of the Pompe Registry was performed to determine types of GAA mutations in registered patients. Genotype correlations were attempted. Results: As of September 2009, 742 patients from 28 countries were enrolled. Seventy percent (517/742) had symptom onset >12 months of age; 23% (170/742) had symptom onset £12 months of age. Data are missing for 7% (55/742) of patients. Mutation data are available for 284 patients (83 IO; 201 LO). Of common mutations, the frequency of c.525delT appears similar between IO and LO patients; however, understanding the contributions of the other mutation in a patient to phenotype is important. p.Asp645Glu occurs more in IO patients. The most frequent mutation in LO patients is c.-32-13T>G, the splice mutation; it is not seen in IO patients with cardiomyopathy, appearing more frequently in older patients with a milder disease progression. Nonsense mutations predominate in IO patients with cardiomyopathy. Conclusions: Because mutations may be rare, the Pompe Registry is essential to record the full clinical picture. Although lack of longitudinal disease information and clean genotype information have hindered establishing genotype-phenotype associations thus far, as the Registry grows and more genetic information is collected, it may provide a more complete description of the clinical spectrum of the disease. As a global database with clinical and genetic information available through a centralized source, the Pompe Registry is an ideal means to do this.

2037/T

The Genetics of Hermansky-Pudlak Syndrome. *R. Hess, R. Fischer, W.A. Gahl, M. Huizing.* Medical Genetics Branch, NHGRI/NIH, Bethesda, MD.

Hermansky-Pudlak syndrome (HPS) is a disorder of lysosome-related organelle (LRO) biogenesis, resulting in oculocutaneous albinism, a bleeding diathesis, and occasional colitis or pulmonary fibrosis. Eight human HPS subtypes are identified (HPS1-8). Since an accurate diagnosis of each subtype has important prognostic and therapeutic implications and also provides insights into the cell biology of LROs, we extensively characterized each HPS subtype. We have studied 260 HPS patients at the NIH Clinical Center. Our molecular analyses indicated that HPS-1 (188 patients, 13 HPS1 mutations) comprises the largest group due to a founder mutation in NW Puerto Rico. HPS-2 results from mutations in AP3B1, encoding the beta3A subunit of adaptor complex-3, a coat protein that mediates vesicle formation. We identified 3 HPS-2 patients, harboring 4 different AP3B1 mutations. We also identified 13 HPS-4 patients (10 HPS4 mutations), 22 HPS-3 patients (10 HPS3 mutations; with founder mutations in Central Puerto Rico and in Ashkenazi Jews), 10 HPS-5 patients (13 HPS5 mutations), and 5 patients with HPS-6 (8 HPS6 mutations). We have not identified any HPS-7 or HPS-8 patients and only one patient/family of each subtype has been reported. Our remaining 12 unclassified HPS patients provide opportunities to identify new HPS-causing genes. Several genes, some corresponding to HPS mouse models that manifest both hypopigmentation and a platelet storage pool deficiency, are good candidates. Any new genetic causes of HPS will aid in elucidating the mechanism by which melanosomes, dense bodies, and lysosomes are created. Our extensive molecular studies allowed for genotype-phenotype analysis. We found that that HPS-1 and HPS-4 patients are at increased risk for developing pulmonary fibrosis and granulomatous colitis. HPS-2 patients have persistent neutropenia and had recurrent childhood infections, and may develop pulmonary fibrosis. HPS-3, HPS-5, and HPS-6 patients are clinically milder, with no apparent pulmonary involvement. These findings reiterate that an accurate diagnosis of each HPS subtype has important prognostic implications.

2038/T

Type 1 Gaucher disease: Early Manifestations in Children Homozygous for the N370S Mutation. *K. Desai, R.J. Desnick, L. Bier, M. Balwani.* Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, NY.

Type 1 Gaucher disease (GD1; deficient acid β-glucosidase), is the most common lysosomal storage disorder, and is particularly prevalent in the Ashkenazi Jewish (AJ) population, in which >70% of affected patients are homozygous or heteroallelic for the N370S mutation (carrier frequency 1 in 17). To date, the clinical manifestations of N370S homozygotes have not been characterized in infancy or early childhood and there are no specific monitoring guidelines for presymptomatic children with this typically later-onset phenotype. Therefore, we analyzed our experience monitoring twenty-one N370S/N370S infants and children diagnosed before age 3 years who were evaluated at baseline and at regular follow up visits. Data from clinical, laboratory and imaging assessments were used to monitor disease progression. The 21 patients included nine females and 12 males aged 3 months to 11.5 years. On review of systems, no symptoms suggestive of GD1 were reported. Physical exam did not reveal any GD1 related manifestations except for a palpable spleen tip in one 2.5 year old patient. There was no evidence of growth retardation; growth ranged from the 10th to 95th percentile for age. Mild speech delay and/or motor delay occurred in 18% of the children. Laboratory evaluation of 20 patients revealed that 10% were mildly anemic (mean hemoglobin 10.1 g/dl) due to iron deficiency and one patient had transient thrombocytopenia (129,000 u/L). Coagulation studies of nine patients revealed 67% had a prolonged aPTT (35.4-39.9 sec) on at least one occasion, and 22% had a persistently prolonged aPTT with normal Factor XI levels. Among the 11 (aged 2.5 - 11.5 years) who had imaging studies, 82% had mild hepatosplenomegaly which was preceded by splenomegaly in two cases. Chitotriosidase activity levels varied markedly among patients and increased in all patients with age (10 months to 11.5 years), reaching 5072 nmol/hr/ml (normal <180 nmol/hr/ml) in a 5 year old male. Angiotensin-converting enzyme levels ranged from 8 to 271 IU/L (normal 8-52 IU/L). Thus, Gaucher disease manifestations in N370S homozygous children can include mild visceromegaly, an abnormal coagulation profile, and biomarker elevation. As newborn screening for these disorders becomes a reality, practical screening and management guidelines are needed for presymptomatic pediatric patients affected with GD1.

2039/T

***AMPD2* plays an important role for regulation of lipid and glucose metabolism.** *T. Morisaki[1,2], J. Cheng[1], K. Toyama[1,2], T. Hirase[1], M. Ikawa[3], M. Okabe[3], H. Morisaki[1].* 1) Dept Bioscience & Genetics, Natl Cerebr & Cardiovasc Ctr Res Inst, Suita, Osaka, Japan; 2) Dept Mol Pathophysiol, Osaka Univ Grad Sch Pharm Sci, Suita, Osaka, Japan; 3) Genome Info Res Ctr, Osaka Univ, Suita, Osaka, Japan.

The regulation of liver energy metabolism in lipid and glucose is crucial for defense to obesity, insulin resistance and type 2 diabetes. AMP deaminase (AMPD) deaminates AMP to IMP and play a key role in stabilization of energy charge. AMPD is expressed at high levels in skeletal muscle, and muscle AMPD (AMPD1) deficiency, which is one of the most common enzyme defects especially in Caucasian, has been reported to be associated with symptoms of a metabolic myopathy, though not all of individuals with this defect exhibit symptoms. In addition to AMPD1, AMPD2 and AMPD3 are known to be expressed in tissues other than skeletal muscle, and AMPD2 gene is widely expressed in non-muscle tissues including liver. The precise function of AMPD2 is not known partly because no AMPD2 deficiency has been reported in human. Recently, we established a murine model for AMPD2 deficiency and found that AMPD2 plays an important role in liver lipid and glucose metabolism. AMPD2 deficient mice exhibited reduced body weight and fat accumulation, down-regulated blood glucose level, and increased insulin sensitivity, but did not show any change of the calorie intake or the spontaneous motor activity. AMPD2 deficient mice also exhibited attenuation of high-fat-diet-induced phenotypes including obesity and insulin resistance. In liver, the decrease of AMPD activity, the increase of AMP, and the increase of the phosphorylated AMP-activated protein kinase (AMPK) and the phosphorylated acetyl-CoA carboxylase were observed. The expression of PPAR gamma, PGC-1beta was down-regulated and the expression of leptin receptor was increased in the liver of those mice. Furthermore, the expression of G-6PC and PEPCK in liver was decreased in those mice. Based on these results, we conclude that AMPD2 is a novel metabolic molecule regulating lipid and glucose metabolism by changing the AMP level, resulting in AMPK phsphorylation and further effects. We hope this gene as a promising target for an effective treatment of obesity, insulin resistance or type 2 diabetes.

2040/T

Variation in STARD13 may contribute to type 2 diabetes and obesity. *L. Bian, R.L. Hanson, Y.L. Muller, J. Perez, B. Kaur, J. Mack, S. Kobes, W.C. Knowler, C. Bogardus, L.J. Baier.* PECRB, NIDDK, NIH, Phoenix, AZ.

The Pima Indians of Arizona have a high prevalence of type 2 diabetes and obesity. To identify genetic variation that contributes to both diseases in this population, we completed a genome-wide association study (GWAS) using the Affymetrix 1-million SNP chip. Three SNPs in genotypic concordance (rs521868, rs523656, and rs2858809) that map within introns of STARD13 were associated with early-onset type 2 diabetes (n = 634, P = 1.2 × 10-5) and BMI (n = 974, P = 5.9 × 10-5). STARD13 encodes StAR-related lipid transfer protein 13 that plays an important role in lipid transport and metabolism. To follow-up on this GWAS observation, the putative promoter, all exons, and exon-intron boundaries of STARD13 were sequenced in 24 Pima Indians; 38 variants were identified and 22 were selected as tag SNPs (using pair-wise r2 ≥0.8 to indicate redundance). Additional tag SNPs (n = 21) across intronic regions were selected from GWAS data. All 43 tag SNPs were genotyped in a population-based sample of 3,501 full-heritage Pima Indians for diabetes/obesity analysis. Six SNPs were associated with type 2 diabetes (P = 0.02-0.0009, OR = 1.16-1.24), 18 SNPs were associated with BMI (P = 0.04-0.0002) and 4 SNPs were associated with both phenotypes. Selected SNPs were genotyped in an additional sample of 3,723 “mixed heritage” Native Americans to assess replication. Comparing these two samples of Native Americans, 2 SNPs replicated for BMI (e.g. rs9536645, had a P = 0.0002 in full heritage Pima Indians, P = 0.04 in “mixed heritage” sample, and P = 0.00003 when samples were combined). None of the diabetes associations independently replicated (P < 0.05) between these 2 Native American samples, although associations remained significant in the combined sample (e.g. rs523656 had a combined P = 0.0009). Among the 6 SNPs associated with diabetes in Pima Indians, 5 were reported in the DIAGRAM meta-analysis for type 2 diabetes in Caucasians and 3 SNPs (rs523656, rs17596576, and rs703232) were associated with diabetes in DIAGRAM (P = 0.002-0.0001, OR = 1.10-1.14). Analysis of rs5236565 in the combined data from Pima Indians, “mixed heritage” Native Americans, and DIAGRAM Caucasians provided the strongest evidence for association with type 2 diabetes (P = 7.4× 10-7, OR = 1.14). We propose that variation within STARD13 modestly increases risk for type 2 diabetes and obesity in Pima Indians, and the diabetes effect may be common to other ethnic groups.

2041/T

Mutations in the SLC22A5 (OCTN2) gene detected in 143 subjects for systemic carnitine deficiency. *F-Y. Li[1], A.W. El-Hattab[1], E.V. Bawle[2], R.G. Boles[3], E.S. Schmitt[1], F. Scaglia[1], L-J. Wong[1].* 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Division of Genetic and Metabolic Disorders, Children’s Hospital of Michigan, Detroit, MI; 3) Division of Medical Genetics, Children’s Hospital Los Angeles, Los Angeles, CA.

Systemic primary carnitine deficiency (CDSP) is an autosomal recessive disorder caused by mutations in the SLC22A5 (OCTN2) gene encoding the plasmalemmal carnitine transporter. The severe form of CDSP was the originally known presentation of the disease and is characterized by progressive infantile-onset cardiomyopathy, weakness, peripheral neuropathy and recurrent hypoglycemic hypoketotic encephalopathy. However, the advent of expanded newborn screening has broadened the clinical spectrum of disease to include asymptomatic newborns and mothers. In this study, the entire coding regions of the OCTN2 gene were sequenced in 143 unrelated subjects who were suspected of having CDSP either as a follow-up of an abnormal newborn screening (NBS) result or as part of a diagnostic work-up when symptomatic. In 70 unrelated infants evaluated because of abnormal NBS results, 48 infants were found to have at least 1 mutation/unclassified missense variant. Thus, sequence analysis detects at least one mutation in 69% (48/70) in NBS positive infants. Thirty-three mothers whose infants had abnormal NBS results, including 21 whose infants were not tested for OCTN2 mutations, were molecularly evaluated and in 28 (85%) of them at least 1 mutation/unclassified missense variant was found. A total of 11 asymptomatic mothers whose infants had abnormal NBS results were found to have 2 mutations. Therefore, OCTN2 sequence analysis is recommended for infants ascertained due to abnormal NBS results and for their mothers. Conversely, 52 unrelated subjects were tested due to clinical indications other than abnormal NBS and only 14 (27%) of them were found to have at least one mutation/unclassified missense variant. Custom designed oligonucleotide array CGH analysis revealed a heterozygous ~1.6 Mb deletion encompassing the entire OCTN2 gene and other genes in one subject who was apparently homozygous for the c.680G>A (p.R227H) mutation upon sequence analysis . Thus, large deletions at the OCTN2 locus may also be considered if by sequencing, an apparently homozygous mutation or only one mutant allele is identified.

2042/T

Thiamine-responsive pyruvate dehydrogenase deficiency in two patients with normal development and muscular symptoms. *E. Naito[1], T. Hashimoto[1], T. Kumagai[2], S. Yamashita[3], Y. Kotani[4], S. Kagami[4].* 1) Dept. of Pediatrics, Japanese Red Cross Tokushima Hinomine Rehab., Komatsushima, Tokushima, Japan; 2) Dept. of Pediatric Neurology, Central Hospital, Aichi Welfare Center for Persons, Aichi, Japan; 3) Dept. of Neurology, Kanagawa Children's Medical Center, Kanagawa, Japan; 4) Dept. of Pediatrics, Institute of Health Biosciences, The University of Tokushima, Graduate School, Tokushima, Japan.

The human pyruvate dehydrogenase complex (PDHC) catalyzes the thiamine-dependent decarboxylation of pyruvate. Thiamine treatment is very effective for some patients with PDHC deficiency. Among these patients, nine mutations of the X-linked pyruvate dehydrogenase (E1) α subunit have been published previously: H44R, V71A, R88S, G89S, C101F, F205L, L216F, R263G, and V389fs. As four mutations (H44R, V71A, R88S, G89S) were in exon 3, exon 3 in the E1 α subunit appears to be important in thiamine-responsive PDHC deficiency. We report the biochemical and molecular analysis of two male patients with clinically thiamine-responsive lactic acidemia, normal development and muscular symptoms (generalized hypotonia and weakness). The PDHC activity was assayed using two different concentrations of TPP. These two patients displayed very low PDHC activity in the presence of a low ($1x10^{-4}$ mM) TPP concentration, but their PDHC activity significantly increased at a high (0.4 mM) TPP concentration. Especially, the PDHC activity of one patient increased to within the normal range. Thus the PDHC deficiency in these two patients was due to a decreased affinity of PDHC for TPP. Therefore, in order to diagnose this type of thiamine-responsive PDHC deficiency and to prevent a diagnostic error, it is necessary to measure the activity of PDHC in the presence of a low ($1x10^{-4}$ mM) as well as a high TPP concentration. The DNA sequence of these two male patients' X-linked E1 α subunit revealed a point mutation (L260Q in exon 8 and A349T in exon 11). Treatment of both patients with thiamine resulted in a reduction in the serum lactate concentration and clinical improvement of muscular symptoms, suggesting that these two patients have a thiamine-responsive PDHC deficiency due to a point mutation in the E1 α subunit gene.

2043/T

A STUDY OF WILSON DISEASE MUTATIONS IN SPAIN. *C. Solis-Villa[1], L. Hierro[2], C. Lacambra[3], P. Jara[2].* 1) Genetics, University Hospital La Paz, Madrid, Spain; 2) Hepatology, University Hospital La Paz, Madrid, Spain; 3) Internal Medicine, Hospital Severo Ochoa, Leganés, Madrid, Spain.

A STUDY OF WILSON DISEASE MUTATIONS IN SPAIN Wilson disease (WD) is an autosomal recessive disorder of copper transport, characterized by toxic accumulation of copper in the liver and subsequently in the brain and other organs. The disease is caused by a large number of mutations in the ATP7B. We present the genetic screening results of 320 WD patients and relatives from 146 different families mainly from Spain. Mutation screening of the entire coding region and the intron-exon boundaries was undertaken by direct sequencing. In order to simplify and to avoid allele drop-out, we carried out long-range PCR of exons 6-9 and exons 10-14 and then sequenced exon by exon. The rest of the coding region was amplified exon by exon. Recently, we have implemented high resolution melting (HRM) to screen mutations in exons 15 through 21, with promising results. We have found 14 novel mutations and 30 mutations previously described. By far the most frequent mutation is p M645R, which accounts for 33% of the mutated alleles followed by H1069Q, with 13%. We have found 8 homozygotes for p M 645R and just one homozygote for H1069Q. In some of the patients, either one (10%) or both mutations (15%), could not be identified. Multiplex Ligation-dependent Probe Amplification (MLPA) was performed in order to discard long deletions as the disease-causing lesion, but no alterations were identified The novel mutations are: p Ser59Leu, p Ile161Thr, p Ile381Ser, c523-525 del AA, (exon 2), IVS2(+5) G>A, c1739delA (exon5), p Met665Val, p Met671Val, (exon 7), p Gln799X (exon 9), p Glu847X (exon10) p Asp1289Tyr (exon 18), p Leu1313Arg, p Asn1324Ser (exon 19), p Gly1347-Leu (exon 20). No mutations were found in exons 3, 4 or 11. Our findings corroborate the importance of the genetic testing of Wilson disease, especially for the diagnosis of relatives. More studies are needed to unveil the underlying lesion in those cases where no abnormality was found; perhaps the regulatory regions need to be investigated. Finally, HRM has proven to be a powerful technique to screen for ATP7B mutations inWD.

2044/T

DNA diagnosis of neuronal ceroid-lipofuscinoses (NCLs) in the Czech Republic. Does the novel mutation c.1439T>G (p.V480G) in CLN2 gene lead to the non-classical phenotype? *H. Vlaskova, L. Stolnaya, H. Treslova, H. Jahnova, H. Poupetova, L. Dvorakova, M. Elleder.* Inst Inherit Met Disorders, Charles Univ/1 Fac Med/UnivHos, Prague, Czech Republic.

The neuronal ceroid-lipofuscinoses (NCLs) are a clinically and genetically heterogeneous group of fatal inherited neurodegenerative lysosomal-storage disorders characterized by progressive mental and motor deterioration, and seizures. There are eight types well defined at the molecular level. Fifty seven probands with this diagnosis have been identified during last 35 years in our Institute that serves as a diagnostic center for the Czech Republic and Slovakia (15 mil. inhabitants). The diagnosis was carried out using the classical techniques (histology, electron microscopy) continuously updated by histochemistry, biochemistry and molecular genetics. NCL1 (n=2), NCL3 (n=4), NCL5 (n=1), NCL6 (n=2) represent the minority types, NCL2 and NCL7 being most frequent in the Czech Republic. NCL7 (n=22) has high incidence in the Roma population. Common missense substitution c.881C>A (p.T294K; 36/44 alleles) and splice mutation c.754+2T>A (7/44 alleles) are prevalent in this group. In the NCL2 group (n=26) the prevalent mutation was of the nonsense type c.622C>T (p.R208X) in 32 of 50 mutant alleles. Twenty four patients had the classical late infantile phenotype while two of them displayed atypical phenotype: In the first of the patients the disease course was of a variant juvenile type with onset before age of 6 and prolonged life span until 15 years. He was a compound heterozygote for p.R208X and p.V480G mutations (Elleder et al. 2008: Acta Neuropathol 116: 119). The second patient with disease onset at the age of 8 is alive, but bedridden, aged 25. Because of the mixed ultrastructural pattern in the initial skin biopsy we first analyzed the genes for the variant late infantile forms (CLN5 - CLN7) however, the results were negative. Analysis of the CLN2 gene disclosed the same genotype as in the first patient. NCL2 was proved by deficient activity of TPP I. This suggests that the novel mutation p.V480G is to be associated with atypical protracted phenotype. According to the information from the Rare NCL Gene Consortium (RNGC) the mutation c.1439T>G (p.V480G) is unique in CLN2 gene. Support: VZ MSM CR 0021620806, VZ MZ CR 64165.

2045/T

Expanded Clinical Spectrum and Neonatal Hyperammonemia Associated with Mutations in TMEM70. *J. Wang[1], O.A. Shchelochkov[1,2], F.Y. Li[1], H. Zhan[1], J.A. Towbin[3], J.L. Jefferies[3], L.C. Wong[1], F. Scaglia[1].* 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Division of Genetics, Department of Pediatrics, University of Iowa, IA; 3) Departments of Pediatrics, Human Genetics and Cardiovascular Sciences, University of Cincinnati College of Medicine, Cincinnati, OH; 4) 4Department of Pediatrics, Baylor College of Medicine, TX.

Mitochondrial disorders are a large and genetically heterogeneous group of disorders posing a significant diagnostic challenge. Only approximately 10-20% of patients have identifiable alterations in their mitochondrial DNA (mtDNA). The remaining ~80-90% of affected patients likely harbor mutations in nuclear genes, most of which are still poorly characterized, and therefore not amenable to efficient screening using currently available molecular methods. Here we present a patient, who has been followed since birth after presenting with neonatal hyperammonemia, Reye-like syndrome episodes, and ventricular tachyarrhythmia. Initial biochemical work-up revealed mild orotic aciduria and significant amounts of 3-methylglutaconic (3-MGC) and 3-methylglutaric (3-MGA) acids in the urine. Muscle biopsy demonstrated the presence of ragged-red fibers and non-specific structural abnormalities of mitochondria. The activities of respiratory chain enzymes (complexes I-IV) showed no deficiency. Mutational analysis of the entire mitochondrial genome did not reveal deleterious point mutations or large deletions. Long-term follow-up was significant for a later-onset hypertrophic cardiomyopathy, muscle weakness, and exercise intolerance. Although she had frequent episodes of Reye-like episodes in infancy and early childhood, mostly triggered by illnesses, these symptoms improved significantly with the onset of puberty. In the light of recent reports linking cases of type IV 3-methylglutaconic aciduria (3-MGCA) and hypertrophic cardiomyopathy to mutations in TMEM70, we proceeded with sequencing analysis of this gene. We identified one previously reported splice site mutation, c.317-2A>G and a novel, unclassified missense variant, c.494G>A (p.G165D) in an evolutionarily conserved region predicted to be deleterious. This variant was not identified in 100 chromosomes of healthy control subjects and 200 chromosomes of patients with cardiomyopathies. In comparison to the previously reported cases, we note that our patient had normal growth parameters and cognitive development, absence of structural heart defects, no dysmorphic features, improvement of symptoms with age, and persistence of hypertrophic cardiomyopathy.

2046/T

Recombinant human NAGLU-IGF2, a potential enzyme replacement therapy for Sanfilippo B syndrome. *S. Kan, C.S. Sinow, K. Haitz, A.K. Todd, S.Q. Le, L. Troitskaya, P.I. Dickson, B. Tippin.* Medical Genetics, LA Biomedical Research Institute (LA Biomed), Torrance, CA.

Mucopolysaccharidosis type IIIB (MPS IIIB; Sanfilippo B syndrome) is a lysosomal storage disorder (LSD) characterized by the defective degradation of heparan sulfate due to lack of α-N-acetyl-glucosamindase (NAGLU). MPS IIIB-affected individuals exhibit mild somatic symptoms, but severe central nervous system degeneration. Enzyme replacement therapy (ERT) has been successfully applied to treat many LSDs, like MPS I, II and VI, Fabry, Gaucher, and Pompe syndromes. However, attempts to use a similar strategy for ERT of MPS IIIB have failed due to inadequate uptake of poorly phosphorylated recombinant human NAGLU (rhNAGLU) via the insulin-like growth factor/mannose-6-phospate (IGF2/M6P) receptor.

In order to enhance the therapeutic potential of rhNAGLU, a modified construct with a C-terminally fused IGF2 receptor binding site and a c-myc tag was created and expressed in Chinese hamster ovary cells. The rhNAGLU-IGF2 was purified by Concavalin A and c-myc affinity chromatography and its biochemical properties were characterized. The purified rhNAGLU-IGF2 showed significantly higher intracellular uptake into MPS IIIB fibroblasts after 4 hours incubation compared with native urinary NAGLU (K_{uptake} of 47 nM, vs 730 nM, respectively). 80% of the cellular uptake was inhibited by IGF2 peptide and 40% was inhibited by neutralizing anti-IGF2 receptor antibody but no cellular uptake inhibition was observed by M6P, suggesting that rhNAGLU-IGF2 enters cells through the IGF2 binding site of the IGF2/M6P receptor. Moreover, rhNAGLU-IGF2 was taken up by several different human neuronal cell lines by the same mechanism. Further *in vivo* assays in MPS IIIB cells were conducted. The colocalization of rhNAGLU-IGF2 with lysosomes by confocal microscopy confirmed its lysosomal targeting. When taken up by cells rhNAGLU-IGF2 reduced GAG storage by 50% demonstrating its cellular physiological activity has potential to treat diseases

The novel rhNAGLU-IGF2 fusion protein shows high-affinity uptake into cells *in vitro* and, reduces GAG storage in MPS IIIB fibroblasts. Additional *in vivo* studies of the effect of intracerebroventricular rhNAGLU-IGF2 treatment on GAG and ganglioside storage in MPS IIIB mice are underway as well as evaluation the enzyme distribution and its half-life.

2047/T

Glycosylation-Independent Lysosomal Targeting of Acid α-Glucosidase Enhances Glycogen Clearance from Muscle Tissue of Pompe Mice. *J.H. LeBowitz[1], J.A. Maga[1], J. Zhou[1], R. Kambampati[1], S. Peng[1], D. Cloutier[2], R.N. Bohnsack[3], A. Thomm[1], S. Golata[1], P. Tom[1], N.M. Dahms[3], B.J. Byrne[2].* 1) ZyStor Therapeutics, Inc., Milwaukee, WI; 2) Powell Gene Therapy Center, College of Medicine, University of Florida, Gainesville, FL; 3) Department of Biochemistry, Medical College of Wisconsin, Milwaukee, WI.

We have used a peptide-based targeting system to improve lysosomal delivery of acid α-glucosidase (GAA), the enzyme deficient in patients with Pompe disease. ZC-701 is a recombinant fusion protein containing the GILT (Glycosylation Independent Lysosomal Targeting) tag fused to the N-terminus of amino acid residues 70-952 of human GAA. The GILT tag is derived from insulin-like growth factor II (IGF-II), a high affinity ligand for the 300 kDa cation-independent mannose-6-phosphate receptor (CI-MPR). This receptor, also known as the IGF-II receptor, shows dual ligand specificity in mammals, binding both IGF-II and proteins containing bis-mannose-6-phosphate (M6P) phosphorylated oligosaccharides with high affinity. We reasoned that the presence of the GILT tag, a high affinity ligand for the CI-MPR, on every ZC-701 enzyme molecule would enable more efficient lysosomal delivery of each ZC-701 molecule to cells possessing the receptor. This should ultimately result in more efficient clearance of the storage product, glycogen, from the lysosome of muscle cells. Our results show that in a cell-based uptake assay, ZC-701 was taken up by L6 myoblasts 25-fold more efficiently than was recombinant human GAA (rhGAA) based on measurement of K_{uptake}, the concentration at which half maximal uptake occurs. Once delivered to the lysosome, the mature form of ZC-701 was indistinguishable from rhGAA and persisted with a half-life indistinguishable from rhGAA. Furthermore, after a four week regimen of enzyme replacement therapy, ZC-701 was significantly more effective than rhGAA in clearing glycogen from skeletal muscle tissue of Pompe mice. The study compared 5 mg/kg and 20 mg/kg doses of ZC-701 and of rhGAA administered in four weekly doses. One week after the last injection, the mice were sacrificed and several muscle tissues were assayed for glycogen content using a biochemical assay. In each tissue, ZC-701 was found to reduce glycogen more effectively than rhGAA. ZC-701 demonstrated a dose response in some tissues but in others, 5 mg/kg was as effective as 20 mg/kg in reducing glycogen. 5 mg/kg ZC-701 cleared more glycogen than did 20 mg/kg rhGAA in all tissues indicating at least four-fold greater potency of ZC-701. We conclude that ZC-701 possesses many characteristics one would desire in an improved enzyme replacement therapy for treatment of Pompe disease patients.

2048/T

Assessment of the utility of globotriaosylceramide as a surrogate marker based on randomized controlled trials in Fabry disease. *R. Schiffmann[1], K. Nicholls[2], A. Mehta[3], J.T.R. Clarke[4,5], R.D. Steiner[6], M. Beck[7], B.A. Barshop[8], W.J. Rhead[9], M. West[10], N. Nair[11], R. Martin[11], P. Huertas[11].* 1) Inst Metabolic Disease, Baylor Res Inst, Dallas, TX; 2) Royal Melbourne Hospital, Parkville, VIC, Australia; 3) Royal Free Hospital and University College Medical School, London, UK; 4) Division of Clinical & Metabolic Genetics, Hospital for Sick Children and University of Toronto, Toronto, Canada; 5) Service de génétique médicale, Centre hospitalier universitaire de Sherbrooke, Sherbrooke, Canada; 6) Oregon Health & Science University, Portland, OR, USA; 7) Center for Lysosomal Storage Diseases, University of Mainz, Mainz, Germany; 8) University of California San Diego School of Medicine, La Jolla, CA, USA; 9) Medical College of Wisconsin, Milwaukee, WI, USA; 10) Department of Medicine, Dalhousie University, Halifax, NS, Canada; 11) Shire HGT, Cambridge, MA, USA.

Background: Fabry disease is an X-linked disorder of glycosphingolipid metabolism caused by a deficiency of the lysosomal enzyme α-galactosidase A. In male Fabry patients, kidney and cardiac dysfunction becomes apparent by the third decade of life, and without treatment often progresses to end-stage disease. Conflicting data exist on the utility of globotriaosylceramide (Gb3) as a surrogate biomarker in studies assessing stage of disease or response to specific therapies such as enzyme replacement. Goal: We present an analysis of the relationship of plasma and urinary Gb3 to the estimated glomerular filtration rate (eGFR) and left ventricular mass index (LVMI) in adult male patients who were enrolled in three prospective, randomized, placebo-controlled, clinical trials of agalsidase alfa and their open-label extension studies. Methods: eGFR was calculated using the Modification of Diet Renal Disease (MDRD) equation in 116 adult male patients. Gb3 was measured by HPLC in 24-hour urine sediment and in plasma. The association between plasma Gb3 and urine Gb3 and estimated glomerular filtration rate [eGFR] and MRI left ventricular mass indexed to height [LVMI]) was assessed by calculating the Pearson correlation coefficients (the strength of linear association between two variables). The resulting estimates were tested for statistical significance at the 0.05 level of significance. The robustness of the results was verified by measuring the Spearman correlation coefficient (rank-transformed data). The Pearson and the Spearman correlations were calculated for both the observed values and the changes from baseline at selected time-points up to month 30. eGFR and change in eGFR or LVMI were analyzed separately as outcome variables. Baseline proteinuria status was investigated as a potential covariate. Results: Current Gb3, baseline Gb3, and absolute Gb3 change were not found to be associated with any of the outcomes. Conclusion: These data indicate that plasma or urinary Gb3 cannot be used as a surrogate for GFR or LVMI in patients with Fabry disease undergoing enzyme replacement therapy.

2049/T

Normal oxidative phosphorylation in intestinal smooth muscle of childhood chronic intestinal pseudo-obstruction. *D. Chretien[1], L. Galmiche[1,2], F. Jaubert[2], F. Sauvat[3], S. Sarnacki[3], O. Goulet[4], Z. Assouline[1], V. Vedrenne[1], N. Boddaert[5], N. Brousse[2], A. Munnich[1], A. Rotig[1].* 1) INSERM U781 and Department of Genetics, Hôpital Necker-Enfants Malades and Assistance Publique-Hôpitaux de Paris, Faculty of Medicine, Université Paris Descartes, Paris, France; 2) Department of Pathology and Tumorothèque; 3) Department of Paediatric Surgery; 4) Department of Paediatric Gastroenterology; 5) Department of Paediatric Radiology.

Chronic intestinal pseudo-obstruction (CIPO) is a severe disease of the digestive tract motility. In pediatric population, CIPO remains of unknown origin for most patients. CIPO is also a common feature in the course of mitochondrial oxidative phosphorylation disorders related for some patients to mutations in TYMP, POLG1, mtDNA tRNAleu(UUR) or tRNAlys genes. We hypothesized that CIPOs could be the presenting symptom of respiratory chain enzyme deficiency and thus we investigated oxidative phosphorylation in small bowel and/or colon smooth muscle of primary CIPO children. We studied 8 children with CIPO and 12 pediatric controls. We collected clinical, radiological and pathological data and measured respiratory chain enzymatic activity in isolated smooth muscle of the small bowel and/or the colon. We also sequenced TYMP, POLG, mtDNA tRNAleu(UUR) and tRNAlys genes. No respiratory chain enzyme deficiency was detected in CIPO children. In myogenic CIPO, respiratory enzymes and citrate synthase activities were increased in small bowel and/or colon whereas no abnormality was noted in neurogenic and unclassified CIPO. Levels of enzyme activities were higher in control small bowel than in control colon muscle. Sequencing of TYMP, POLG, mtDNA tRNAleu(UUR) and tRNAlys genes and POLG gene did not reveal mutation for any of the patients. These data indicate that oxidative phosphorylation deficiency is not a common cause of childhood CIPO. Differences between control small bowel and control colon muscle probably reflect normal distinct motility and metabolic activity.

2050/T

Genetic and intestinal cell-specific contributions to the pathogenesis of necrotizing enterocolitis. *M.H. Premkumar[1], A. Erez[2], E. Munivez[2], S.C.S. Nagamani[2], G. Sule[2], J. Zhang[2], C. Rosales[3], B. Lee[4].* 1) Pediatrics/ Neonatology, Baylor College of Medicine, Houston 77030, TX; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas, 77030; 3) Department of Pathology, Texas Children's Hospital, Houston, Texas, 77030; 4) Howard Hughes Medicine Institute.

Necrotizing enterocolitis (NEC) is an intestinal emergency of premature infants with a high mortality and severe morbidity. The etiopathogenesis of NEC is not known. NO (nitric oxide) is one of the important mediators of NEC, but the exact mechanism of the role of NO is unclear. We hypothesize that cell specific dysregulation of NO metabolism in the intestine can differentially contribute to prevalence of NEC. NO is generated by the conversion of arginine to citrulline by Argininosuccinate lyase (Asl), the only enzyme in the body capable of generating arginine. We have previously generated Asl hypomorphic mice which exhibit generalized Asl deficiency, resulting in global deficiency of NO. The phenotype is characterized by severe growth restriction, shortened life span, and increased free radical injury. Via homologous recombination, we generated a conditional null allele of Asl. Cell specific KO of Asl was generated in smooth muscle, enterocyte and macrophage by breeding with transgenic mice expressing Cre recombinase in the respective cells. A double KO of eNOS and smooth muscle Asl was also generated. A mouse model of NEC was established in these mutant and wild type mice by subjecting premature mouse pups to exclusive formula feed, hypoxia and hypothermia. The severity of NEC was graded based on histopathology. Inflammatory mediators, protein and mRNA expression of Asl, nitrites and nitrosylation were measured using standard techniques. **Results**: Asl levels on Western blot and RT-PCR analysis were lower in the tissue specific Asl KOs in smooth muscle and macrophages compared to wild type. The incidence, severity of NEC and survival rates did not differ significantly among smooth muscle Asl Kos and double KOs of eNOS/smooth muscle Asl in comparison to the wild type suggesting that the smooth muscle and the endothelial NO contribute minimally to the pathogenesis of NEC. In macrophage KOs, the in vitro cytokine response to stimulation with lipopolysaccharide (LPS) was significantly blunted compared to wild type suggesting the possible role of iNOS in the pathogenesis of NEC.

2051/T

A rare mutation in GOT1 determines asparate aminotransferase (AST) levels in OOA. *H. Shen, C. Damcott, S. Chai, R. Yang, H. Hu, Q. Gibson, D. Gong, B. Mitchell, A. Shuldiner.* School of Med, EDN, Univ Maryland, Baltimore, MD.

Asparate aminotransferase (AST) catalyzes the reversible conversion between aspartate/alpha-ketoglutarate and oxaloacetate/glutamate, pivotal steps in metabolism of amino acid, glucose, and lipid. We have recently carried out a genome wide association study of serum AST levels in 862 Amish participants of the HAPI Heart Study and identified significant association of AST levels with a cluster of SNPs located on chromosome 10q24.1 (peak association was rs17109512; P value = 5.46E-14), in the vicinity of GOT1, the gene encoding cytosolic AST. The frequency of rs17109512 was rare (MAF = 0.006) The 10 heterozygotes had significantly lower AST levels compared to the 852 homozygotes for the common allele (mean of AST: 10.0+/- 2.8 vs. 18.8+/-5.2). Given the low frequency of the associated SNP, its proximity to GOT1, and the very large effect size of the association, we hypothesized that rs17109512 is tagging a functional SNP in GOT, most likely a mutation affecting the protein sequence of AST. We sequenced the gene and identified an in-frame deletion of three nucleic acids encoding asparagine at position 389 in GOT1 gene. Further genotyping of Del389Asn in other Amish samples (n=1600) identified an additional 20 carriers (MAF = 0.006). The variant was not detected in 647 outbred Caucasians. Asn at codon 389 is conserved among known mammalian cytosolic ASTs. The AST activity of recombinant proteins carrying the Del389Asn mutation was significantly lower than the recombinant protein carrying the wide-type allele. In conclusion, we discovered a rare in-frame deletion in GOT1 gene encoding cytosolic AST enzyme in the Old Order Amish. This finding will help us to understand the biology of AST in human intermediary metabolism.

2052/T

Prevalence of lysosomal storage disorders in India: Our experience. *J. Sheth[1], M. Mistri[1], N. Oza[1], U. Dave[1], P. Gambhir[2], R. Shah[1], F. Sheth[1].* 1) Biochem & Molec Bio, Inst Human Gen, Ahmedabad, India; 2) Sasoon General Hospital, Pune, India.

With the population of 1.2 billion, birth rate of about 29 million per year and consanguineous marriages in many parts of the country storage disorders are considered to be high in India. However, due to overlapping clinical phenotypes, lack of therapeutic options for majority of LSDs, the subject remains an investigative with lesser interest from the clinician. On the other hand recent availability of enzyme replacement therapy for some of the storage disorders there is a growing interest among clinicians in an early diagnosis of these diseases. Present study was carried out in 604 children in the age range of 3 months to 12 years with variable phenotypes like coarse facial features, hepatomegaly, neuroregression, epiphyseal skeletal abnormality, corneal clouding, cherry red spot, impaired motor neuron function/hypotonia, respiratory complications with progressive muscular weakness and regression of learned skill/mile stone. All were investigated for seven mucopolysaccharide disorders, glycolipid and lipid storage disorders (Tay Sachs, NPD, Gaucher), storage of sulphatides (MLD and Krabbe), glycogen storage (Pompe), defects in lysosomal transporters (Sialic acid storage disorder) and lysosomal trafficking protein abnormality (Mucolipidosis and NPD-C). Enzyme study was carried out from leucocytes, plasma and skin fibroblasts using fluorometric and spectrophotometric substrate. 208/604 (34.44%) children wear found to have storage disorders like MPS (36.53%), glycolipid and lipid storage (38.46%), sulphatides accumulation (10.57%), glycogen storage (9.66%), lysosomal transporter abnormality (1.93%) and lysosomal trafficking protein abnormality (2.9%). Impaired motor neuron function/hypotonia and neuroregression were the most common phenotype observed in 58.65% and 42.78% children with storage disorders. While other phenotypes were hepatosplenomegaly (28.36%), coarse facial features (29.32%), cherry red spot(32.21%), regression of learned skill/delayed mile stone (32.21%), skeletal abnormality (17.78%), respiratory complications with progressive muscle weakness (9.6%) and corneal clouding (2.4%). Our study suggests that glycolipid and lipid storage disorders are the most common storage disorders in India followed by MPS and information can be utilized for the national screening programme of storage disorders like Gaucher and NPD A/B to offer an early therapeutic intervention with improved prognostication.

2053/T

A common splicing mutation in Korean patients with classical galactosemia. *G.H. Kim[1,3], B.H. Lee[1,2,3], C.K. Cheon[4], J.M. Kim[3], M. Kang[3], J.H. Kim[3], S.H. Yang[5], J.H. Choi[2,3], H.W. Yoo[1,2,3].* 1) Med Gen Clinic & Lab, Asan Med Ctr, Seoul, Korea; 2) Department of Pediatrics, Asan Medical Center Children's Hospital, Seoul, Korea; 3) Genome Research Center for Birth defects and Genetic Diseases,Asan Medical Center Children's Hospital, Seoul, Korea; 4) Pusan National University, Beomeo-ri, Mulgeum-eup, Yangsan-si, Gyeongnam, Korea; 5) Institute of Metabolism,Green Cross Reference Laboratory, Seoul, Korea.

Classical galactosemia is caused by a galactose-1-phosphate uridyltransferase (GALT) deficiency. Newborn screening programs have been established for the early diagnosis of galactosemia. We describe here four Korean children who were identified during newborn screening and confirmed to have classical galactosemia based on enzymatic and molecular genetic analysis. Patients were followed until the age of 0.9-10.8 years (median, 3.4 years), and all four patients showed normal growth and development. Asians have been reported to have mutations distinct from those of Caucasians and African Americans, indicating that *GALT* gene mutations are ethnically diverse. Our four patients had a total of five mutations, two of which were previously known and three were novel (p.R200X, p.E363K and c.252+1G>A) mutations. Interestingly, c.252+1G>A, which leads to skipping of exon 2, was observed in three of the four patients (3 of 8 alleles), indicating that this mutation may be common in Koreans with classical galactosemia. Screening for classical galactosemia in 158,126 Korean newborns identified only 1 individual (Patient 3). In conclusion, our findings provide further evidence for the ethnic diversity of classical galactosemia, which may be rarer in Koreans than in other populations.

2054/T

A novel mitochondrial tRNA lysine variant, m.8358A>G, associated with exercise intolerance, muscle weakness, and fatigue. *V. Venegas[1], M. Landsverk[1], J. Wang[1], D. Boué[2,4], Z. Sahenk[3,5], L-J. Wong[1].* 1) Dept Mol and Hum Genetics, Baylor Col Medicine, Houston, TX; 2) Department of Pathology; 3) Departments of Pediatrics and Neurology, The Ohio State University, Columbus, OH; 4) Department of Laboratory Medicine, Nationwide Children's Hospital, Columbus, OH; 5) Center for Gene Therapy, Neuromuscular Program, Columbus, OH.

Mutations in the mitochondrial genome contribute to a variety of muscular disorders. More than half of the known mitochondrial mutations are located in tRNA genes, which comprise only 10% of the mitochondrial genome. Here we report a novel mitochondrial DNA variant in tRNA Lys at nucleotide 8358 (m.8358A>G). At the time of testing, the proband was a twelve year old male of European Caucasian decent presenting with muscle weakness, fatigue and elevated creatine kinase (CK) levels. Histology noted fiber type (size) disproportion, with only small type 1 fibers intermixed with essentially normal sized type 2 fibers in the absence of any fiber type predominance, as well as rare (<1%), COX-negative fibers by histochemistry. Analysis of muscle respiratory chain enzymes revealed a reduction of activity for complex I (NADH dehydrogenase) and complex IV (cytochrome c oxidase). Sequence analysis of the entire mitochondrial genome in a muscle specimen of the proband revealed an apparently homoplasmic m.8358A>G variant. Blood samples from the proband's asymptomatic siblings and mother identified heteroplasmic m.8358A>G in his brother and mother, and homoplasmic m.8358A>G in his sister, age 15 at the time of testing. Recently, the great aunt of the proband, age 58, presented with fatigue, muscle spasms, exercise intolerance, headaches and hypothyroidism. Sequence analysis of this individual also showed homoplasmic m.8358A>G. Allele-specific oligonucleotide hybridization and heteroplasmy quantification using ARMS PCR confirmed the results obtained by sequencing. This m.8358A>G variant is located in the acceptor stem of tRNA Lys. The A at nucleotide position m.8358 is normally paired with a U at nucleotide position m.8300 and this variant therefore changes a U:A pairing to a U:G mispairing. Multiple tRNA Lys mutations have been identified in this region. This change has not been reported in either the MitoMap or mtDB databases, and has not been found in any of the individuals in our database whose whole mitochondrial genome was analyzed (>1500). The collective data suggest that the m.8358A>G variant is likely to be pathogenic. However, age at onset of disease appears to vary and the apparent homoplasmy in the proband's asymptomatic sister suggests other factors may affect penetrance. Studies of this variant in other tissues from family members may further elucidate its pathogenicity.

2055/T

Hyposialylation in a novel murine glomerulopathy. *S. Kakani[1], J. Poling[1], T. Yardeni[1], H. Dorward[1], I. Manoli[1], C. Ciccone[1], A. Astiz-Martinez[1], P. Zerfas[2], D. Darvish[3], E. Tian[4], K. Ten Hagen[4], W. Gahl[1], M. Huizing[1].* 1) Medical Genetics Branch, National Human Genome Research Institute, Bethesda, MD; 2) Division of Veterinary Resources, Office of Research Services, National Institutes of Health, Bethesda, MD; 3) HIBM Research Group, Encinco, CA; 4) Developmental Glycobiology Unit, NIDCR, NIH, Bethesda, MD.

Hereditary inclusion body myopathy (HIBM) is an autosomal recessive myopathy associated with mutations in the *GNE* gene, which encodes the bifunctional, rate-limiting enzyme in sialic acid (SA) biosynthesis, UDP-GlcNAc 2-epimerase/ManNAc kinase. Since Gne knockout mice are embryonic lethal, we created a knock-in HIBM mouse mimicking the M712T founder mutation. Over 95% of mutant mice died before postnatal day 3 (P3) with unexpected glomerular disease involving podocyte foot process effacement, glomerular basement membrane splitting, and hyposialylation of podocalyxin, the primary podocyte sialoprotein. Oral intake of the sialic acid precursor ManNAc increased survival of mutant pups beyond P3 to ~50%, with improvements in glomerular architecture and podocalyxin sialylation. We examined the glycosylation status of the renal glycocalyx in this mouse model, which may assist in identification of related human renal disorders due to hyposialylation. Paraffin embedded kidney sections from untreated (embryonic, P2) and ManNAc treated (P6) mice were stained with a variety of lectins and antibodies (WGA-FITC, PNA-FITC, Jacalin-488, HPA-FITC, Podocalyxin). Abnormal, increased podocyte staining in mutant kidneys was found for the lectins PNA (for terminal galactose), Jacalin (for terminal galactose on O-linked glycans), and HPA (for terminal GalNAc). These abnormal patterns were partially restored after ManNAc feeding. Podocalyxin, although hyposialylated in mutant mice, appeared to be correctly localized to podocyte membranes in all samples. The lectin profiles suggested hyposialylation of predominantly O-linked glycans in the glomerular glycocalyx of mutant *Gne* M712T knock-in mice. We hypothesize that when a shortage of SA occurs (i.e., through reduced *Gne* activity), N-linked glycans will be preferentially sialylated over O-linked glycans. Although ManNAc treatment does not completely restore glomerular glycocalyx sialylation, it re-sialylates and restores the glycocalyx sufficiently for survival beyond P3. Hyposialylation has been indicated in some human glomerulopathies, including minimal change nephrosis, membranous glomerulonephritis, focal segmental glomerulosclerosis, and IgA nephropathy. The murine lectin staining panel can now be applied to human samples for identification of glomerular hyposialylation. Moreover, ManNAc might be considered as a future treatment in humans afflicted by these renal diseases.

2056/T

Targeting mitochondrial disorders with custom array CGH. *T. Lewis, E. Lyon, R. Mao.* R&D, Molecular Genetics, ARUP Instutitue for Experimental and Clinical Pathology, Salt Lake City, UT.

BACKGROUND: Mitochondrial disorders are the most common group of metabolic disorders with an estimated prevalence of 1/5000. Mitochondrial disorders are clinically variable, affect single or multiple organs, present at any age and with any mode of inheritance creating a diagnostic challenge. An estimated 1500 genes encode for mitochondrial proteins; 37 encoded by the mtDNA while the remainder are encoded by nuclear DNA. As mutations can originate in either mtDNA or nuclear genes encoding organelle proteins, determining which of the many genes are responsible for the disease in the patient can be invasive, expensive, time-consuming, and labor-intensive. METHODS: To assist in laboratory evaluation, a custom CGH array was designed to cover 105 nuclear genes involved in mitochondrial biosynthesis as well as the entire mitochondrial genome. Probes were tiled at an average of 15bp throughout each nuclear gene including 500bp of flanking 5' and 3' material and spanning the mitochondrial genome. Nine samples with known deletions or duplications in the genomic regions covered on the array were analyzed using SignalMap2.5. RESULTS: Nine samples with known copy number changes were evaluated with the custom-designed mitochondrial array. In addition to concordant results for the known variations, potential new variant regions were observed. In the MFN2 gene, an 800bp copy number change in the mitofusin 2 (MFN2) gene was observed in two of nine samples (Log2 -0.50 and -0.59, 72 probes) and is of interest as it spans exon 18 encoding for the second coiled-coil domain. Three samples showed a copy number change in intron 3 of the PC gene and in intron 7 of the LARS gene, a deletion was observed in one patient with boundaries that precisely matching a LINE element. Four of the nine samples harbored a previously reported variation in introns 4 of the PDSS1 gene. CONCLUSIONS: A high-density custom array has been designed for detecting copy number changes in 104 nuclear genes implicated in mitochondrial disorders and the mitochondrial genome. The use of targeted array CGH holds great promise as a screening and diagnostic tool for mitochondrial disorders. The ability to examine greater than 100 genes at once, will allow the discovery of copy number changes in genes targeted for their role in mitochondrial disorders.

2057/T

ABCG2/BCRP encodes a high-capacity urate transporter and its common variant increases serum uric acid levels in humans. *I. Hiroki[1], H. Matsuo[1], T. Takada[2], K. Ichida[3,4], T. Nakamura[5], A. Nakayama[1,6], K. Suzuki[7], T. Hosoya[4], H. Suzuki[2], N. Hamajima[8], N. Shinomiya[1].* 1) Dept. Integrative Physiol., National Defense Med. College, Tokorozawa, Japan; 2) Dept. Pharm., Univ. Tokyo, Tokyo, Japan; 3) Dept. Pathophysiol., Tokyo Univ. Pharm. Life Sci., Tokyo Japan; 4) Dept. Intern. Med., Jikei Univ. School Med., Tokyo, Japan; 5) Lab. Math.,National Defense Med. College, Tokorozawa, Japan; 6) Dept. Defense Med., National Defense Med. College, Tokorozawa, Japan; 7) Dept Publ. Health, Fujita Health Univ., School Health Sci., Aichi, Japan; 8) Dept Preventive Med., Nagoya Univ. Grad. School Med., Aichi, Japan.

The ATP-binding cassette, subfamily G, member 2 (ABCG2/BCRP) gene encodes a multi-specific transporter which is expressed on the apical membrane in several tissues such as kidney and intestine. It was also reported that the ABCG2 gene locates in a gout-susceptibility locus (MIM 138900) on chromosome 4q, which was demonstrated by a genome-wide linkage study. Recent genome-wide association study revealed that ABCG2 relates to serum uric acid (SUA) and gout, while its mechanism was still unclear. Since the nucleotide analogs which is exported by ABCG2 is structurally similar to urate, we hypothesized that ABCG2 would be a urate secretion transporter and therefore relate to SUA. Transport assays were performed with the use of membrane vesicles prepared from ABCG2-overexpressing cells, and revealed that ABCG2 is a high-capacity urate transporter in the presence of ATP (calculated Km: 8.24 ± 1.44 mM). ABCG2 mutation with Q141K, a high-frequent variant, reduced its urate transport by approximately half, which could be accounted for by its decreased protein expression. Consequently, quantitative trait locus analysis of 739 Japanese individuals showed that the Q141K variant of ABCG2 increased SUA levels as the number of minor alleles of Q141K increased (P = 6.60 x 10-5), and when adjusted for sex, the corrected P value is 2.02 x 10-6. We also genotyped more than 2,000 individuals from a random sample of Japanese population and found that dysfunctional genotypes of ABCG2 occupied approximately 50% of them. These findings imply that ABCG2 has an essential physiological role to control SUA levels through urate secretion in vivo, and that its common dysfunctional variant increases SUA.

2058/T

Identification of a possible major genetic variant for hypokalemic periodic paralysis among Filipinos. *J.B. Nevado[1], K. Fabular[2], G. Facun[2], M. Falagne[2], J. Firme[2], M. Gabatino[2], D. Gauiran[2], L. Go[2], M. Gomez[2], E. Gonzales[2], L. Gonzales[2], J. Granada[2], P. Grullo[2], C. Hipolito[2], V. Igualada[2], N. Illescas[2], R. Imperio[2].* 1) University of the Philippines-Manila, Manila, Philippines; 2) University of the Philippines College of Medicine, Manila, Philippines.

Hypokalemic periodic paralysis (HOKPP) is a condition characterized by repeating episodes of ascending general loss of motor functions attributed to critically low blood potassium levels with intracellular shifting. Generally prevalent worldwide, the condition is far more common and severe among Southeast Asians, which suggests the presence of different genetic predisposition. Thus, we screened for genetic variations in CACNA1S in Filipinos with HOKPP. We recruited 10 adults with recurrent episodes of HOKPP in the past 3 years, without blood acidemia, and thyroid and renal function. Amplification and sequencing of the CACNA1S exons revealed a consistent deletion in 3 bases within exon 11 that produces frameshift variant present in all patients, but which balances out after 5 codons only. The changes are located in the second ion transport domain and the wild-type neutral-amino acid set (MSIFN from position 278) is replaced with predominantly positive and cysteine-containing set (CHLH), which can increase the channels's resistance to the inflow of calcium cations. Therefore, the described changes in the CACNA1S gene in Filipinos with HOKPP may perturb calcium homeostasis that can result to intracellular potassium retention.

2059/T

Preliminary Fluorometric and Colorimetric Assay in the Diagnosis of Metachromatic Leukodistrophy and Krabbe Diseases in Colombia. *I. Arévalo Vargas[1], M. Jay Garcia[1], P. Guerrero[2], J. Uribe[1].* 1) Universidad de los Andes, Bogotá D.C., Colombia; 2) Neuróloga Pediatra, Hospital Militar Central, Bogotá D.C., Colombia.

Inborn errors of metabolism are a group of hereditary diseases that consist in the storage of metabolites due to deficiency of an enzyme in a metabolic pathway. Specifically, Metachromatic Leukodystrophy (MLD) and Krabbe (KD) diseases are autosomal recessive disorders of sphingolipid metabolism that affect the nervous system, these diseases cause severe demyelination and are characterized by a deficiency of Arylsulfatase A (ASA) and β-Galactosylcerebroside (β-Galsyl) lysosomal enzymes, which generate an accumulation of galactosylcerebroside and sulfatides respectively. The reference values for these diseases in Colombia population are unknown. The aim of this study was to develop a compared colorimetric (COL) and fluorometric (FL) technique for MLD and KD.

We measured ASA activity with para-nitrocatechol sulfate for COL and 4-methylumbelliferyl sulfate (4-MUS) for FL, while β-Galsyl activity with Trinitrophenylamino-lauroylgalactocerebroside (TNPAL-Galactocerebroside) for COL and 6-hexadecanoylamino-4-methylumbelliferyl-β-D-galatoside (HMU-βGal) for FL. We analyzed 50 samples for ASA 38 controls and 12 patients with white matter damage and 23 samples were analyzed for β-Galsyl 18 controls and 5 patients. We use as enzyme control β-galactosidase (β-gal) and all activities were expressed in nmol/mg protein/h.

Activity ASA controls (n=38) ranged 0.8-3.4 for FL assay, while in COL technique ranged 100-500, and of the 12 individuals that were associated with white matter damage 3 showed a lower activity in both assays ranged 0.41-0.47 for FL and 32-55 for COL assay. Results obtained β-Galsyl activity in controls (n=23) in range of 2 to 5 for COL and 0.1 to 3 for FL, and a prospective patient with Krabbe with a lower activity 1.85 for COL and 0.034 for FL. The enzyme control βgal allowed to verify the sample quality, because with this we could confirm that samples weren't deficient in more than one enzyme.

This study established the reference values of controls for high-risk screening in Colombia. Both techniques are reliable for detection and early diagnosis of MLD and KD, nevertheless FL assay is 100 fold more sensitive than COL technique.

2060/T

Treatment Trends for Mucopolysaccharidosis I: Findings from the MPS I Registry. *P. Arn[1], R. Giugliani[2], T. Okayuma[3], F. Wijburg[4] on behalf of the MPS I Registry Board of Advisors.* 1) Division of Genetics, Nemours Children's Clinic, Jacksonville, FL; 2) Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil; 3) National Center for Child Health and Development, Tokyo, Japan; 4) Academic Medical Center, Amsterdam, The Netherlands.

OBJECTIVE: We analyzed how treatment approaches to MPS I have evolved over time in the MPS I Registry (www.mpsiregistry.com), an international observational database that collects data relevant to disease progression, treatment, and outcomes of MPS I patients. METHODS: Data entered into the Registry as of March 2009 were analyzed. Treatment allocation (hematopoietic stem cell transplantation [HSCT], enzyme replacement therapy [ERT] with laronidase, both ERT and HSCT, and neither treatment) was determined overall and in relation to year of diagnosis/treatment. Also analyzed were median ages at diagnosis and at first disease-specific treatment in relation to year of treatment initiation. RESULTS: Among the 227 Registry patients who began treatment <2003 (when ERT became available commercially), 24% received ERT only and 76% HSCT. Among the 535 patients who began treatment ≥2003, 75% received ERT alone and 25% underwent HSCT. The proportion of patients who received neither treatment declined from 16% among patients diagnosed <2003 to 9% among patients diagnosed ≥2003. The proportion of HSCT patients who also received ERT was 6% among patients who began treatment <2003, 62% (34/55) in 2003-2004, 76% (28/37) in 2005-2006, and 93% in 2007-2009 (40/43). Among all transplanted patients, median age at first treatment was under age 2 for all year ranges, and among ERT-alone patients was 12.3 in patients who began treatment <2003 (n=55) and 2.8 for those who began treatment in 2009 (n=16).The median interval between diagnosis and first treatment declined in transplanted patients by 0.4 years and in ERT-alone patients by 5.8 years from <2003 to 2007-2009. CONCLUSIONS: Over time, data from the MPS I Registry show a decreasing proportion of untreated patients, an increasing proportion of transplanted patients also receiving ERT, a decreasing age at first treatment among ERT-alone treated patients, and a decreasing interval between diagnosis and treatment in all patients.

2061/T

Genetic screening study of Niemann-Pick disease type C (NP-C) in adults with neurological/psychiatric signs (ZOOM). *P. Bauer[1], H.H. Klünemann[2], F. Sedel[3], D. Linden[4], J.E. Wraith[5], M.C. Patterson[6], M. Pineda[7], J. Priller[8], A. Muller[9], H. Chadha-Boreham[9], C. Remy[9], D.J. Balding[10].* 1) Medical Genetics, Univversity of Tuebingen, Tuebingen, Germany; 2) Regensburg University Clinic for Psychiatry and Psychotherapy, Regensburg, Germany; 3) Pitié Salpêtrière Hospital, Paris, France; 4) Bangor University, Bangor, UK; 5) Manchester Academic Health Science Centre, St Mary's Hospital, Manchester, UK; 6) Mayo Clinic, Rochester, MN, USA; 7) Hospital Sant Joan de Déu, Barcelona, Spain; 8) Neuropsychiatry, Charité-Universitätsmedizin, Berlin, Germany; 9) Actelion Pharmaceuticals Ltd, Allschwil, Switzerland; 10) Institute of Genetics, University College London, London, UK.

A gene sequencing analysis recently found an NP-C prevalence of 6.4% among undiagnosed adolescent and adult psychiatric patients. To investigate further the extent to which adult psychiatric patients might be affected by NP-C, an international genetic screening study has been designed to evaluate NP-C in patients with psychosis or early-onset dementia.

The first phase of this multicentre observational study is cross-sectional, aimed at evaluating the prevalence of NP-C in selected psychiatric patients who will be recruited from around 50 psychiatric and neurological reference centres across the EU and USA. Consecutive adult patients aged 18-50 years will be included according to five criteria, which specify different combinations of psychosis and/or early-onset dementia plus pre-defined neurological or visceral symptoms. Psychosis is defined according to ICD-10 and DSM-IV criteria. Blood samples will be collected from all recruited patients for *NPC1* and *NPC2* gene mutation analysis (direct sequencing and multiplex ligation-dependent probe amplification). Data on patients' clinical status and history will be captured electronically by secure web-based reporting. As well as evaluating NP-C prevalence, analyses of the cross-sectional data will assess NP-C phenotypes and possible genotype-phenotype associations. Next, a retrospective case-control phase will explore non-genetic predictors of NP-C that may be useful as diagnostic tools.

This screening study will provide valuable data on the prevalence of latent NP-C among adults with neurological/psychiatric signs, and will strengthen knowledge of the clinical manifestations of adult NP-C.

2062/T

Effect of miglustat on disease progression in Niemann-Pick disease type C (NP-C): a multicentre retrospective observational cohort study. *R. Giorgino[1], M.C. Patterson[2], M. Pineda[3], F. Sedel[4], C. Luzy[1], J.E. Wraith[5].* 1) Actelion Pharmaceuticals, Allschwil, Switzerland; 2) Mayo Clinic, Rochester, MD, USA; 3) Hospital Sant Joan de Déu, Barcelona, Spain; 4) Hôpital Pitié Salpêtrière, Paris, France; 5) Manchester Academic Health Science Centre, St Mary's Hospital, Manchester, UK.

NP-C is a rare and devastating genetic disorder characterized by a range of progressive, disabling neurological manifestations. A clinical trial demonstrated that miglustat stabilized key parameters of neurological disease progression in children and adults with NP-C over 24 months. We present data from a subset of miglustat-treated patients who were included in a previous observational cohort study assessing the natural history of NP-C.

Physicians managing patients with NP-C in clinical practice settings were asked to retrospectively assess patients' neurological disease progression using a disability scale at diagnosis and up to 4 follow-up visits, including both pre- and post-treatment evaluations. The disability assessment[1] evaluated patient function on 4 key parameters of disease progression (ambulation, manipulation, language and swallowing), rated on a modified scale from 0 (best) to 1 (worst). Individual disability parameter scores and a composite score (including all 4 parameters) were assessed.

A total of 19 miglustat-treated patients were included in the observational cohort. The mean±SD patient age was 13.6±5.9 years at diagnosis and 18.7±8.0 years at miglustat initiation. The mean±SD period between diagnosis and treatment was 4.9±4.1 years. The median (range) duration of miglustat therapy was 1.2 (0.2-3.0) years. Mean (95%CI) composite scores increased (indicating disease progression) from 0.18 (0.13,0.23) at diagnosis to 0.48 (0.39,0.57) at last pre-treatment visit. After miglustat treatment, the mean (95%CI) score was 0.44 (0.34,0.35), indicating stabilization. Similarly, individual parameter scores for ambulation, manipulation, language and swallowing increased between diagnosis and last pre-treatment visit, and were consistently reduced after miglustat therapy.

These data indicate continuous neurological disease progression in NP-C patients before treatment and stabilization of disease-specific neurological parameters after miglustat therapy.

1. Pineda et al. Mol Genet Metab 2009;98:243-9.

2063/T

Guidelines for the evaluation and management of children with Fabry disease. *R.J. Hopkin[1], M. Banikazemi[2], D.P. Germain[3], M. Mauer[4], A. Tylki-Szymanska[5], D.G. Warnock[6], W.R. Wilcox[7].* 1) Department of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 2) Department of Clinical and Molecular Genetics, Columbia University College of Physicians and Surgeons, New York, NY; 3) University of Versailles, Hôpital Raymond Poincaré, Garches, France; 4) Department of Pediatrics, University of Minnesota Medical School, Minneapolis, MN, United States; 5) The Children's Memorial Health Institute, Warsaw, Poland; 6) Department of Medicine, University of Alabama at Birmingham, Birmingham, AL; 7) Medical Genetics Institute, Cedars-Sinai Medical Center Los Angeles, CA.

Fabry disease is an X-linked lysosomal storage disorder, characterized by decreased or absent activity of lysosomal α-galactosidase A. As a result of this enzyme deficiency, globotriaosylceramide (GL-3) and other glycosphingolipids accumulate within various tissues and eventually impair vital organ function, putting patients at risk of developing renal failure, cardiovascular dysfunction, and stroke. The initial signs and symptoms of Fabry disease, including neuropathic pain in the extremities, hypohidrosis, angiokeratomas, and gastrointestinal discomfort, generally appear during childhood. More serious complications of Fabry disease can also occur during childhood, including proteinuria, valvular dysfunction, conduction abnormalities, left ventricular hypertrophy and arrhythmia. In view of the growing recognition that significant manifestations of Fabry disease can occur during childhood, an international group of physicians who have pediatric patients enrolled in the Fabry Registry developed a set of specific guidelines for assessing Fabry disease in children based on evidence from the Fabry Registry and published literature. A summary of the general types of recommended assessments is shown below.

Summary of Recommended Pediatric Assessments

Medical and Family History	Measured Glomerular Filtration Rate
Enzyme Activity and Genotype	Albuminuria and Proteinuria
Physical Exam	Quality of Life, Fatigue, Pain
Cardiac, Audiologic, Cranial MRI	Antibodies, Plasma GL3 Levels

This new Pediatric Minimum Recommended Schedule of Assessments will increase the medical community's awareness of the burden of Fabry disease in children, assist with monitoring , and improve the outcome for these young patients.

2064/T

Glucocerebrosidase alternative promoter has features and expression characteristic of housekeeping genes. *M. Hrebicek[1], E. Svobodova[1], L. Mrazova[1], O. Luksan[2], L. Stolnaja[1], M. Jirsa[2], L. Dvorakova[2].* 1) Institute of Inherited Metabolic Disorders, Charles University, Prague, Czech Republic; 2) Laboratory of Experimental Hepatology, Institute of Clinical and Experimental Medicine, Prague.

Glucocerebrosidase deficiency is the cause of Gaucher disease. Glucocerebrosidase transcripts show alternative splicing at their 5' ends, suggesting that a fraction of the transcripts originates at an alternative upstream promoter (P2) located 2.6 kb upstream of the first ATG. Currently, there are five alternative GBA transcripts in the databases, one of which is transcribed from the downstream (P1) promoter and four others apparently from the P2 promoter. The alternative transcripts from the putative upstream promoter contain one or two extra exons (exon -2 or exons -2, -1, respectively), but the first ATG codon and predicted amino-acid sequence are the same as in the transcript from the downstream P1 promoter. Our goal was to confirm that the putative P2 functions as a promoter and to study its properties. The in-vitro Dual-Luciferase Reporter Assay (Promega) was used to verify that the region 1 kb upstream of exon -2, which contains the presumed P2, can function as a promoter. The constructs with the sequence of P1 promoter exhibited the highest activity of luciferase (17, 82 ± 1,1 relative luciferase units) in HEP-G2 cells, while the P2 construct reached 3, 01 ± 0, 43 relative luciferase units. Four constructs carrying serial deletions of P2-containing construct pGL4 -353/-1509 were created to delineate the P2 promoter. The region from -1311 bp to -1509 bp (counted from the first ATG) likely contains a negative regulator of transcription activity. Three major initiation sites (328, 361 and 394 bp upstream of the first ATG) were identified by 5'RACE. Although faint RT-PCR products, which may represent promoter upstream transcripts (PROMPTs), were amplified up to position -481 bp, quantitative RT-PCR using TaqMan probes (Applied Biosystems) confirmed that the -328, -361 and -394 positions are the main transcription initiation sites. The expression of P2-originating transcripts measured by quantitative RT-PCR in twenty different tissues revealed similar levels relative to two known housekeeping genes, as well as to transcripts from P1. The P2 contains an unmethylated CpG island, multiple Sp-1 consensus binding sites and unlike P1 does not contain a TATA-box and CAAT boxes. Together with its expression pattern these features underscore the housekeeping character of the P2 promoter of GBA gene. Whether the promoter may play a role in the pathogenesis of Gaucher disease remains to be elucidated. Supported by GAUK 121407, VZ MšM ČR 0021620806.

2065/T

Wolman Disease (LIPA p.G87V) Genotype Frequency in Patients of Middle Eastern Descent. *D. No[1,2], Y. Valles-Ayoub[1], S. Esfandiarifard[1], P. Sinai[1], Z. Khokher[1,2], G. Lee[1], D. Darvish[1].* 1) HIBM Research Group, Reseda, CA; 2) Los Angeles Mission College, Sylmar, CA.

Wolman disease is a rare inherited condition caused by Lysosomal acid lipase (LIPA, LAL) deficiency. Infants with Wolman disease are healthy and active at birth but develop symptoms of severe malnutrition in the first few months of life. Harmful amounts of lipids accumulate in the spleen, liver, bone marrow, intestine, adrenal glands, and lymph nodes. Almost all affected infants die before the age of one. Although worldwide incidence is estimated at 1/350,000 newborns, Wolman disease seems to occur at a higher frequency in the Middle Eastern community of the Los Angeles area. Identifying high risk populations for this rare genetic disease may have significant impact for those working on gene therapy targeting lysosomal enzyme deficiencies. As a validation study, we analyzed 165 DNA specimens of Middle Eastern origin by automated sequencing of Exon 4 of LIPA, in search of p.G87V (ggc>gtc). This mutation is also described as p.G66V due to post-translational modification of the LAL protein (coded for by LIPA) that cleaves off the first twenty-one amino acids. For LIPA p.G87V, a heterozygous frequency of 3/165 (1.82%) was discovered. Thus, we estimate that as high as 1 in 12,100 newborns of Middle Eastern couples may be at risk. Additional studies are required to confirm and further validate the higher frequencies seen in our sample pool, and to determine if people of Middle Eastern descent have a higher risk for Wolman disease.

2066/T

Updated results from a Phase 2 study of eliglustat tartrate, a novel, investigational oral compound for Gaucher disease type 1 (GD1). *J. Peterschmitt[1], E. Lukina[2], N. Watman[3], E.A. Arreguin[4], G. Pastores[5], M. Iastrebner[6], M. Dragosky[6], H. Rosenbaum[7], M. Phillips[8], M. Kaper[1], T. Singh[1], A.C. Puga[1].* 1) Genzyme Corp, Cambridge, MA; 2) National Research Center for Haematology, Moscow, Russia; 3) Hospital Ramos Mejia, Buenos Aires, Argentina; 4) Instituto Mexicano del Seguro Social Hospital de Especialidades, Col. La Raza, Mexico; 5) New York University, New York, USA; 6) Instituto Argentino de Diagnostico y Tratamiento, Buenos Aires, Argentina; 7) Rambam Medical Center, Haifa, Israel; 8) Sha'are Zedek Medical Center, Jerusalem, Israel.

Introduction: Eliglustat tartrate (Genz-112638) is a novel oral inhibitor of glucosylceramide synthase under investigation for the treatment of GD1. Aim: Report updated 2-year efficacy and safety data from an ongoing Phase 2 study. Methods: This open-label, uncontrolled, multicenter Phase 2 clinical trial of eliglustat tartrate (50 or 100 mg bid depending on plasma level) enrolled 26 untreated adults with GD1. Efficacy results included changes from baseline in spleen and liver volumes, hemoglobin and platelet levels, bone mineral density (BMD) and other skeletal findings (reviewed centrally), and achievement of GD1 therapeutic goals for anemia, thrombocytopenia and organomegaly. Results: New 30-month hematologic, visceral and biomarker data will be available for presentation. Data are currently available for up to 20 patients who completed 2 years of treatment. Mean hemoglobin level increased by 2.1±1.5 g/dL and platelet count by 81.5±56.0%; mean spleen volume decreased by 52.4±10.7% and liver volume by 23.9±12.8% (all P<0.001). No bone crises or reductions in mobility were reported. Bone marrow infiltration by Gaucher cells was reduced (8/18 patients) or stable (10/18 patients). There were no new lytic lesions or bone infarcts. Existing lytic lesions remained stable; of 7 existing infarcts, 1 improved and 6 remained stable. Mean lumbar spine BMD increased by 7.8±10.6% (P= 0.010), DXA T-Score by 0.6±0.8 (P=0.012), and DXA Z-Score by 0.6±0.7 (P=0.003), with major gains among osteoporotic/osteopenic patients. After 2 years, most patients met short-term therapeutic goals published by Pastores et al (*Semin Hematol* 2004). Overall, 85% (17/20) of patients met established therapeutic goals for ≥3 of 4 parameters. Eliglustat tartrate was well tolerated. The most common adverse events (AEs) through 2 years were viral infections (6 patients), and urinary tract infections, increased blood pressure, and abdominal pain (3 patients each). Eight drug-related AEs, all mild, occurred in 6 patients. Discussion: In the Phase 2 study, eliglustat tartrate has shown promising efficacy and safety as a potential oral substrate-reduction therapy for GD1 with continued improvements in hematologic, visceral, and bone parameters after 2 years. Two controlled Phase 3 registration studies are underway in untreated patients (ENGAGE) and in patients switching from enzyme replacement therapy (ENCORE). Another Phase 3 study will compare different dose frequencies of eliglustat (EDGE).

2067/T

The emerging phenotype of long-term infantile Pompe survivors. *S.N. Prater, S.G. Banugaria, L.E. Case, J.F. Mackey, M.M. Canfield, S.L. DeArmey, P.S. Kishnani.* Duke University Medical Center, Durham, NC.

The natural history of untreated infantile Pompe disease is limited as the median age of death in this lethal disorder is approximately 12.7 months. With the advent of enzyme replacement therapy (ERT) with alglucosidase alfa (Myozyme®), the clinical course of the disease has improved significantly. Improved survival has also led to a number of unanswered questions regarding the clinical course of treated disease. Currently, there is a paucity of data in the published literature pertaining to the physical and cognitive phenotype of older infantile Pompe survivors. Herein we report a series of 9 (7 M, 2 F) of the oldest infantile Pompe survivors treated with ERT ranging from 4 to 10 years of age. All patients had symptom onset within the first 6 months of life with the presence of severe cardiomyopathy. Median age at start of ERT was 4.6 months (range 0.3 - 6.3 months). Patients received alglucosidase alfa at 20 - 40 mg/kg biweekly and all patients tolerized to ERT. At most recent follow-up, all 9 patients were ambulatory and none required invasive ventilation. The majority of patients had some residual motor weakness (neck flexor and dorsiflexor weakness, myopathic facies). Additional notable findings included ptosis, hypernasal speech, velopharyngeal weakness, sensorineural hearing loss and osteopenia. Despite hypernasal speech and some expressive language delays, these children are cognitively intact. Although there are some phenotypic similarities to late-onset patients, there also exist key distinguishing features. This series represents the largest cohort of long-term survivors of infantile Pompe disease. It provides a contextual basis for the emerging phenotype and relevant issues in clinical management. Data obtained thus far are encouraging. Continued systematic follow-up is needed, however, to better characterize this emerging phenotype and to allow for improved patient management.

2068/T

Evaluation of the apoptotic effect of globotriaosylceramide (Gb3)on normal mononuclear cells: implication for Fabry disease. *P. Rozenfeld, N. De Francesco, C. Fossati.* Dept Immunolgia, Univ Natl de La Plata, Buenos Aires, Argentina.

Fabry disease is an X-linked lysosomal storage disorder (LSD) due to deficiency of the enzyme alpha-galactosidase A, resulting in intracellular deposition of globotriaosylceramide (Gb3). Plasma Gb3 concentration in Fabry disease patients varies between 2 to 50 μM, while in normal individuals values are below 1,2 μM. In order to analyze a possible pathophysiologic role of Gb3 in Fabry disease, we aim to investigate weather addition of Gb3 on normal cells induces apoptosis. Peripheral blood mononuclear cells (PBMC) from normal control individuals were treated in culture with different concentrations of Gb3 (range: 0 a 40 μM) during different times (0 to 48 hs). Apoptosis levels were analyzed by Annexin V and TUNEL by flow cytometry. Moreover, mithocondrial membrane depolarization by the reagent JC-1 was measured. The treatment of PBMC with Gb3 at different times resulted in an increase of apoptosis, with maximum levels at 6hs. The dose response curve with Gb3 showed a positive effect on apoptosis. These results show a direct effect of pathological Gb3 levels on PBMC. This result would suggest a causal relationship on apoptosis in Fabry disease that could be related to pathophysiology of this disorder.

2069/T

International disease registry for Niemann-Pick disease type C: preliminary data. *B. Schwierin[1], E. Mengel[2], F.A. Wijburg[3], M. Pineda[4], J.E. Wraith[5], M.T. Vanier[6], A. Muller[1], M. Silkey[1], R. Giorgino[1], M.C. Patterson[7].* 1) Actelion Pharmaceuticals Ltd, Allschwil, Switzerland; 2) Villa Metabolica, University of Mainz, Mainz, Germany; 3) Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands; 4) Hospital Sant Joan de Déu, Barcelona, Spain; 5) Manchester Academic Health Science Centre, St Mary's Hospital, Manchester, UK; 6) INSERM Unit 820, Lyon, France; 7) Mayo Clinic, Rochester, MN, USA.

A disease registry was started in Europe in September 2009 to evaluate the long-term disease course of Niemann-Pick disease type C (NP-C) in clinical settings.

All patients with a diagnosis of NP-C are eligible for inclusion irrespective of treatment. Demographics, disease characteristics and treatment data are collected. Patients are monitored using a disability scale (Pineda et al. Mol Genet Metab. 2009;98:243-9) that evaluates ambulation, manipulation, language and swallowing; rating from 0 (best) to 1 (worst).

Eighteen patients (median age [range] 13.8 [1.9-46.3] years; 50% female) were enrolled as of March 2nd 2010. Seventeen patients were confirmed as receiving miglustat therapy (mean exposure 1.83 years). Early visceral involvement was recorded in 4/9 (44%) evaluable patients. Most patients (15/18 [83%]) were diagnosed by biochemical or biochemical/genetic testing. Age at diagnosis ranged from 1.7-44.2 years. All patients had neurological manifestations at enrolment. Median ages at first neurological manifestation were: 0.4 years in early-infantile (aged 6 months to <2 years, n=1), 4.9 years in late-infantile (2 to <6 years, n=3), 10.1 years in juvenile (6 to <15 years, n=10), and 39.6 years in adolescent/adult patients (≥15 years, n=3). The median (95%CI) composite disability score among 15 evaluable patients was 0.35 (0.23, 0.55) at enrolment. Low numbers of patients had normal ambulation (2/15 [13%]), language (3/15 [20%]) and manipulation (3/16 [19%]) at enrolment; 7/16 (44%) had normal swallowing.

This registry will provide valuable information on the long-term progression of functional neurological impairments and treatment outcomes in NP-C.

2070/T

MCP-1 deficiency reduces macrophage infiltration and aggravates demyelination and paralysis in mouse model of Krabbe disease. *J.S. Shen[1, 2], X.L. Meng[1, 2], S. Kawagoe[3], K. Shimada[4], Y. Eto[3], T. Ohashi[2, 3].* 1) Institute of Metabolic Disease, Baylor Research Institute, Dallas, TX; 2) Department of Gene Therapy, Jikei University School of Medicine, Tokyo, Japan; 3) Department of Genetic Diseases & Genome Science, Jikei University School of Medicine, Tokyo, Japan; 4) Department of Anatomy, Jikei University School of Medicine, Tokyo, Japan.

Krabbe disease is a severe demyelinating disease caused by genetic deficiency of a lysosomal enzyme β-galactocerebrosidase. Infiltration of PAS positive macrophages in the nervous system is the hallmark of Krabbe disease. However, the exact role of these microglia/macrophages in pathogenesis of this disease remains to be elucidated. Monocyte chemoattractant protein-1 (MCP-1) is upregulated significantly in the brain of animal models of Krabbe disease, suggesting that MCP-1 may contribute to cell infiltration. To assess the role of microglia/macrophages in Krabbe disease, twitcher mice, the murine model for Krabbe disease were mated with MCP-1 knockout mice to generate MCP-1 deficient twitcher mice. Compared to MCP-1 intact (+/+) twitcher, MCP-1 deficient (-/-) twitcher mice showed worsened clinical symptoms including general motor functions and muscle strength. The onset of paralysis of hind limbs also occurred earlier in MCP-$1^{-/-}$ twitcher than MCP-$1^{+/+}$ twitcher. There was significant reduction of the number of PAS^+ macrophages and Mac-1 (a marker of monocyte/macrophage)-immunoreactive cells in MCP-1 deficient twitcher mouse brain. Consistent to the worsened neurological symptoms, immunohistochemistry and quantitative RT-PCR showed significantly reduced myelin basic protein and other myelin related proteins in both the central and peripheral nervous systems in MCP-$1^{-/-}$ twitcher compared to MCP-$1^{+/+}$ twitcher mice. This study demonstrated that MCP-1 plays important role in recruitment of macrophages to demyelinating lesions and that microglia/macrophages infiltration plays protective, rather than pathogenic, role in Krabbe disease.

2071/T

Hypodontia of mandibular permanent canines and long with curved tooth roots are newly recognized finding of Mucopolysaccharidosis type VI. *P. Tanpaiboon[1], P. Dejkhamron[2], P. Kantaputra[3].* 1) genetics and metabolism, children's national medical center, Washington, DC; 2) Department of Pediatrics, Faculty of Medicine, Chiang Mai university, Chiang Mai 50200 Thailand; 3) Division of Pediatric Dentistry, Department of orthodontics and Pediatric Dentistry, Faculty of Dentistry Chiang Mai University, Chiang Mai 50200 Thailand.

Mucopolysaccharidosis VI (MPS VI) is a very rare autosomal recessive lysosomal storage disease caused by deficiency of the enzyme Arylsulfatase B. Lacking or decreasing enzyme activity leads to accumulation of dermatan sulfate. Phenotype is highly variable and involves multiorgan systems. Oral manifestations have been reported including thick lips, high-arched palate, enlarged alveolar process, gingival hyperplasia, opened mouth and enlarged tongue. The previously reported dental anomalies consist of delayed tooth eruption, enamel hypoplasia, malocclusion, multiple dentigerous cysts, and malformed teeth. Here, we report a Thai MPS VI with new dental findings. A 17 yr-old girl was referred to genetics clinic due to coarse facies and short stature. She also had symptoms of left sided heart failure. She is a freshman and can live independently. Her medical problems were first brought to medical attention when she was 9 yr-old. From the medical record at that time, she had short stature, joint stiffness, and cloudy cornea. The time line of each problem was unclear and she had not received any diagnosis or treatment. Family history was significant with a 9 yr-old brother who also had similar clinical phenotypes but with less stiffness of joints and no heart problems. Both parents were healthy and denied consanguinity. Physical examination showed body weight 20 kg, height 102 cm, macrocephaly, coarse facies and cloudy cornea. Mitral stenosis, aortic stenosis murmurs were detected. She had umbilical hernia, hepatosplenomegaly, limitation of spine-joints movement and claw hands. Skeletal survey demonstrated dysostosis multiplex. Echocardiography showed severe mitral valve stenosis and mild aortic stenosis. Enzyme analysis indicated Arylsulfatase B deficiency confirming diagnosis with MPS VI. Oral manifestations consist of congenital missing of the mandibular permanent canines, maxillary constriction, posterior crossbite, anterior openbite, long and curved tooth roots, and widening of alveolar process. Hypodontia and long tooth roots have never been described in patients with MPS VI. In conclusion this is the first time that hypodontia and long tooth roots are reported in a patient with MPS VI. It is noteworthy that mutation of Arylsulfatase B which led accumulation of dermatan sulfate subsequently caused hypodontia and long tooth root in this patient. These findings have raised the question of the role of dermatan sulfate in tooth development.

2072/T

TNFα contributes to inflammation in the pathogenesis of Sandhoff disease. *E.J. White[1], H. Abo Ouf[1], S.A. Igdoura[1,2].* 1) Department of Biology; 2) Department of Pathology & Molecular Medicine, McMaster University, Hamilton, Ontario, Canada.

Mutations in the hexb gene can produce a deficiency in the lysosomal enzymes beta-hexosaminidase A and beta-hexosaminidase B, which leads to the accumulation of GM2 ganglioside and related glycolipids in neurons. The hexb-/- mouse provides a model of the inherited human disorder, known as Sandhoff disease, with progressive neurodegeneration leading to paralysis and seizures. In addition to the GM2 storage disorder that is intrinsic to disease progression, increases in pro-inflammatory immune cell function are hypothesized to contribute to the pathogenesis with TNFα signaling playing a key role. Previous work has demonstrated that the ablation of TNFα delays the onset of neurodegeneration in hexb-/- mice. The contribution of innate and adaptive immune responses in the onset and progression of Sandhoff disease is still unclear. The first aim of this study is to examine the impact of hexb deficiency as well as hexb and TNFα combined deficiencies on the immunophenotypes of peripheral inflammatory cells. In addition, the second aim is to examine cytokine production in bone marrow derived macrophages (BMDMs) from wild type, hexb-/- and hexb-/-tnfα-/- mice in response to treatment with LPS, poly I:C or GM2 ganglioside. Our findings indicate that hexb and TNFα combined deficiencies result in an increase in the frequency of peripheral blood $CD19^+$ B cells, $CD8^+$ cytotoxic T cells as well as $NK1.1^+$ NK and $NK1.1^+CD3^+$ NKT cells when compared to hexb deficient and wild type mice. Interestingly, hexb-/- mice show an overall decrease in all examined immunophenotypes of peripheral blood cells when compared to wild type mice. Furthermore, BMDMs from hexb-/- and hexb-/-tnfα-/- mice produce significantly lower levels of MCP-1 and IL-10 in response to LPS whereas IL-1β levels are only reduced in BMDMs from hexb-/-tnfα-/-. On the other hand, IL-12p70 expression was elevated in BMDMs from hexb-/- mice. These data point to an effect of TNFα on the abundance of peripheral blood cells during neuroinflammation. In addition, the LPS response observed in BMDMs reflects a role for both hexb and TNFα as modulators of macrophage function. Our data highlights the importance of TNFα during the robust neuroinflammatory cascade in Sandhoff disease.

2073/T

Few females develop anti-α-galactosidase A IgG antibodies in response to agalsidase beta treatment: data from the Fabry Registry. *W.R. Wilcox[1], D.J. Gruskin[2], D.G. Warnock[3].* 1) Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, CA; 2) Genzyme Corporation, Cambridge, MA; 3) Department of Medicine, University of Alabama at Birmingham, Birmingham, AL.

Fabry disease results from a genetic deficiency of lysosomal α-galactosidase A (αGAL). The lack of αGAL enzymatic activity impairs the catabolism of globotriaosylceramide (GL-3) and other glycosphingolipids, which then accumulate within various cell and tissue types. Enzyme replacement therapy (ERT) with agalsidase beta, a form of recombinant human αGAL, is administered via intravenous infusions. Like all therapeutic proteins, agalsidase beta can potentially cause immunogenicity. Seroconversion status and peak anti-αGAL immunoglobulin G (IgG) antibody titers were evaluated in patients enrolled in the Fabry Registry. As of April 2, 2010, a total of 827 Fabry Registry patients who were treated with agalsidase beta as their only source of ERT had reported post-baseline anti-αGAL IgG titer data. Four hundred sixteen of 573 males (73%) eventually developed anti-αGAL IgG antibodies, with a median peak titer value of 3200 (minimum 100, maximum 819200). In contrast, only 31 of 254 females (12%) seroconverted and their median peak titer value was 200 (minimum 100, maximum 6400). Males were less likely than females to have tolerized (defined as being seronegative after seroconversion, at time of the most recent anti-αGAL IgG test); only 47 of 416 males tolerized (11%, 95% CI: 8%-15%), compared to 17 of 31 females (55%, 95% CI: 36%-72%). There were no statistically significant differences in endogenous (in vitro) levels of plasma or leukocyte αGAL enzyme activity between patients who had seroconverted and patients who had not. However, only a subset of patients who had seroconverted reported corresponding plasma or leukocyte αGAL enzyme activity data (222 of 416 males [53%] and 15 of 31 females [48%]). Genotype data were available for 27 of the 31 females who seroconverted. Most genotypes were unique and there were no clear similarities in genotype among the females who seroconverted. In summary, relatively few Fabry Registry females developed antibodies against agalsidase beta. Those females who did develop antibodies had much lower peak titer values than males and were likely to tolerize.

2074/T

Clinical phenotype in a mother and her son with a novel mutation for Fabry disease. *S. Yang, K. O'Brien, C. Tifft.* NHGRI, National Institute of Health, Bethesda, MD.

Fabry Disease (FD) is an X-linked lysosomal storage disease caused by mutations of the alpha-galactosidase A (GLA) gene resulting in a deficiency of the alpha-galactosidase A (alpha-GAL-A) enzyme. Male hemizygotes with classical Fabry disease often show the complete spectrum of symptoms beginning in mid childhood while female heterozygotes demonstrate a variable phenotype ranging from that seen in male patients to asymptomatic. We describe the case of a 45-year-old African American female with 12 year history of hypertension who developed rapid elevation in serum creatinine with insignificant proteinuria leading to renal biopsy for a diagnosis. The biopsy showed distended cytoplasm, myelinoid fibures, and zebra bodies in the podocytes consist with Fabry disease. At the time of her initial genetic evaluation, she denied any clinical symptoms of Fabry disease and there were no findings on physical exam to support the diagnosis. The family history was likewise negative. However, the molecular analysis of the GLA gene revealed a novel missense mutation in exon 6 (c806 T>A; p.Val269Glu) of unknown clinical significance. A detailed workup at the NIH Clinical Center was initiated which revealed multi-organ system involvement including left ventricular hypertrophy, increased Gb3 (globtriaosylceramide) excretion, whorl-like corneal opacities (cornea verticillata), hypohidrosis, and possible mild acroparesthesias (peripheral neuropathy). The patient's 23-year-old asymptomatic son was also tested and found to carry the V269E mutation. His AGA activity of 5.6 pmol/punch/hr was well within the range for patients with Fabry disease (2.0-12.0). Our findings conclude that the V269E mutation may be associated with a milder clinical course; however, close clinical monitoring remains important. Enzyme replacement therapy (ERT) has been discussed and offered to both patients.

2075/T

A missense variation in human Fibroblast Growth Factor 23 (FGF23) gene that induces functional alteration and shows association with renal phosphate leak in calcium nephrolithiasis. *T. Esposito[1], G. Mossetti[2], D. Rendina[2], G. De Filippo[2], A. Perfetti[3], S. Magliocca[1], P. Formisano[3], F. Gianfrancesco[1], P. Strazzullo[2].* 1) Inst Gen & Biophysics, Italian Natl Res Council, Naples, Italy; 2) Dep. of Clinical and Experimental Medicine, Federico II University Medical School, Naples, Italy; 3) Dip. di Biologia e Patologia Cellulare e Molecolare, Università di Napoli "Federico II", Naples, Italy.

Approximately 20% of patients with calcium nephrolithiasis and normal parathyroid function show a reduced rate of renal tubular phosphate reabsorption and reduced serum phosphate concentration (i.e. renal phosphate leak). We have previously demonstrated that in these patients circulating levels of fibroblast growth factor 23 (FGF23), a hormone regulating phosphate homeostasis, were significantly higher compared to stone formers without renal phosphate leak and to healthy controls. We detected a C716T (T239M) non-synonymous change in the FGF23 gene in stone formers with renal phosphate leak. The C716T allele and genotype frequencies were significantly higher compared to controls [C vs. T allele frequencies (p= 0.03), genotype frequencies (p=0.007)], and to stone formers without renal phosphate leak (p=0.024 and p=0.002, respectively). In the study population as a whole, subjects carrying the T239M missense variation showed levels of serum phosphate and TmPi/GFR significantly lower compared to subjects without the variation. The major intracellular signaling pathway of FGF was shown to be the Ras/MAPK pathway. FGF-23 was found to significantly phosphorylate ERK, and then we tested the ability of FGF23 wt and FGF23-239M to induce ERK phosphorylation. Our data clearly showed that both proteins (wt and 239M) induced dose dependent activation of ERK protein, and that the mutated protein (FGF23-239M) gives higher activation when compared to wt protein. These data could suggest that in presence of the mutated protein (FGF23-239M) there is a major inhibition of the sodium-phosphate co-transporter operating in the proximal tubule, resulting in renal phosphate leak that we observed in our patients. Our results highlight a novel significant association between the T239M missense variation in the FGF23 gene and calcium nephrolithiasis with renal phosphate leak.

2076/T

Generation and Characterization of a Mouse Model of Polyglucosan Body Disease. *H.O. AKMAN, W.J. CRAIGEN.* Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Background: The synthesis of glycogen is catalyzed by the sequential actions of two enzymes: (i) glycogen synthetase, which attaches glucosyl units to linear chains of glycogen; and (ii) glycogen branching enzyme (GBE), which attaches a short branch of glucosyl units to a linear chain. Glycogen storage disease type IV (GSD IV) (OMIM 232500) is an autosomal recessive disorder caused by GBE deficiency, leading to the accumulation of an abnormal amylopectin-like polysaccharide (polyglucosan) in multiple tissues, including liver, heart, skeletal muscles, and the central nervous system. A late-onset clinical variant, known as adult polyglucosan body disease (APBD), causes a neurodegenerative disorder simulating amyotrophic lateral sclerosis (ALS). The two naturally occurring animal models of this disorder, American quarter horses and Norwegian forest cats, are not practical laboratory animals. The mechanism for polyglucosan associated pathology is poorly understood and there are no treatment strategies for the disease. Method: We have developed a mouse model for APBD by introducing a common adult disease allele, Y329S, into the mouse GBE1 gene by homologous recombination in embryonic stem cells. In addition, we have generated a null allele by deleting exon 7 of the GBE gene. Results: GBE1 activity in muscle and liver is decreased to 6% and 30% of control values in mice homozygous and heterozygous, respectively, for the Y329S mutation. The Y329S mutation does not cause polyglucosan accumulation in heterozygous animals, and they appear otherwise normal, however homozygous animals have severe polyglucosan accumulation in muscle and liver, and to a lesser extent in peripheral nerves. Conclusion: Introduction of the Y329S mutation associated with APBD into the mouse GBE1 gene has generated a mouse model for polyglucosan body disease. This animal model is suitable for investigating the pathogenesis and possible novel treatments of polyglucosan accumulation.

2077/T

Getting to the heart of the matter: Cardiac involvement in Glycogen Storage Disease type III. *S.L. Austin[1], A.D. Proia[2], J. Butany[3], S.B. Wechsler[1,4], P.S. Kishnani[1].* 1) Div of Medical Genetics, Dept of Pediatrics, Duke Univ Med Ctr, Durham, NC; 2) Dept of Pathology, Duke Univ Med Ctr, Durham, NC; 3) Dept of Pathology, Toronto General Hospital, Toronto, Canada; 4) Div of Cardiology, Dept of Pediatrics, Duke Univ Med Ctr, Durham, NC.

Cardiac involvement is a feature common to several glycogen storage diseases (GSD) such as GSD II, III, and IX. A significant amount is known about extent of cardiac involvement in Pompe disease (GSD II) where glycogen accumulation affects both the structure and the function of the heart. We are beginning to better recognize the level of cardiac involvement in the other GSDs, particularly GSD III. GSD III is a rare disease of variable clinical severity affecting primarily the liver, heart, and skeletal muscle. It is caused by deficient activity of glycogen debranching enzyme which is a key enzyme in glycogen degradation. Unlike other GSDs, glycogen accumulation in GSD III is structurally abnormal, which is thought to cause liver fibrosis/cirrhosis. Cardiac involvement in GSD III is typically thought of as left ventricular hypertrophy (LVH) with very slow progression. Life threatening cardiac arrhythmias have been occasionally reported and there are a few cases of cardiac transplant. Given the improved long term survival of this population, cardiac symptoms continue to be revealed. We recently reported on cardiac wall thickness and left ventricular mass as measured by echo over time in individuals with GSD III. To further our understanding of the impact of glycogen accumulation on heart structure and function in this group, we examined cardiac tissue of three individuals with GSD IIIa: two after cardiac transplant (27 yrs and 40 yrs) and one deceased (unknown cause; 36 yrs). In the individuals with cardiac transplant, cardiac function was greatly impaired prior to transplant. Histology revealed fibrosis. There was marked LVH as well as right ventricular hypertrophy. In contrast, the individual who died of an unknown cause only had minimal LVH. However, heart tissue may provide a clue for the possible cause of death. The cardiac conduction system was abnormal with glycogen accumulation within the specialized muscle cells of the SA and AV nodes, the penetrating bundle of His, and the right bundle branch. The degree of glycogen accumulation within the conduction system was less than in the cardiac myocytes. The glycogen accumulation in the conduction system could have served as a substrate for a lethal arrhythmia. Additional cardiac pathology findings will be presented. The lessons learned from carefully studying these individuals will lead to better clinical management for our patient population with GSD III.

2078/T

Brain function in individuals with PKU treated with Kuvan: Evidence from functional magnetic resonance imaging. *S.E. Christ[1], D. Peck[2], A.J. Moffitt[1], R. Hillman[2].* 1) Psychological Sciences, University of Missouri, Columbia, MO; 2) Child Health, University of Missouri School of Medicine, Columbia, MO.

Background: Phenylketonuria (PKU) is a genetic disorder characterized inefficient metabolism of phenylalanine. Early and continuous dietary control prevents the severe neurologic and cognitive consequences once associated with PKU. Kuvan (sapropterin dihydrodichloride, BH4) represents a new supplemental pharmacologic treatment for PKU. In the present study, the researchers utilized functional MRI to examine neurocognitive functioning in individuals with and without PKU. The potential impact of Kuvan treatment on neural activity in PKU was also explored.

Methods: Brain imaging data was collected from 7 individuals with early-treated PKU (mean age = 21.9 years) immediately before treatment with Kuvan and then again after 4 weeks of Kuvan treatment. For comparison purposes, data was also collected from 5 non-PKU individuals (mean age = 20.0 years). At each timepoint, neural activity was recorded during performance of a working memory task.

Results: Analysis of the pre-treatment data revealed PKU-related irregularities in neural activation in prefrontal cortex (PFC) and other brain regions, $F(1, 10) > 5.53$, $p < .05$ FDR-corrected. At the 4-week evaluation, two participants had responded to Kuvan with a >20% reduction in phenylalanine levels. Both also showed improved activation for a region in orbitomedial PFC. Findings for other brain regions were mixed.

Conclusion: The present results provide evidence of brain dysfunction in individuals with early-treated PKU. Whereas the initial findings on Kuvan treatment are promising, additional data is needed to fully evaluate its benefits for brain function in PKU.

2079/T

The Different Protein Functions of Glycerol Kinase are Important in Metabolism and Adipocyte Formation. *K.M. Dipple[1,2], L.S. Parr[1], G. Sriram[3].* 1) Dept Human Genetics, UCLA, Los Angles, CA; 2) Dept of Pediatrics, UCLA, Los Angeles, CA; 3) Dept of Chemical and Biomolecular Engineering, University of Maryland, College Park, MD.

Glycerol kinase (GK) catalyzes the phosphorylation of glycerol and is at the interface of carbohydrate and lipid metabolism. GK is the causative gene for GK deficiency (GKD), an X-linked inborn error of metabolism for which there is no genotype-phenotype correlation. GK also has alternative protein functions including the ATP-stimulated translocation promoter (ASTP). GK also plays a role in adipogenesis and the N288D mutation in GK has been associated with obesity and diabetes. We hypothesize that investigating the roles of GK in other metabolic pathways and understanding its alternate functions will explain the complexity of GKD as well as its role in obesity. In order to examine this, we performed metabolic flux analysis in lipid and central carbon metabolism in GK-overexpressing H4IIE cells. In addition, we examined the GK ASTP activity and the ability of the cells to differentiate into adipocytes in Gyk (mouse GK ortholog) knockout (KO) mouse embryonic fibroblasts (MEFs) infected with mouse Gyk, E. coli GK, or the N288D Gyk mutant. Metabolic flux analysis showed that GK-overexpressing H4IIE cells had significantly altered fluxes through central carbon and lipid metabolism. This included flux thru the pentose phosphate pathway and increased production of lipids including stearic acid. Oil Red O staining confirmed the higher lipid reserves. There was an equal contribution of glycerol to carbohydrate metabolism in all cell lines, suggesting that GK's alternate functions are important for these observations. In MEFs, the E. coli GK lacks ASTP activity but retains the enzymatic activity; and do not differentiate into adipocytes (similar to Gyk KO MEFs,). Additionally, the N288D mutation, loses phosphorylation activity, retains ASTP activity, and cells differentiate similar to wild type preadipocytes. Therefore the GK phosphorylation activity does not play a role in adipogenesis while the ASTP activity does. In conclusion, GK overexpression globally affects both carbohydrate and lipid metabolism in rat liver cells and GK is required for normal adipocyte differentiation. In addition, the ASTP function appears to play a greater role in adipocyte differentiation and lipogenesis than the phosphorylation activity. This work shows the importance of understanding all of the cellular protein activities of GK, in order to understand the variability in the GKD pathogenesis.

2080/T

Linking cholesterol biosynthesis and human behavior. *C. du Souich[1, 2, 3], M.J.M. Nowaczyk[4], A. König[5], F.L. Raymond[6], K.W. McLarren[2, 3], R. Larstone[7], J. Livesley[7], R. Friedlander[7], M.A. Marra[8], C.F. Boerkoel[1, 2, 3].* 1) Medical Genetics, Children's and Women's Health Centre of BC, University of British Columbia, Vancouver, BC, Canada; 2) The Rare Disease Foundation, Vancouver, BC, Canada; 3) Child and Family Research Institute, Vancouver, BC, Canada; 4) Pathology & Molecular Medicine and Pediatrics McMaster University, Hamilton, ON, Canada; 5) Universitätsklinikum Gieβen und Marburg GmbH, Standort Marburg, Marburg, Germany; 6) Cambridge Institute for Medical Research University of Cambridge, UK; 7) Department of Psychiatry, University of British Columbia, Vancouver, BC, Canada; 8) BC Cancer Agency Genome Sciences Centre, Vancouver, BC, Canada.

INTRODUCTION: Several epidemiological studies have established an association between cholesterol levels and human behavior. The molecular bases of human behavior however, have largely eluded identification in part due to the type of diagnostic criteria used. Disorders of behavior are amalgams of symptoms and signs that are the product of several underlying causal mechanisms. Therefore, dissection of human behavior into unidimensional, homogenous traits should facilitate identification of causation.

METHODOLOGY: Testing this, we identified an X-linked disorder of cholesterol biosynthesis with intellectual and behavioral abnormalities that establishes this link. CK syndrome presents in affected males with intellectual disability, microcepahly, seizures, cortical malformations and dysmorphism. Affected females have normal intellect and physical appearance but segregate high levels of callousness, an important trait of psychopathy. To establish this link further, we extend these results to other disorders of cholesterol biosynthesis including Smith-Lemli-Opitz syndrome and Congenital hemidysplasia with ichthyosiform erythroderma and limb defects (CHILD) syndrome.

RESULTS: Partial loss-of-function mutations of NSDHL (NAD(P)H steroid dehydrogenase-like) cause abnormal personality traits. Findings in other cholesterol metabolism disorders support the link between cholesterol and human behavior and shed light on a possible biological mechanism causing psychiatric disease.

CONCLUSION: We establish a genetic link between human behavior and cholesterol biosynthesis, a long recognized epidemiological association. We also demonstrate that dissection of human behavior into homogenous traits allows ready identification of genetic factors modulating behavior and offers insight into a possible mechanism of disease.

2081/T

HUMAN ARGININOSUCCINIC ACIDURIA IS A HUMAN MODEL OF NITRIC OXIDE INSUFFICIENCY. *A. Erez[1], S. Nagamani[1], M. Premkumar[1], Y. Chen[1,7], O. Shchelochkov[1], P. Campeau[1], A. Mian[1], T. Bertin[1,7], J. Black[2], H. Garg[6], H. Zeng[3], Y. Tang[6], M. Summar[3], W. O'Brien[1], W. Mitch[4], J. Aschner[3], J. Marini[5], N. Bryan[6], B. Lee[1,7].* 1) Baylor College of Medicine, Houston, TX; 2) Department of Pathology, Texas Children's Hospital, Houston, TX; 3) Department of Pediatrics and Center for Human Genetics Research, Vanderbilt, University Medical Center, Nashville, TN; 4) Department of Medicine and Division of Nephrology, Baylor College of Medicine, Houston, TX; 5) Department of Pediatrics/ Nutrition, USDA/ARS Children's Nutrition Research Center, Baylor College of Medicine; 6) Brown Foundation Institute of Molecular Medicine, University of Texas - Houston Health Science Center; 7) Howard Hughes Medical Institute, Houston, TX.

Nitric Oxide (NO) is an essential signaling molecule for diverse physiological and disease processes. In humans, the urea cycle disorder argininosuccinic aciduria (ASA) caused by deficiency of argininosuccinic acid lyase (ASL) shows systemic features that in part reflect global dysregulation of NO homeostasis due to deficiency of intracellular arginine synthesis and inability to utilize extracellular arginine. We generated and characterized a novel Asl hypomorphic mouse model in which endogenous, intracellular Arginine production is impaired resulting in decreased NO production and a multi system dysfunction. We used this mouse model to understand the mechanism underlying human ASL deficiency phenotype and to assess new treatment modalities. We found that ASL is essential for a novel complex assembly necessary for NO synthesis. We next verified our mouse studies in a case study of patients with ASL deficiency. ASA patients have decreased biochemical markers and dynamic measures of NO production despite elevated arginine level resulting from their treatment. In addition, ASA patient's fibroblasts cannot utilize extracellular arginine for NO synthesis. Hence, we hypothesized that supplementing ASA patients with NO donors as a direct replacement of the NO deficiency, will have a beneficial effect. We further studied a 15 year old boy diagnosed with ASA complicated by hypertension for more than ten years. He was unresponsive to multiple anti-hypertensive regiments including diuretics, ACE inhibitors, calcium channel blockers, and beta-blockers. Recently, he proceeded into a hypertension crisis. As a proof of principal to our recently described novel role of ASL in nitric oxide synthesis, we treated the patient with Isosorbide dinitrate as an NOS independent NO donor. The patient showed a dramatic clinical response with normalization of the blood pressure over a three day treatment with weaning of all other anti-hypertensive medications. This case highlights the central role of ASL in nitric oxide regulation, demonstrates that ASL deficiency is a human genetic model of NO insufficiency, and provides a potential novel therapy for these patients that need to be further studied in controlled trials.

2082/T

ETFDH mutations are the major cause of myopathy with multiple acyl-CoA dehydrogenase deficiency (MADD). *C. Gellera[1], B. Castellotti[1], V. Pensato[1], C. Antozzi[2], B. Garavaglia[1], G. Uziel[3], S. Di Donato[1], M. Rimoldi[1], F. Taroni[1].* 1) Unit of Genetics of Neurodegenerative and Metabolic Diseases, IRCCS Carlo Besta Neurological Institute, Milano, Italy; 2) Unit of Neurology and Neuromuscular Disorders, IRCCS Carlo Besta Neurological Institute, Milano, Italy; 3) Department of Child Neurology, IRCCS Carlo Besta Neurological Institute, Milano, Italy.

Glutaric Aciduria type II (GAII) and Multiple Acyl-CoA Dehydrogenase Deficiency (MADD) include a wide spectrum of autosomal recessive diseases caused by defects in mitochondrial electron transfer system and fatty acid catabolism. The clinical presentation range from a severe encephalomyopathy of childhood to a mild myopathic form of adult frequently responsive to riboflavin treatment (RR-MADD). These disorders can be due to defects of Electron Transfer Flavoprotein (ETF) or ETF dehydrogenase (ETFDH) but for some cases an as yet unidentified riboflavin metabolism alteration is also supposed. We have studied 23 index cases, in which the biochemical profile, a characteristic pattern of medium- and long-chain esterified carnitines in plasma, and the clinical presentation were suggestive of MADD. The onset was variable from childhood to adult age including severe and very mild presentations. Five patients had a positive familial history for the presence of an affected sibiling. Acyl-CoA dehydrogenase activities for short-, medium- and long-chain fatty acids were markedly reduced in the analyzed cases (10/23) showing a residual activity between 10% and 50%. A pathological pattern of urinary dicarboxylic acids was detected during metabolic crisis. About 50% of patients showed a marked improvement of clinical conditions and the normalization of biochemical parameters after treatment with riboflavin. ETFDH gene analysis demonstrated the presence of 15 different mutations (13 missense, 1 nonsense, and 1 splicing mutation) on both alleles either in homozygous or in compound heterozygous form, in 16/23 patients (70%). The large majority of mutations (13/15) were novel. Four out of 23 patients resulted negative for the analysis of ETFDH, ETFA and ETFB genes indicating genetic heterogeneity, albeit limited. In 3/23 index cases, we identified mutations on one allele only and we excluded the presence of intragenic rearrangements. Interestingly, in two of these families, which carried the same mutation, segregation of the mutation with the disease in two generations indicated an autosomal dominant transmission. These results would suggest that a dominant-negative mechanism might underlie the disease in a subgroup of patients. Our study indicates that (1) mutations in ETFDH gene are the major cause of MADD, (2) MADD myopathy may present as an autosomal dominant trait, and (3) other genes are likely to be implicated in the pathogenesis of this rare metabolic disease.

2083/T

2,4-dienoyl CoA reductase deficiency: a novel neurometabolic disease. *M. Gucsavas-Calikoglu[1], D. Frazier[1], S. Young[2], D. Millington[2].* 1) Pediatrics, University of North Carolina School of Medicine, Chapel Hill, NC; 2) Biochemical Genetics Laboratory, Duke University Medical Center, Durham, NC.

Mitochondrial 2,4-dienoylCoA reductase is a key enzyme for the beta-oxidation of unsaturated fatty acids. A deficiency of this enzyme was reported in 1990 by Roe et al. in a black female infant with hypotonia, hyperlysinemia, hypocarnitinemia, and decadienoylcarnitine in urine and blood. She died of respiratory acidosis at age four months. We now report a second case in a Hispanic male. Our patient presented at age 8 weeks with failure to thrive, microcephaly and central hypotonia. Plasma and urinary 2,4-decadienoylcarnitine(C10:2); plasma, CSF and urine lysine(LYS); blood and CSF lactates were elevated. Urine organic acids showed increased methylmalonic, glutaric and urine amino acids showed increased LYS. Incubation of skin fibroblasts with C18:2 showed an accumulation of C10:2 six fold greater than control cells. Very long chain fatty acids and CSF neurotransmitters were normal. Treatment consisted of dietary LYS restriction, caloric support, provision of medium chain fatty acids and carnitine. At age 6 mos, he had social smile but did not sit. At age 18 months, he had nystagmus and abducens palsies, hypertonia and clonus. He developed choreoathetosis and intermittent lactic acidosis with minor illnesses. At 30 mos, he had pancreatitis. He developed gram negative sepsis after gastric tube placement and experienced further neurological decline. At 36 mos, he had renal tubular acidosis. At 4 =BD years, he had severe encephalopathy, dystonia, spastic quadriplegia, cortical blindness, epilepsy and episodic central apnea. He died at age 5 with aspiration pneumonia. Multiple MRIs were consistent with progressive leukodystrophy, generalized cerebral atrophy, ventriculomegaly, and bilateral basal ganglia T2 abnormalities. Autopsy showed ventriculomegaly and severe white matter gliosis particularly in frontal lobes and basal ganglia. The biochemical etiology of this disorder is currently unknown and the relationship between elevated C10:2 and LYS is unclear. Dietary LYS restriction was not successful in preventing neurological decline. Progressive encephalopathy, dystonia, RTA, intermittent lactic acidosis, the presence of leukodystrophy and basal ganglia lesions on MRI and gliosis on autopsy suggest that 2, 4-dienoyl CoA reductase deficiency results in a neurometabolic disease. Possible mechanisms may involve mitochondrial dysfunction with oxidative damage and disturbed lipid peroxidation. The disorder may be incompatible with survival.

2084/T

Molecular confirmation of VLCAD deficiencies in newborns. *M. Landsverk, F. Scaglia, L-J. Wong.* Dept of Mol and Hum Genetics, Baylor College of Medicine, Houston, TX.

Acyl-CoA dehydrogenases catalyze the initial step in the beta-oxidation of fatty acids in the mitochondrial matrix. Deficiencies in acyl-CoA dehydrogenases are autosomal recessive disorders that result in a decreased ability to oxidize fatty acids, thereby leading to metabolic dysfunction. Very-long chain acyl-CoA dehydrogenase (VLCAD) targets 14 to 20 carbon long-chain fatty acids. VLCAD deficiency (VLCADD) can generally be grouped into three phenotypes: a severe, early-onset, form with a high incidence of cardiac and multiorgan failure; a milder childhood form usually presenting with hypoketotic hypoglycemia; and an adult myopathic form with intermittent rhabdomyolysis, muscle cramps and exercise intolerance. Early detection through newborn screening (NBS) by measuring acylcarnitines using tandem mass spectrometry has increased the number of patients identified with VLCADD, and has allowed for the identification of milder clinical phenotypes. An increase in acylcarnitine C14:1 is suggestive for VLCADD. However, acylcarnitine profiles alone cannot confirm a diagnosis since initial elevations of C14:1 on NBS may also reflect neonatal stress. Conversely, normal biochemical testing on follow-up of abnormal NBS for VLCADD does not rule out the disorder since C14:1 levels tend to normalize over time. Molecular identification of deleterious mutations in the ACADVL gene is necessary to confirm the diagnosis. From January 2007 to October 2009 we analyzed 175 individuals, ages 6 months and younger, presenting with abnormal NBS or clinical indications suggestive of VLCADD for mutations in ACADVL. We identified previously reported mutations as single heterozygotes in 36 individuals, compound heterozygotes in nine, and homozygotes in 5. An additional 13 individuals harbored known mutations as compound heterozygotes with unclassified variants. We also identified 6 novel frameshift mutations leading to premature stop codons, 1 novel indel, 1 novel in-frame deletion, and 4 novel splice-site mutations, all heterozygous. Unclassified variants were found as single and compound heterozygous in 21 and 8 individuals respectively. However, we found no known mutations or unclassified variants in the coding exons of ACADVL in 66 (38%) individuals tested for VLCADD. These results underscore the necessity of mutation analysis of the ACADVL gene when VLCADD is suspected in children under the age of 6 months based on suggestive clinical indication or positive NBS results.

2085/T

Mechanistic study for mucopolysaccharidosis VII using induced pluripotent stem (iPS) cells. *X. Meng[1, 2, 4], J. Shen[1, 2, 4], Sh. Kawagoe[2], T. Ohashi[1, 2], R.O. Brady[3], Y. Eto[2].* 1) Department of Gene Therapy, The Jikei University School of Medicine, 3-25-8 Nishi-Shinbashi, Minato-Ku, Tokyo, Japan 105-8461; 2) Department of Genetic Diseases & Genome Science, The Jikei University School of Medicine, 3-25-8 Nishi-Shinbashi, Minato-Ku, Tokyo, Japan 105-8461; 3) National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892; 4) Current address: Institute of Metabolic Disease, Baylor Research Institute, 3812 Elm Street, Dallas, TX 75226.

Mucopolysaccharidosis VII (MPS VII) is a genetic metabolic disorder, caused by a genetic defect for a lysosomal enzyme, β-glucuronidase (GUSB). The substrates of the enzyme, glycosaminoglycans (GAGs), accumulate in multiple tissues. Affected patients show dysostosis multiplex such as skeletal dysplasia, hepatosplenomegaly and mental retardation. Hydrops fetalis occurs frequently suggesting presence of abnormalities in prenatal development in this disease. The pathogenesis of this disease is largely unknown. MPS VII mouse is a naturally-occurring murine model of MPS VII, having null mutation in GUSB gene causing biochemical and pathological abnormalities closely resemble that of MPS VII patients. Percentage of MPS VII mouse newborns born from heterozygote mating is significantly lower than expected 25%, suggesting intra-uterine loss of some affected embryos. To study the pathogenesis of MPS VII, we generated induced pluripotent stem (iPS) cells from MPS VII mouse (MPS VII-iPS) by retrovirally transduction of Oct3/4, Sox2 and Klf4 into tail tip fibroblasts. Generated iPS cells express ES cell marker genes and were pluripotent as demonstrated by in vitro differentiation study and teramota formation in nude mice. Notably, MPS VII-iPS cells showed markedly impaired ability to from embryoid bodies (EBs) in vitro compared with normal mouse derived iPS cells. MPS VII-EBs exhibited elevated level of hyaluronan and its receptor CD44, and markedly reduced expression levels of E-cadherin and cell-proliferating marker. Partial correction of enzyme deficiency in MPS VII-iPS cells led to improved EB formation and reversal of aberrant protein expression. These data indicate a potential mechanism for the partial lethality of MPS VII mice in utero and suggest a possibility of abnormality in embryonic development of human MPS VII. Our observations shed new light on the effect of defective lysosomal enzyme on embryogenesis and demonstrated the unique promise of iPS cells for studying the pathogenesis of genetic disorders.

2086/T

Quality of Life in Adults with Glycogen Storage Diseases I and III. *C.M. Milleson, S.L. Austin, P.S. Kishnani.* Division of Medical Genetics, Department of Pediatrics, Duke University Medical Center, Durham, NC.

Glycogen storage diseases (GSD) are an inherited group of conditions resulting in glycogen accumulation in various organs. The liver, kidney, and intestines are most affected in GSD I, while the liver, heart and skeletal muscles are affected in GSD III. With increasing survival rates, individuals with both disorders have chronic, long term complications and a significant disease burden. Despite the severity of these diseases, there has been no previous research published on quality of life (QOL) in adults with GSD I or III. Our objective was to examine QOL in adults with GSD I and III, to determine if there are notable differences between the groups. The Short-Form Health Survey (SF-36) was used to measure physical, mental, and social functioning. The sample consisted of 21 individuals with GSD I (19-48 yrs, mean=31 yrs) and 11 individuals with GSD III (23-56 yrs, mean=40 yrs). Descriptive statistics were calculated for the demographics and the eight scale scores. A Mann-Whitney test was used to compare the medians of the GSD I and III scale scores. Scores were also compared to a normed US population. Individuals with GSD III reported poorer quality of life than those with GSD I or the general US population. In physical domains, individuals with GSD III were greatly impaired, while those with GSD I were only minimally impaired. In mental and emotional domains, individuals with GSD I and III fared well—better than the general US population. Additionally, social functioning was significantly affected for individuals with GSD I and III when compared to the general US population. QOL in adults with GSD III was more impaired in physical domains than GSD I, possibly due to the additional symptom of muscle involvement in GSD III. The mental or emotional QOL domains are not impaired by the diseases, but rather, may suggest protective coping mechanisms. Despite positive emotional health, social functioning is an area of concern for individuals with GSD I and those with GSD III. This may be due to the frequency of their feeding schedule or due to other disease-related factors. Areas of future research interest include exploring whether there are protective benefits from having GSD I or III that facilitate positive emotional outcomes, examining if the physical and emotional outcomes of individuals with GSD I and III change over time, and determining if there are additional or improved ways in which individuals GSD I and III can be supported by medical providers.

2087/T

Detection of ACADVL and SLC22A5 Deletions and Duplications by MLPA. *M. Procter[1], K. Tuin[3], R. Mao[1,2].* 1) ARUP Laboratories, Salt Lake City, UT; 2) University of Utah Department of Pathology, Salt Lake City, UT; 3) MRC-Holland, Amsterdam, The Netherlands.

Background: Very long-chain acyl-coenzyme A dehydrogenase deficiency (VLCAD) and Primary Carnitine Deficiency (PCD) are fatty acid oxidation disorders that prevent the body from converting certain fats to energy, proving particularly problematic in times of fasting. VLCAD is caused by pathogenic variations in the ACADVL gene. PCD is caused by defects in the SLC22A5 gene, which encodes the protein OCTN2. Both disorders are autosomal recessive and 80-85% of cases of each disorder are caused by mutations in the respective gene. However, the remaining 15-20% of cases show no mutations and are presumed to be caused by complete or partial deletions of the ACADVL gene (for VLCAD) or the SLC22A5 gene (for PCD). We evaluated a Multiplex Ligation-dependent Probe Amplification (MLPA) assay for detection of deletions and duplications in ACADVL and SLC22A5 for use in our molecular laboratory. Methods: An MLPA assay for ACADVL and SLC22A5 was developed by MRC-Holland at the request of ARUP. MLPA is a semi-quantitative multiplex PCR approach to determine the relative copy numbers of each of 20 ACADVL exons and 10 SLC22A5 exons. Genomic DNA was hybridized overnight with probe mix, probes were ligated, and the fragments were amplified using universal dye-tagged primers. PCR products were subjected to fragment analysis by capillary electrophoresis and results analyzed by GeneMarker v1.85 software. To determine accuracy, DNA from 21 whole blood samples was collected from volunteers who denied any personal or family history of VLCAD or other metabolic disorders. DNA was extracted from the whole blood using MagNAPure extraction and tested using this MLPA assay. Additionally, because samples with deletions were not available for validation, we used amplicon mapping to ascertain the specificity of the MLPA probes. This was done by combining amplicons for exons together and testing them in MLPA reactions in place of genomic DNA. Results and Conclusions: All 21 accuracy samples gave no deletion MLPA results, with excellent between-run and within-run specificity for each probe data point. For individual probe specificity, one ACADVL MLPA probe and 3 SLC22A5 MLPA probes lay outside the amplicon boundaries and could not be immediately tested for specificity. The remaining 26 probes were detected in the appropriate exon amplicons. Our evaluation showed this assay to be reliable and robust in the detection of deletions and duplications in each exon of the ACADVL and SLC22A5 genes.

2088/T

Mutational screening of ALG(Glycogen Debranching Enzyme) gene in glycogen storage disease type III. *CF. Sargin Ozkaya[1], AE. Manguoglu[1], V. Uygun[2], R. Ertan[2], G. Luleci[1], S. Berker Karauzum[1].* 1) Department of Medical Biology and Genetics, Akdeniz University Medical Faculty; 2) Department of Pediatric Gastroenterology, Antalya, Turkey.

Ten patients who were followed in Akdeniz University Medicine Faculty Department of Pediatric Gastroenterology Hepatology and Nutrition, and diagnosed as GSD type 3 with liver biopsy and enzymatic analyses in Erasmus University Clinical Laboratory of Genetics in Rotterdam (Holland) were participated to the study. The age, consanginuity, residency, somatic development data, clinical, biochemical and echocardiographic data were compared with enzimatic activity of the patients. Mutation analyses resulted with a missense mutation in exon29 c.13428 A>G (Gln-Arg) in one patient, a missense mutation in exon 6 c.1628 G>T (Gly-Val) in three patients, a missense mutation in exon 6 c. 1646 A>T (Tyr-Phe) in the two of the same three patients, a nonsense mutation in exon31 c.15047 C>T (Gln-Stop) in one patient, a polymorphism in two patients in exon31 c.14956 G>A (Val-Val) that does not cause any change in protein production. Two splice site mutations were found in the first base of exon 12 c. 4362G>A in one patient and in intron 16 c.6970 A>G in an other patient. In this study, we followed ten patients with GSD type 3 which can be referred for Turkish people. We observed no serious clinical problem in these cases. Mutation analyses resulted with three missense mutation, one nonsense mutation, two splice site mutations and two silent mutations which were accepted as polymorphism. A further study is necessary to state these findings as mutation.

2089/T

The *TAT* gene in tyrosinemia type II: A mutational update. *G. Scherer[1], S. Adler[1], E. Bausch[1], L. Peña-Quintana[2], K. Fumic[3], N.G. Dépraz[4], Y. Giguère[5], P. Huppke[6], G.A. Mitchell[7], E. Mönch[8], E.R. Trimble[9], D. Trump[10], C. Vianey-Saban[11].* 1) Inst Human Genetics, Freiburg, Germany; 2) Unit of Pediatric Gastroenterology, Hepatology and Nutrition, Complejo Hospitalario Universitario Insular Materno-Infantil, University of Las Palmas de Gran Canaria, Canary Islands, Spain; 3) Clinical Institute of Laboratory Diagnosis, Zagreb University School of Medicine, Zagreb, Kroatia; 4) Pédiatrie Moléculaire, Centre Hosp. Univ. Vaudois, Lausanne, Switzerland; 5) Laboratoire de Dépistage Néonatal, Service de Biochimie Médicale, CHUQ, Québec, Canada; 6) Kinderklinik und Poliklinik, Georg-August-Universität, Göttingen, Germany; 7) Université de Montréal, Service de Génétique Médicale, Montréal, Canada; 8) Charité University Medical Center, Campus Virchow-Klinikum, Berlin, Germany; 9) Department of Clinical Biochemistry, Royal Victoria Hospital, Belfast, UK; 10) Department of Medical Genetics, Addenbrooke's Hospital, Cambridge, UK; 11) Centre de Biologie Est, CHU Lyon, Bron, France.

Tyrosinemia type II (Richner-Hanhart syndrome, RHS; MIM 276600) is a rare autosomal-recessive disorder characterized by keratitis with photophobia, palmoplantar hyperkeratosis, variable mental impairment, and elevated blood tyrosine levels. The disorder results from deficiency in the liver-specific enzyme tyrosine aminotransferase (TAT). The 11 kb *TAT* gene at 16q22 encompasses 12 exons encoding a 454 amino acid protein. Starting with our initial publication (Natt et al., PNAS 89:9297, 1992), *TAT* mutations have up to now been identified in 18 RHS families totalling 18 different mutations: one deletion, three frameshift, three splice, three nonsense, and eight missense mutations. We now identified 15 missense mutations and two identical R297X nonsense mutations in 14 RHS families from 10 different countries. Of the 15 missense mutations, one (R119W) has been described previously, three recurred twice, and one was observed in three families from Las Palmas, Canary Islands, not known but likely to be related. The nine novel missense mutations are: L76Q, A147V, T209I, A237P, K280R, M375R, D389N, P406L, R417Q. The 17 different TAT missense mutations known thus far all affect conserved amino acid residues and highlight their importance for transaminase function. Notably, the K280R substitution affects the lysine residue to which the cofactor pyridoxal phosphate binds during catalysis. Comparison of the clinical, biochemical and molecular findings for all RHS patients with identified *TAT* gene mutations does not reveal any clearcut genotype-phenotype correlation. Our study raises the number of different mutations in the *TAT* gene to 28, emphasizing the great mutational spectrum in this rare metabolic disorder.

2090/T

Diagnostic tests for hereditary renal hypouricemia. *I. Sebesta[1,2], B. Stiburkova[2].* 1) Institute of Clinical Biochemistry; 2) Institute of Inherited Metabolic Disorders, Charles University, First Faculty of Medicine, Prague, Czech Republic.

Introduction: Primary hereditary renal hypouricemia is a genetic disorder characterized by defective renal urate reabsorption with complications such as nephrolithiasis and exercise-induced acute renal failure. The known causes are: defects in the SLC22A12 gene, which encodes the human urate transporter 1 (hURAT1), and also impairment of the recently identified voltage urate transporter (URATv1), encoded by SLC2A9 (also called GLUT9) gene, which plays probably a major role in urate homeostasis as was revealed by recent study of knockout mice. Diagnosis is based on two markers: hypouricemia (< 119μmol/l) and increased fractional excretion of uric acid (> 10%). Therapy requires alkalization of urine, drinking plenty of water, and abstention of strenuous exercise. To date, the cases with mutations in hURAT1 gene have been reported in East Asia only. More than one hundred Japanese patients have been described and this number is unique worldwide. Hypouricemia is sometimes overlooked, therefore we have set up the flowchart for this disorder. Methods: The patients were selected for molecular analysis from 620 Czech hypouricemic patients. These cases were found from 3 600 blood and urine samples. Serum and urinary uric acid and creatinine were determined. The sequence analysis by automated DNA sequencer (Applied Biosystems 3100) of the coding region of SLC22A12 and SLC2A9 genes were performed after informed consent. Results: The proposed scheme was as follows. Secondary causes of hyperuricosuric hypouricemia were excluded as the first step. The estimation of :1) serum uric acid , 2) excretion fraction of uric acid, 3) and analysis of hURAT1 and URATv1 genes follow then. Using this flow chart we were able to find 3 transition and 4 deletions in SLC22A12 gene, yet unpublished, in 6 Czech patients. In addition, one family with one nucleotide insertion in URATv1 gene was found. Three patients were suffering from acute renal failure and urate nephrolithiasis. Conclusions: Our finding of the defect in URATv1 gene gives further evidence that SLC2A9 is a causative gene of primary renal hypouricemia and supports the important role of SLC2A9 in regulation of serum urate levels in humans. Hereditary renal hypouricemia is still unrecognized condition and probably not wide spread in Japan and Korea only. (Supported by grant VZMSM0021620806, Czech Republic).

2091/T

Biotinidase Analysis: The Quest Diagnostics Experience. *R. Sharma[1], D. Salazar[1], R.M. Lobo[1], W. Sun[2], C.M. Strom[1,2], J.A. Neidich[1].* 1) Biochemical Genetics, Quest Diagnostics Nichols Institute, San Juan Capistrano, CA; 2) Molecular Genetics, Quest Diagnostics Nichols Institute, San Juan Capistrano, CA.

Biotinidase deficiency is an autosomal recessive disorder that manifests with central nervous system symptoms including seizures and mental retardation and skin symptoms such as dermatitis. Biotinidase is the enzyme involved in releasing biotin from biocytin and biotinyl peptides, the products of carboxylase degradation. Biotin is a cofactor for 4 biologically important carboxylases: propionyl CoA carboxylase, β-methylcrotonyl CoA carboxylase, pyruvate carboxylase, and acetyl CoA carboxylase. These enzymes are involved in gluconeogenesis, fatty acid synthesis, and amino acid catabolism. Since August 2008, we have analyzed more than 1600 patient samples for biotinidase. From studies of enzyme activity alone, 22 patients were identified as deficient, 21 as partially deficient, and 26 as possible carriers. Mutation analysis was performed on 4 of 22 patients identified as deficient: 2 patients were homozygous for the H447Y mutation; 1 patient had Q456H in conjunction with both A171T and D444H; and the fourth patient had A171T in conjunction with both D444H and the novel mutation R538S. Mutation analysis was also performed on 9 of 23 patients identified as partially deficient; each patient was found to have a mutation previously associated with partial enzyme deficiency. All 9 patients had the D444H partial-deficient mutation, along with either the Q456H mutation (3 patients) or D444H, D444H-A171T, 97_104delinsTCC, 459G>A, R5538C, or R157H (the remaining 6 patients, respectively). Out of 27 patients identified as possible carriers, mutation analysis was performed on 6: 5 patients had either the K176N, R538S, R157H, or D444H mutation; 1 patient had both the D444H and R538C mutations. In our sample group, D444H was the most common mutation associated with apparent partial enzyme deficiency. We suggest that mutation analysis in conjunction with biotinidase enzyme analysis may be helpful in the diagnosis and subsequent treatment of patients with biotinidase deficiency.

2092/T

A new case of *cblF* disease detected by newborn screen and a follow up on a 14-year-old patient. *M. Shinawi[1], D.S. Rosenblatt[2], D. Watkin[2], L. Spreitsma[1], D. Dietzen[1,3], O. Oladipo[3].* 1) Pediatric, Genet. & Genomic Med., Washington Univ, St. Louis, MO; 2) Department of Human Genetics, McGill University, Montreal, Qc, , Canada; 3) Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO.

BACKGROUND: The *cblF* defect is due to disturbed lysosomal release of cobalamin (Cbl) into the cytoplasm. The disease is very rare and the *cblF* patients have variability in age of onset, presenting symptoms, response to treatment, and long-term complications. The *LMBRD1* was identified as the causative gene for *cblF* and it has been shown that the majority of mutated alleles carry the deletion c.1056delG. **METHODS:** Clinical and biochemical characterization of a new patient with *cblF* and a follow up of a 14-year-old patient with *cblF* who initially presented with failure to thrive (FTT) and feeding difficulties. We compare the clinical findings in our patients with previously reported cases. **RESULTS:** The new patient had C3 elevation on newborn screen. Confirmatory tests revealed hyperhomocysteinemia, hypomethioninemia, methylmalonic aciduria, decreased serum Cbl, and complementation analysis showed *cblF* defect. The patient was small for gestational age (SGA), exhibited dysmorphic features and mild developmental delays, and had trigonocephaly and ventricular septal defect. There was complete biochemical normalization within 2 weeks of parenteral Cbl treatment and her feeding difficulties and FTT improved. The other patient presented at 4 weeks of life and was homozygous for the 1056delG mutation. She has not been regularly followed by a metabolic service and was treated only with monthly cyanocobalamin shots. The patient has never experienced metabolic decompensation. Her height was at 1st percentile. She was an average 8th grade student with no behavioral concerns and had regular menstrual cycles. Her hyperhomocysteinemia and methylmalonic aciduria significantly improved after switching to weekly hydroxycobalamin. **CONCLUSIONS**: Patients with the *cblF* defect exhibit variable expressivity and long-term outcomes. Of the 14 *cbl*F confirmed patients, 8 showed FTT, 5 were SGA, 5 developed feeding difficulties, 5 were reported with congenital heart defects, 6 had low serum Cbl at presentation, and 4 presented with elevated C3 on newborn screen. 3 of the 14 died during first year of life (2 due to cardiac surgery complications and one was an unexpected death) and 7 exhibited variable degrees of developmental delays. The SGA and increased incidence of birth defects are intriguing features and suggest a prenatal effect. The thorough investigation of additional *cblF* patients will shed light on the clinical course, neurological outcome, and efficacy of treatment.

2093/T

Methylmalonic acidemia and optic nerve atrophy: reversal of sub-acute loss of visual function with anti-oxidant therapy. *J.L. Sloan[1], N.S. Hauser[1], I. Manoli[1], W.M. Zein[2], K. Bowles[2], K. O'Brien[3], B.P. Brooks[2], C.P. Venditti[1].* 1) Genetics and Molecular Biology Branch, NHGRI, Bethesda, MD; 2) Ophthalmic Genetics and Visual Function Branch, NEI, Bethesda, MD; 3) Medical Genetics Branch, NHGRI, Bethesda, MD.

Isolated methylmalonic acidemia (MMA) results primarily from a defect in the mitochondrial enzyme methylmalonyl-CoA mutase (MUT). Patients present with recurrent metabolic crises and multisystemic complications including growth retardation, chronic renal failure, pancreatitis and developmental delay despite optimal management. Studies in MMA knock out mice and patient tissues have suggested that mitochondrial dysfunction plays a significant role in the pathophysiology of MMA. Optic nerve atrophy (ONA) has been described as a rare complication of the disorder, and the pathophysiology is unknown. Patients with isolated MMA evaluated through NIH study 04-HG-0127 (clinicaltrials.gov identifier: NCT00078078) "Clinical and Basic Investigations of Methylmalonic Acidemia and Related Disorders" underwent comprehensive ophthalmologic evaluation. We report four *mut^0* patients with ONA out of the 56 with isolated MMA followed in our protocol. All four patients were males, and presented with a decreased visual acuity at the ages of 7, 22, 23 and 24 years. Vision loss was bilateral but asymmetric and the progression was variable. Best-corrected vision ranged from 20/40 to 20/800 and in two patients visual evoked potentials were severely diminished and delayed. There were no apparent biochemical or environmental triggers shared by the patients and none had an acute metabolic decompensation during the onset of the symptoms. Each carried two mutations in the MUT gene. Two patients were negative for the Leber Hereditary Optic Neuropathy mitochondrial mutations. Patient 4 was a 24 year-old male, 4 years status-post cadaveric kidney transplantation, whose vision progressively worsened from 20/20 to 20/40 OS and 20/125 OD over a 4-week period. Daily oral coenzyme Q10, vitamin E, ascorbic acid, thiamine, and intravenous infusions followed by oral N-acetylcysteine were employed. The patient's visual acuity improved to 20/25 OD and 20/20 OS over one month, although the nerve fiber layer thickness remained decreased. Our experience suggests that 1) ONA is a late onset complication of isolated MMA and therefore all patients should have periodic thorough ophthalmologic evaluation and counseling about the symptoms of ONA. 2) Anti-oxidant therapy can be used to at least partially reverse the functional visual effects of optic nerve pathology in MMA patients in the acute setting.

2094/T

Evidence of a Frequent 2-Methylbutyryl-CoA Dehydrogenase (ACADSB) Mutation Among Somalian Immigrants in the USA and Questions About its Role in Autism Spectrum Disorder. *E.H. Smith[1], D. Gavrilov[1,2,3], K. Raymond[1,3], P. Rinaldo[1,3], S. Tortorelli[1,3], D. Matern[1,3], D. Oglesbee[1,2,3].* 1) Biochemical Genetics Laboratory, Mayo Clinic, Rochester, MN; 2) Molecular Genetics Laboratory, Mayo Clinic, Rochester, MN; 3) Department of Medical Genetics, Mayo Clinic, Rochester, MN.

Background. 2-methylbutyryl-CoA dehydrogenase (2MBD) deficiency is an autosomal recessive disorder of L-isoleucine metabolism that is detectable by elevated 2-methylbutrylcarnitine (C5) levels in bloodspots through newborn screening (NBS) by tandem mass spectrometry. Previous studies described a mutation, c.303+3A>G, in the 2MBD gene (ACADSB), in four children of East African descent. Of these, two unrelated children from Somalia were homozygous for this alteration and displayed autistic features. Minnesota is home to the largest Somalian population in the Western hemisphere and autism rates in this group are three times that of the general population. **Methods.** In order to assess a possible correlation between 2MBD deficiency and autism, we surveyed cases of 2MBD deficiency identified by NBS between 2004-2009 for Somalian ancestry and retrospectively analyzed a subset of negatively screened cases from 2009-2010 for the presence of c.303+3A>G. **Results.** Molecular analysis confirmed homozygosity for c.303+3A>G in four newborns and two carriers of the mutation, all of whom were of Somalian descent. Two infants (one carrier and one c.303+3A>G homozygotic individual) were retrospectively identified from negatively screened NBS cases. Significant overlap was observed for C5 levels between confirmed carriers of this mutation and homozygotic individuals. Clinical follow up is available for only one infant, who is developmentally normal at two months of age without treatment. **Conclusions.** From previous observations, and from our identification of six additional unrelated infants, the c.303+3A>G mutation is common to the Somalian population within the USA. Current cut-off values for NBS C5 levels are insufficient to detect all cases of 2MBD deficiency due to c.303+3A>G homozygosity. While the question of whether 2MBD deficiency is a contributor to autism spectrum disorder remains to be elucidated, ongoing studies on the natural history of this metabolic condition, and mutation analysis of autistic patients with Somalian ancestry, will aid in determining the proportion of c.303+3A>G in autism.

2095/T

A mouse model of creatine deficiency: Effects on growth and metabolite levels. *C. STROMBERGER[1], H.O. AKMAN[2], W.J. CRAIGEN[2].* 1) Department of Radiotherapy and Radiological Oncology, Charité University Medicine, Berlin, Germany; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston TX 77030.

Creatine deficiency is a recently recognized inborn error of metabolism that can be diagnosed either by magnetic resonance spectroscopy or by measuring analytes in blood and urine. It can be caused by defects either in creatine biosynthesis or transport. Two enzymes catalyze creatine synthesis from arginine and glycine; arginine glycine amidinotransferase (AGAT) and guanidinoacetate methyltransferase (GAMT). Each exhibits intellectual impairment, and in the latter case epilepsy, but it is surprising that a lack of creatine phosphate, an energy reservoir in high concentration in brain and muscle, is otherwise a viable deficiency state. In order to better understand the requirement for creatine and the underlying mechanisms that may account for the intellectual disability associated with its deficiency, we generated a knockout model of AGAT deficiency using a conditional knockout strategy that allows for the tissue specific deletion of the gene. Mice globally lacking the AGAT activity are live born and survive to adulthood, but exhibit growth retardation. Deficient mice are fertile, but blood amino acid levels demonstrate a severe reduction in creatine levels, and, surprisingly, arginine concentrations in blood are reduced, suggesting that the AGAT activity provides a net contribution to arginine stores during development. This mouse model will be valuable in defining the neurologic phenotypes in AGAT deficiency, and may provide novel insights into the role of creatine in other tissues such as myocardium and skeletal muscle.

2096/T

Novel HADHB mutations, including an exonic deletion, in a neonate with mitochondrial trifunctional protein deficiency. *S.F. Suchy[1], H. Shuhaiber[2], R. Busin[1], S. Aradhya[1], W.K. Chung[2].* 1) GeneDx, Gaithersburg, MD; 2) Columbia University, New York, NY.

The mitochondrial trifunctional protein is a multi-enzyme protein that catalyzes the last three steps of beta-oxidation. It is comprised of 4 alpha and 4 beta subunits encoded by the *HADHA* and *HADHB* genes, respectively. Mitochondrial trifunctional protein (MTP) deficiency leads to a wide clinical spectrum of disease ranging from severe neonatal/infantile cardiomyopathy, acute hepatic dysfunction and early death, to mild chronic progressive sensorimotor polyneuropathy with episodic rhabdomyolysis. Mutations in *HADHA* are more common than *HADHB*. An affected male was delivered to G1P1 mother at 28 weeks gestation via emergency section due to mother's acute fatty liver of pregnancy, oligohydramnios and increased decelerations in the fetus. The baby's birth weight was 867 g, birth length was 34 cm, and head circumference was 25 cm. His Apgar scores were 6 and 8 at 1 and 5 minutes, respectively. The newborn screen was abnormal with elevated C16-OH (0.99 μmol/L, normal< 0.1 μmol/L) and C18:1-OH (1.23 μmol/L, normal< 0.1 μmol/L), consistent with LCHAD/MTP deficiency. Follow-up plasma acylcarnitine profile revealed an elevated C12-C18 hydroxylated and non-hydroxylated acylcarnitine. In vitro interrogation of beta-oxidation in fibroblasts with radiolabeled palmitate demonstrated significant elevations of C14, C16, C16-OH and C18 suggesting either LCHAD or trifunctional protein deficiency. Molecular testing failed to identify a mutation in HADHA. Sequencing *HADHB* revealed that the patient was heterozygous for a novel IVS4-1 G>T mutation. Deletion/duplication testing by exon-level array CGH identified a deletion of exon 4 in *HADHB*. This is the first report of an exon deletion in *HADHB*. Spiekerkoetter et al. (2003) described the largest series of 15 patients from 13 families with *HADHB* mutations and demonstrated that the most common mutations are missense mutations. Our patient has two novel, uncommon mutations: a splice site mutation and deletion of a single exon. This case demonstrates the value of exon-level deletion/duplication analysis, and highlights the significance of maternal history of acute fatty liver disease of pregnancy in patients carrying a fetus with MTP deficiency.

2097/T

NSDHL deficiency and disease: methyl sterols or cholesterol? *J. Trinh[1, 3], M. Morimoto[1, 3], G. Hendson[2, 3], K. McLarren[1, 3], C. du Souich[1, 3], C.F. Boerkoel[1, 3].* 1) Department of Medical Genetics, University of British Columbia, Child & Family Research Institute, Vancouver, BC, Canada; 2) Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada; 3) Rare Disease Foundation, Vancouver, BC, Canada.

BACKGROUND: NAD(P)H steroid dehydrogenase-like (NSDHL) is an X-linked gene that encodes for a 3β-hydroxysteroid dehydrogenase in the cholesterol biosynthetic pathway. Loss-of-function mutations of NSDHL cause CK and CHILD syndromes. CK syndrome patients have syndromic intellectual disability. Their dysmorphic features include microcephaly, thin build, a long and thin face, epicanthic folds, almond-shaped eyes, upslanting palpebral fissures and micrognathia. Their intellectual disability can be attributed to cortical malformations and seizures. A thorough examination of the expression of NSDHL in human tissues has never been done making delineation of the cell autonomous and non-autonomous aspects of NSDHL deficiency difficult. HYPOTHESIS: NSDHL deficiency causes the features of CK and CHILD syndromes by cell autonomous and non-autonomous mechanisms. METHOD: We profiled NSDHL expression in human male and female tissues by immunohistochemistry, qRT-PCR, and immunoblotting. We also compare this expression profile to that of the mouse Nsdhl and other enzymes in the cholesterol biosynthetic pathway. RESULTS: Our preliminary results show expression of NSHDL in steroidogenic, epithelial, and neuronal cells. CONCLUSION: This expression profile suggests that NSDHL loss-of-function mutations have both cell autonomous and cell non-autonomous effects. Based on comparison of NSDHL expression to that of other enzymes in the cholesterol biosynthetic pathway, we propose that some features of CK and CHILD syndromes are caused by the accumulation of methyl sterols rather than cholesterol deficiency alone.

2098/T

Reliable determination of phenylketonuria genotypes predictive of sapropterin dihydrochloride response. *J. Utz[1], C. Pham Lorentz[1], D. Markowitz[1], K.D. Rudser[2], C.B. Whitley[1].* 1) Adv Therapies, Pediatrics, Univ Minnesota, Minneapolis, MN; 2) Biostatistics, Univ Minnesota, Minneapolis, MN.

Background: Sapropterin dihydrochloride (SAP), a synthetic tetrahydrobiopterin (BH4), works as a chaperone of the phenylalanine hydroxylase enzyme (PAH) in phenylketonuria (PKU) by facilitating folding of PAH into its most active conformation and stabilizing the active conformation. No standard pharmacogenetic tests exist to identify responsive genotypes. Previous studies have failed to identify genotypes that consistently predict response, but they were weakened by varied: 1) SAP/BH4 doses; 2) response definitions; 3) duration; 4) phenylalanine (PHE) test times at varied protein catabolic states; 5) control of dietary PHE. START (sapropterin therapy actual response test) protocol is a double-blind, placebo-controlled, 4-week clinical diagnostic test that obviates the confounders aforementioned. START results were evaluated for response-genotype correlates and trends in molecular characteristics. Results: Sixty PKU patients completed START, with 44 patients' mutations known and 35 different genotypes represented. Thirty patients (50%) were responsive to SAP. Alleles singly associated with response include Y414C (7/7 patients, 5 genotypes) and I65T (8/8 patients, 5 genotypes) . Mutant allele R408W, located at the crux of a strategic hinge site for activating folding in the wild type, is associated with non-response in 16/20 (80%)patients and 11/15 (73%) genotypes. Straight-chain amino acids substituted with aromatic amines had low residual activity and were associated with non-response in 16/20 (80%) patients, 11/15 (73%) genotypes. Substitution with lower molecular weight amino acids was associated with high residual activity and responsiveness in 23/26 (88.5%) patients. Conclusions: The START protocol provides a consistent pharmacogenetic test to reliably identify genotypes associated with SAP responsiveness and augments understanding of the chaperone role of SAP/BH4. To date, START has identified 22 genotypes predictive of response. Response trends were associated with mutant locus at activating PAH fold sites and spatial folding changes incurred by substituted amino acid size and configuration. Substitution with aromatic amines is associated with non-response. Ongoing study of BH4 response via START protocol will provide a reliable pharmacogenetic test for improving understanding of how PKU mutations impact PAH protein folding dynamics and will enhance understanding of PKU disease and its management.

2099/T

Niemann-Pick Type C Disease in Australia: Data From Four Decades of Diagnosis. *M. Walterfang[1,2], M. Fahey[3,4], D. Bratkovic[5,6], M. Fietz[5,6], D. Velakoulis[1,2], E. Storey[7,8].* 1) Neuropsychiatry Unit, Royal Melbourne Hospital, Parkville, Victoria, Australia; 2) Melbourne Neuropsychiatry Centre, University of Melbourne, Parkville, Victoria, Australia; 3) Department of Neurology, Monash Children's, Monash Medical Centre, Clayton, Victoria, Australia; 4) Department of Pediatrics, Monash University, Clayton, Victoria, Australia; 5) Metabolic Unit, Women's and Children's Hospital, Adelaide, South Australia, Australia; 6) SA Pathology, Adelaide, South Australia, Australia; 7) Neurology Unit, Alfred Hospital, Prahran, Victoria, Australia; 8) Department of Neurology, Monash University, Clayton, Victoria, Australia.

Niemann-Pick type C (NPC) disease is a well-characterised protean neurovisceral disorder that is caused by mutations to NPC1 and NPC2, whose products function as intracellular sterol chaperones. NPC presents from neonates to adults, with presentations ranging from cholestatic jaundice in infancy to mental retardation, seizures, ataxia, psychiatric illness and dementia through childhood and adulthood. Australia's sole lysosomal storage disease reference laboratory serves the Asia-Pacific region; diagnosis via filipin staining and esterification began in the 1990s and stored samples were retrospectively analysed for biochemical characterisation. Data from all patients diagnosed since 1980 (n=84) were analysed; the majority (n=60) of patients were from Australia. Age of diagnosis ranged from the neonatal period to 49 years of age, with "classical" biochemical phenotypes diagnosed at a mean age of 7 and "variant" phenotypes at a mean age of 25. Over four decades, there was a year-on-year increase in the rate of diagnoses, with 40 patients diagnosed in the last decade, particularly the adult-onset form. There was a significant correlation between esterification rate ($r^2=0.3$, $p<0.001$) and filipin staining percentage ($r^2=0.3$, $p<0.0001$) and age of diagnosis. In patients genotyped, I1061T was the most common causative mutation, and seen in patients up to the age of 30; S954L was associated with later-onset disease. This analysis presents a large regional cohort diagnosed through a single laboratory and illustrates the wide phenotypic variation of disease, but suggests that there are correlations between genotype, biochemical and clinical phenotypes. Increased recognition of the disease, particularly adult-onset forms, appears to be responsible for the increasing proportion of variant illness diagnoses.

2100/T

SECONDARY BIOTIN DEFICIENCY OBSERVED IN TWO JAPANESE INFANTS DUE TO CHRONIC USE OF HYPOALLERGIC INFANT FORMULA. *Y. Watanabe[1,2], T. Ohya[2], T. Ohira[2], T. Fukui[3], T. Watanabe[3], T. Inokuchi[1], M. Yoshino[2], T. Matsuishi[1,2].* 1) Research Institute of Medical Mass Spectrometry, Kurume University School of Medicine, Fukuoka, Japan; 2) Department of Pediatrics and Child Health, Kurume University School of Medicine, Kurume, Japan; 3) School of Human Science and Environment, University of Hyogo, Himeji, Japan.

Background: Biotin is a water-soluble vitamin and is widely distributed in many foods. Biotin functions as a cofactor for acetyl-CoA carboxylase (AC), methylcrotonyl-CoA carboxylase (MCC), propionyl-CoA carboxylase (PCC), and pyruvate carboxylase (PC), and is important in fatty acid synthesis, amino acid metabolism, and gluconeogenesis. Symptoms of biotin deficiency include dermatitis, hypoglycemia, characteristic organic acidemia, seizures, and developmental delay. Although biotin deficiency is believed to be rare, there have been several reports of symptomatic infants with secondary biotin deficiency in Japan because biotin is not supplemented in infant special formula such as hypoallergenic infant formula including lactose free formula. Objectives: To raise awareness of importance of supplementing biotin in infant special formula by reporting infants diagnosed with biotin deficiency due to the typical biochemical profiles consistent with biotin metabolism defects including biotinidase deficiency and holocarboxylase synthetase deficiency (HCS). Case report: Patient 1 is an 8-month-old Japanese female, ex-33 week premature infant, with seizures and history of idiopathic gastric rupture. Hypoallergenic formula was implemented since age 20 days. Her physical exam was unremarkable. Laboratory studies including lactic acidemia, a low free and a high C5OH carnitine, and elevated urine 3-hydroxyisovaleric acid and 3-methylcrotonylglycine, prompted us to study biotin metabolism defects. Biotinidase and HCS were measured normal. Plasma and urine biotin were measured below the reference ranges: plasma: free 0.3 ng/ml ref. 0.4-1.1; urine < 2 ref: 4-25. Patient 2 is an 11-month-old, ex-33 week premature, Japanese male. He was solely fed with lactose free hypoallergenic formula until 7 months when small solid foods were started. His physical exam was unremarkable but mild metabolic acidosis was noted. Laboratory studies including plasma and urine biotin levels, plasma acylcarnitines and urine organic acids were consistent with biotin deficiency. The enzymes related to biotin metabolism were normal. Conclusion: Hypoallergenic infant formula has recently been more commonly used. Pre-symptomatic infants with biotin deficiency might be not rare in Japan. Supplementation of biotin in hypoallergenic infant special formula is essential to promote infant's well-being.

2101/T

DPM1 mutations in a patient with dystrophic muscle and abnormal alpha-dystroglycan. *A.C. Yang[1], B. Ng[2], S.A. Moore[3], J.S. Rush[4], K.M. Raymond[5], C.J. Waechter[4], H.H. Freeze[2], L. Mehta[1].* 1) Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, NY; 2) Sanford Children's Health Research Center, Sanford-Burnham Medical Research Institute, La Jolla, CA; 3) Department of Pathology, The University of Iowa, Iowa City, IA; 4) Department of Molecular and Cellular Biochemistry, Chandler Medical Center, College of Medicine, University of Kentucky, Lexington, KY; 5) Department of Laboratory Medicine and Pathology, Mayo Clinic School of Medicine, Rochester, MN.

Type I congenital disorders of glycosylation (CDG) are rare autosomal recessive diseases involving a spectrum of defects in post-translational modification of proteins via attachment of carbohydrate chains. Patients with CDG type I typically present with neurological manifestations including seizures, psychomotor retardation, cerebellar atrophy, hypotonia, and microcephaly. Other associations include liver disease and coagulation abnormalities. Such disorders result from N-linked glycosylation defects. Patients with severe forms of dystroglycanopathy typically present with muscular dystrophy, brain and eye abnormalities that arise from reduced O-linked mannosylation of alpha-dystroglycan. We describe an infant who initially presented with hypotonia, camptodactyly, severe motor delays, elevated CK, and borderline microcephaly. A muscle biopsy showed necrotizing myopathy and a mosaic pattern of reduced alpha-dystroglycan immunostaining with glycoepitope antibodies suggestive of dystroglycanopathy. Carbohydrate deficient transferrin testing was abnormal and consistent with CDG type I. Phosphomannomutase and phosphomannose isomerase activities were all within the normal range ruling-out CDG-Ia and Ib. No mutations were found in DPM2 or DPM3, but sequencing of DPM1 (dolichol-P-mannose synthase subunit 1) revealed a heterozygous splice site mutation (c.563G/A = R188K) that leads to a premature stop codon as well as a homozygous novel Gly>Val change (c.455 G>T = G152V) of unknown pathogenicity. Enzyme analysis showed a 75% deficiency in DPM1 activity while Km was normal suggesting a decrease in the amount of enzymes. The DPM complex, composed of 3 protein subunits, has previously been shown to have roles in both N-glycosylation and O-mannosylation. There has been only one case reported in the literature that links CDG with dystroglycanopathies: a girl with a homozygous DPM3 mutation who has a mild muscular dystrophy and dilated cardiomyopathy (Lefeber et al, 2009). We present another case of apparent CDG type I with clinical and muscle biopsy findings consistent with dystroglycanopathy. DPM1 mutations are being investigated as the cause. Previously reported patients with DPM1 mutations, classified as CDG-Ie, have been described to have elevated CPK, but have not been evaluated for a dystroglycanopathy. In the future, such investigations will expand the phenotypic spectrum of these rare disorders.

2102/T

Development of Models of Succinyl-CoA Ligase Deficiency and mtDNA Depletion in Mouse and Fruit Fly. *T.R. Donti[1], N.K Hawkins[1], M. Ge[1], K. Eldin[2], B.H. Graham[1].* 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Pathology, Texas Children's Hospital, Houston, TX.

Mitochondrial diseases associated with depletion of mitochondrial DNA (mtDNA) have emerged as an important subset of mitochondrial diseases over the past few years. Mutations in subunits of Succinyl-CoA Ligase (Synthetase), a component of the citric acid cycle, have been associated with mitochondrial encephalomyopathy and mitochondrial DNA depletion. *SUCLA2*, encoding the ADP-specific beta subunit, and *SUCLG1*, encoding the alpha subunit, have both been demonstrated to be mutant in cases of mitochondrial disease with mtDNA depletion. The goal of this project is to develop model systems of Sucinyl-CoA Ligase deficiency to study disease pathogenesis and biology of mtDNA maintenance. A gene trap allele of *Sucla2* (*Sucla2*^{SA}β*geo*) has been isolated in mouse embryonic stem (ES) cells and used to generate transgenic animals. *Sucla2*^{SA}β*geo* homozygotes exhibit recessive lethality with most mutants dying late in gestation (e18.5). Histological analysis of mutant placenta reveals increased mineralization and mutant embryos are approximately 25% smaller than wild type littermates. Mutant placenta and embryonic brain, heart and muscle show varying degrees of mtDNA depletion (20-60%), while there is no appreciable mtDNA depletion in mutant liver. Mouse embryonic fibroblasts (MEFs) derived from e12.5 embryos show a 50% reduction in mtDNA content after five passages for mutant compared to wild type. In *Drosophila*, a deletion mutant of *Scsalpha* (*CG1065*, *SUCLG1* ortholog) has also recently been isolated through imprecise excision of a P-element inserted next to the gene. Preliminary analyses of this mutant reveal that they are homozygous viable but exhibit neurological dysfunction manifested through increased sensitivity to mechanical stress (increased bang sensitivity). Quantification of mtDNA in these mutants is currently being performed. The characterization and exploitation of models of Succinyl-CoA ligase deficiency promise to provide insights into the pathogenesis of mitochondrial diseases with mtDNA depletion as well as into the biology of mtDNA maintenance.

2103/T

Deoxyguanosine kinase (*DGUOK*) deficiency presenting as neonatal hemochromatosis. *N.A. Hanchard[1], O. Shchelochkov[1,2], E. Brundage[1], E. Schmitt[1], A. Roy[3], M. Finegold[3], L. Wong[1], F. Scaglia[1].* 1) Human and Molecular Genetics, Baylor College of Medicine, Houston, TX; 2) Division of Genetics, Department of Pediatrics, University of Iowa Hospitals and Clinics, Iowa City, IA; 3) Department of Pathology, Baylor College of Medicine, Houston, TX.

Mutations in the nuclear gene deoxyguanosine kinase (*DGUOK*) can result in mitochondrial DNA (mtDNA) depletion, which may present as neonatal liver failure. *DGUOK* deficiency as the underlying diagnosis in the severe hepatic failure of neonatal hemochromatosis (NH), however, is poorly described in the literature and under-recognized in clinical practice. We report an African American female neonate born at term to non-consanguineous parents, who failed her newborn screen for tyrosinemia (tyrosine 800 μmol/L; upper cut-off 500 μmol/L). By two weeks of age she was hospitalized with severe, progressive liver failure and an elevated serum lactate (9.4 mMol/L). Urinary succinylacetone was not observed and plasma amino acids were consistent with hepatic failure. Further evaluation revealed an elevated ferritin (1627 ng/ml; upper limit 391 ng/ml), which, in conjunction with an abdominal MRI suggestive of hepatic siderosis, led to a presumptive diagnosis of NH. Despite intensive treatment, her hepatic dysfunction and coagulopathy worsened, leading to death on day of life 44. Subsequent autopsy demonstrated significant hepatic siderosis as well as extrahepatic iron deposition sparing the spleen, consistent with the diagnosis of NH. The abnormal newborn screen, severe liver dysfunction, and elevated lactate prompted evaluation for a hepatic mtDNA depletion syndrome. Sequencing of the nuclear genes *POLG1*, *MPV17* and *DGUOK* in blood revealed an apparent homozygous change c.572A>G (p.Y191C) in the *DGUOK* gene. This missense variant is predicted to disrupt enzyme function and has been reported in a neonate with the hepatocerebral form of DGUOK-related mtDNA depletion syndrome. Oligonucleotide arrayCGH targeted to genes involved in metabolic and mitochondrial disorders revealed a significant reduction of mtDNA content in blood (approximately 45% of age and tissue matched control). Parental samples were unavailable for analysis; however, a high resolution SNP-array demonstrated an 8 mb region of absent heterozygosity encompassing the *DGUOK* gene, confirming homozygosity of *DGUOK*. The etiology of NH is unclear, but autosomal recessive inheritance is implied; we are thus evaluating other NH cases for evidence of MtDNA depletion. This report underscores the importance of considering *DGUOK* deficiency as a cause of NH, highlights mtDNA depletion in the differential diagnosis of neonatal tyrosinemia, and broadens the ethnic profile of *DGUOK* mutations to include African Americans.

2104/T

A Drosophila model of mitochondrial complex 1 deficiency. *V. Hegde[1,2], B. Duboff[1,2], R. Vogel[2], M. Feany[1,2].* 1) Brigham and Women's Hospital, Boston, MA; 2) Harvard Medical School.

As a group, mitochondrial respiratory chain disorders are one of the most common inborn errors of metabolism. They frequently causes a devastating encephalomyelopathy of infancy, Leigh's disease. Mitochondrial complex 1 deficiency is the most common cause of respiratory chain dysfunction. The pathophysiology of complex I deficiency is poorly understood, at least in part due to the absence of adequate animal models. Here we demonstrate that complex 1 deficiency can be modeled in Drosophila by post-transcriptional silencing (knockdown) of a critical subunit of complex 1. Knockdown flies show many of the features of human mitochondriopathies including a shorter life span, locomotor dysfunction, histological evidence of neurodegeneration, reduced complex 1 activity and electron microscopy findings of large dysmorphic mitochondria. Our results demonstrate that many of the clinical features of mitochondrial complex 1 disease can be modeled in Drosophila, a powerful model organism to study genetic pathways and disease pathophysiology.

2105/T

Mitochondrial-Genetics Diagnostic Clinic Two-Year Experience at the Children's Hospital of Philadelphia. *E. Place, M. Falk.* Division of Human Genetics, Department of Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, PA 19104, USA.

The Mitochondrial-Genetics Diagnostic Clinic at the Children's Hospital of Philadelphia was established in 2007 to facilitate the often complicated diagnostic process for the heterogeneous class of mitochondrial diseases. Here, we review our 2-year clinical experience, focusing on the number and type of diagnoses made. **METHODS**: Since June 2008, 79 new patients ranging in age from 6 weeks to 82 years were evaluated in this Clinic. 2/3 of referrals were for abnormal laboratory results (13), family history (10), seizures (10), GI problems particularly dysmotility (8), developmental delay (6), and muscle weakness (6). All patients underwent clinical evaluation including physical and dysmorphologic examination, medical and family histories, and metabolic screening studies. Genetic diagnostic testing and more invasive testing such as tissue biopsy, LP, and neuroimaging studies were obtained based on individual presentation. **RESULTS**: 14 individuals were found to have genetically-confirmed primary mitochondrial DNA (mtDNA) cytopathies: a boy with severe multi-systemic disease including retinitis pigmentosa, cataracts, and cardiomyopathy (*tRNA-SER(AGY)* C12264T); a mother and 2 daughters with complex I deficiency due to two LHON mutations (*ND4* G11778A heteroplasmy and *ND6* T14484C homoplasmy); 6 individuals with progressive myopathy (*tRNA-LEU* A3288G); 2 siblings with COX deficiency (*tRNA-TRP* 5537_5538insT); and mother and daughter with MELAS (*tRNA-LEU* A3243G). mtDNA mutations of uncertain pathogenesis were identified in 5 individuals (*tRNA-TYR* A5836G, *ND2* C4936T, and *ATP8* C8472T plus *ND2* C4960T). 3 patients had myopathy, global delay, and abnormal respiratory chain studies of unclear genetic etiology. 1 individual had severe mtDNA depletion in skeletal muscle of unknown genetic etiology. Non-mitochondrial diseases subsequently diagnosed in this cohort included central dopamine deficiency, Ulrich muscular dystrophy, myotonia congenita, *CPT II* deficiency, *SEPN1*-related myopathy, Fabry's disease, and Gitelman syndrome. 4 chromosomal abnormalities were diagnosed including *IL1RAPL2* deletion, *MEFC2* deletion, a three-way unbalanced translocation, and isochromosome Xp. **CONCLUSIONS**: The diagnosis of mitochondrial disease is a complex but navigable process. Retrospective review of a 2-year dedicated outpatient experience highlights the wide range of heterogeneous conditions that must be considered in patients for whom mitochondrial disease is suspected.

2106/T

Creation of a Mouse Model for Lowe Syndrome and Dent Disease 2 by Humanizing a Paralogous Modifier Gene. *R. Nussbaum[1,2], E. Chan[1], I. Bernardini[3], Y.-M. Kuo[1], W. Gahl[3], S. Bothwell[1].* 1) Medicine, UCSF, San Francisco, CA; 2) Institute for Human Genetics, UCSF, San Francisco, CA; 3) Medical Genetics Branch, NHGRI/NIH, Bethesda, MD.

The Lowe OculoCerebroRenal syndrome (OCRL) is a pleiotropic X-linked human disorder characterized by congenital cataracts, cognitive disability, and proximal renal tubular dysfunction, particularly low molecular proteinuria and often aminoaciduria, phosphaturia and bicarbonaturia. OCRL is caused by loss-of-function mutations in the *OCRL* gene encoding Ocrl, a type II phosphatidylinositol bisphosphate 5-phosphatase. Mutations in *OCRL* can also cause a proximal tubular disorder known as Dent Disease type 2, in which the disease is limited to the proximal renal tubules. A first attempt to create a mouse model for OCRL/Dent 2 failed when we found that *Ocrl*-mice are unaffected. We reasoned that the disparate phenotype between humans and mice with loss-of-function mutations in *Ocrl*/*OCRL* resulted from differences in how the two organisms cope with loss of the enzyme rather than in differences between the two species in the function of the enzyme itself. We hypothesized that *Inpp5b* and *INPP5B*, which also encode a type II phosphoinositide 5-phosphatase in mice and humans, respectively, might underlie the disparate phenotype in the two species because (1) they are the closest paralogs to *Ocrl* and *OCRL* in the respective genomes of mice and humans, (2) *Inpp5b* has overlapping function in vivo with *Ocrl*, and (3) the two species differ in a number of important ways in how *INPP5B* and *Inpp5b* are expressed. We used a bacterial artificial chromosome containing *INPP5B* to create transgenic *Ocrl*-;*Inpp5b*-/-mice expressing *INPP5B*. All showed reduced post-natal growth, low molecular weight proteinuria, and aminoaciduria when hemizygous for the *INPP5B* insertion but not when homozygous for the insertion. Ophthalmological abnormalities were not present. We have created the first animal model for OCRL/Dent Disease 2 by humanizing a modifier paralog in mice carrying the mutant disease gene.

2107/T

Cytoskeletal alterations in the bladder from male mice with cystinuria. *A. Sahota[1], K. Johnson[2], M. Yang[1], A.O. Emmanuel[1], B. Zheng[1], J.G. Barone[2], L. Serrano[1], J.A. Tischfield[1].* 1) Dept Gen, Rutgers Univ, Piscataway, NJ; 2) Dept Surgery, UMDNJ-RWJMS, New Brunswick, NJ.

Background: There are numerous etiologies of bladder obstruction in humans, which mainly affects males. SLC3A1 encodes a subunit of the transporter system for cystine and dibasic amino acids. Bladders from Slc3a1 knockout male mice age >3 months have cystine stones, hypertrophy, and outlet obstruction, but only a small fraction of cystinuria females age >20 months form stones. These observations suggest that cystinuria male mice may be a good model for bladder obstruction. How obstruction leads to changes in the ultrastructure and physiology of bladder epithelia remains to be determined. We hypothesize that obstruction-induced stretch of the bladder may lead to alterations in the cytoskeleton. Keratins (KRTs) are a large group of intermediate filaments in the cytoplasm of epithelial cells, and several KRTs are epithelial cell lineage markers. Thus, analysis of KRT expression may allow identification of the specific epithelial cell types affected by or involved in bladder obstruction. **Methods:** We examined mRNA expression changes for several KRTs in bladders from 4-5 months old cystinuria and control mice by quantitative real time PCR and for KRTs 7, 14, and 19 as part of an epithelial-to-mesenchyme (EMT) specific PCR array. Frozen bladder sections were analyzed by immunofluorescence to identify the cell tyles expressing KRTs 14 and 17. **Results:** KRTs 5, 7, 8, 18, 19, and 20 were expressed at similar levels in cystinuria and control male mice. The expression of KRTs 14 and 17 was over 30 times higher in bladders from cystinuria male mice compared with cystinuria females or control mice, and over-expression of KRT 14 was confirmed in the EMT PCR array. KRT 14 expression was localized to the basal layer in bladder sections from cystinuria female mice, whereas in cystinuria male mice there was extensive proliferation of the epithelial layer and KRT expression was observed in the apical layer as well as in the submucosal layers. KRT 17 expression was localized to the apical region in cystinuria female mice, whereas expression in cystinuria male mice extended from the apical layer towards the basal layer. **Conclusions:** The dramatic increase in KRT 14 expression in cystinuria males suggests that cells of the basal layer may be involved in the EMT process and this may account for the hypertrophy and increased fibrosis seen in these mice. Changes in KRT 17 expression suggest a change in phenotype from differentiated apical cells to a less mature phenotype.

2108/T

PCSK9: FROM GENE AND VARIANTS TO PROTEIN AND PLASMA LEVELS. *M. Abifadel[1,2], J.P. Rabès[1,3], M. Varret[1], ME. Samson- Bouma[1], E. Bruckert[1], M. Devillers[1], V. Carreau[4], L. Tosolini[1], M. Marduel[1], A. Marques[1], P. Couvert[4], D. Bonnefont-Rousselot[5], M. Guérin[4], A. Carrié[4], A. Prat[6], N. Seidah[6], C. Boileau[1,3].* 1) INSERM U781, Hospital Necker, APHP, Université Paris Descartes, Paris, France; 2) Faculté de Pharmacie, Université Saint-Joseph, Beirut, Lebanon; 3) Laboratoire de Biochimie et de Génétique moléculaire, hôpital Ambroise-Paré, APHP ; Université Versailles-Saint-Quentin-en-Yvelines, UFR médicale Paris-Ile-de-France-Ouest, Boulogne; 4) INSERM UMR S939, hôpital Pitié-Salpêtrière; 5) UF de biochimie des maladies métaboliques, hôpital Pitié-Salpêtrière, APHP, Paris, France; 6) Laboratory of Biochemical Neuroendocrinology, Clinical Research Institute of Montreal, Montreal, QC, Canada.

Hypercholesterolemia is one of the major causes of coronary heart disease (CHD). The genes encoding the low density lipoprotein receptor (LDLR) and its ligand apolipoprotein B (APOB) have been the two genes classically implicated in autosomal dominant hypercholesterolemia (ADH). Our discovery of the implication of PCSK9 in hypercholesterolemia by linkage analysis studies in ADH families with no mutation in the LDLR or in the APOB genes brought to light an unknown actor in cholesterol metabolism that has been extensively investigated since. Several PCSK9 variants have been identified, some of them are gain of function mutations causing hypercholesterolemia by a reduction of LDL receptor levels, while others are loss of function variants associated with a reduction of LDL-C levels and a decrease in the risk of CHD. PCSK9 is a secreted protein that represents now a major therapeutic target for the treatment of hypercholesterolemia and prevention of CHD. By studying further patients with hypercholesterolemia unrelated to the LDLR or the APOB genes, we have recently identified new variants of PCSK9 and studied the effect of these variants on the maturation of the enzyme. Furthermore, we measured PCSK9 concentration, by an ELISA method, in the plasma of patients with familial hypercholesterolemia carrying different mutations of PCSK9 especially the p.S127R, p.F216L, p.R218S mutations that we have already reported. These data further contribute to the characterization of PCSK9 variants and mutations and their impact in cholesterol diseases.

2109/T

Mitochondrial DNA mutations in mexican patients with diabetes mellitus type 2 and their relation with corporal composition and dietary habits. *C. Torres-Pedraza[1,3], J. Becerra-Contreras[1], H.R. Martinez-Ramirez[1], A. Sarralde-Delgado[1], M. Escalante-Pulido[1], K.P.T. Reddy[2], L. Sandoval-Ramirez[1,2].* 1) IMSS, Guadalajara, Mexico; 2) CUCS, Universidad de Guadalajara, Guadalajara, Mexico; 3) Universidad del Valle de Atemajac (UNIVA),Guadalajara, Mexico.

OBJETIVE: To compare the corporal composition and intake habits of diabetic mellitus type 2 patients with and without mutations in the mitochondrial DNA. METHODS: The sequence of 3108-3717 bp region of mtDNA was analyzed by capillary electrophoresis. Mitochondrial DNA was obtained from blood samples of twenty T2DM patients (eight female and twelve men). We applied a 24-hour recall, food frequency and made an anthropometric assessment; the anthropometric measures was performed according to the protocol of the International Society for the Advancement Kinanthropometry (ISAK), which was used a Harpenden calipers (British Indicators Ltd., LondonR) with a sensitivity of 0.1 mm and a constant pressure of 10 mm2, for the seven skinfold evaluation (triccipital, biccipital, subscapular, abdominal, supraspinal, abdominal, iliac crest of). The fat percentage was calculated using the equation of Durning and Womesley and weight by using a scale tanita BC50 model, the diameters were measured with anthropometer. Anthropometric evaluation was done with the help of a specialized software called BORIS. The patient was given food and drink diary which had to do for three days, and returned later. The reminder information 24 hours and the food diary were analyzed at the software nutripac. RESULTS: A new mutation C3393T was detected in one patient (5%) and seven patients presented polymorphisms (4/20 had T3552A, 1/20 had T3394C, 1/20 had C3448A and 1/20 had the T3335C and T3396C polymorphisms). Four patients had a healthy BMI, sixteen (16/20) had overweight and obesity, according to the parameters established by the American Dietetic Association. CONCLUSIONS: All patients had a high percentage of body fat. The calories intake by patients was above their total caloric requirements. All they consumed more than 7% saturated fat, exceeding this rate to three times. Only five individuals were within the recommendations of 50 to 60% of intake carbohydrates, the rest of patients exceeded this percentage, especially who those showing polymorphisms.

2110/T

RRM2B mtDNA Depletion Syndrome presenting with Pyruvate Dehydrogenase Deficiency. *L.A. Wolfe[1,2], J.J McGrath[2], L.J Wong[3], G.D Vladutui[4].* 1) Undiagnosed Diseases Program, NHGRI, Bethesda, MD; 2) Department of Genetics, Yale School of Medicine, New Haven, CT; 3) Baylor College of Medicine, Houston, TX; 4) Robert Guthrie Biochemical & Molecular Genetics Laboratory, Kaleida Health Laboratories & the University, Buffalo, NY.

Purpose: Describe clinical and biochemical features of a male infant presenting with failure to thrive and severe diarrhea in the neonatal period. Case review: A 2-week-old male born to consanguineous Hispanic parents after an uneventful pregnancy and delivery, presented at 2 weeks of age with failure to thrive and severe diarrhea was admitted to the hospital for further evaluation. Laboratory evaluation revealed normal acylcarnitine profile, urine organic acids, and CK. Plasma lactic acid (15 mmol/L, normal <2.2), and pyruvate (0.48 mmol/L, normal 0.03-0.10) were elevated with a mild increase of alanine (435.4 uM, normal 142-421). Due to a high L: P ratio (33), a mitochondrial electron transport chain (ETC) disorder was suspected. Common mtDNA point mutations and deletions were negative. Sequencing analysis of 5 genes (SUCLG1, DGUOK, POLG1, SUCLA2, TK2) responsible for mtDNA depletion, complex IV assembly genes (SURF1, SCO1, SCO2, COX 10), SDH subunits A-D, and complex I assembly genes (C6ORF66, NDUFA1) were all negative. Methods used: prospective evaluation and clinical biochemical testing. A novel hemizygous unclassified missense variant, c.677G>A (p. R226H) in the PDHA1 gene was detected. On muscle biopsy, abnormal mitochondria with proliferation were noted. Profound deficiencies in complexes IV and II-III were detected with reduced complexes I and I-III activities. Meanwhile, deficiency in pyruvate dehydrogenase complex (PDC) activity was detected in blood lymphocytes. The patient was started on carnitine and ubiquinol, which improved his diarrhea, and he was discharged home. Subsequently, the patient's clinical condition continued to decline and he became ventilator dependent. MtDNA depletion syndrome was suspected and sequence analysis of the RRM2B gene, encoding a newly discovered p53-inducible ribonucleoside reductase subunit was performed. A homozygous c.109_110delAA (p. K37EfsX14) mutation was revealed. Results: Although an ETC deficiency may be secondary to the PDC defect, it is not known if mtDNA depletion would cause PDC deficiency. Thus, the clinical significance of p. R226H in PDHA1 remains unclear. This case underscores the complications in diagnosing mitochondrial ETC disorders.

2111/T

Novel Mutation in the Human Sucrase-Isomaltase Gene. *Z. Wu, S. Uhrich, C.R. Scott.* Department of Pediatrics, University of Washington, Seattle, WA 98195-6320.

Sucrase-isomaltase (SI) enzyme complex is a heterodimeric membrane-bound glycoprotein that resides on the intestinal brush border. It is comprised of two homologous subunits, sucrase and isomaltase. This enzyme is implicated in the digestion of dietary starch and disaccharides. Congenital deficiency of the SI complex causes a disorder resulting in maldigestion and malabsorption. This disorder is called congenital sucrase-isomaltase deficiency (CSID). The clinical phenotype is genetically transmitted as autosomal recessive disorder. To assist in understanding the genetic basis of CSID and to correlate genotype to phenotypic expression, we have established a diagnostic procedure that involves nucleotide sequencing of the SI gene. The SI gene is located on chromosome 3 (3q25.2-q26.2), spans about 100 kb, contains 48 exons and encodes a protein containing 1827 amino acids. Amino acids from 1 to 1007 form the isomaltase domain, whereas from 1008 to 1827 form the sucrase domain. Mutations occurring in any regions of the SI gene may have effects on both enzyme activities. We have examined DNA samples from eight patients who were diagnosed with CSID by bowel biopsy or positive hydrogen breath test. 18 molecular variations within the SI gene were identified in these patients that are interpreted to be responsible for CSID. By comparing our results with the mutation database (http://www.hgmd.cf.ac.uk) and SNP database (http://genome.ucsc.edu), 8 new variations are identified: one deletion (c.545delT), one presumed splicing error (IVS10 -2a>g) and six missense mutations (1) Q307X (c.975C®T), (2) E613X (c.1893G®T), (3) L741P (c.2278T®C), (4) R1124X (c.3427C®T), (5) I1378S (c.4189T®G) and (6) Y1417X (c.4307T®G). It may now be cost effective to use sequencing of the SI gene, rather than anesthesia, intestinal biopsy and enzyme assays, for confirmation of the diagnosis of CSID.

2112/T

Genetic techniques for newborn screening for cystinosis. *T. Vilboux[1], M.R. Erdos[2], R. Kleta[3], J.Z. Balog[4], I. Bernardini[1], M. Huizing[1], W.A. Gahl[1,4].* 1) Medical Genetics Branch, National Human Genome Research Institute, NIH, Bethesda, MD; 2) Genome Technology Branch, National Human Genome Research Institute, NIH, Bethesda, MD; 3) Centre for Nephrology, University College London, London, UK; 4) Office of the Clinical Director, Medical Genetics Branch, NIH, Bethesda, MD.

Cystinosis is an autosomal recessive disease characterized by lysosomal accumulation of cystine due to a defective lysosomal cystine transporter, cystinosin. Cystine deposits in various organs lead to renal failure, hypothyroidism, insulin-dependent diabetes, muscle deterioration, swallowing difficulty, retinal blindness, and cerebral involvement. The prevalence of cystinosis approximates 1/160 000. The causative gene, *CTNS* (12 exons), located on 17p13, encodes cystinosin, a 367 amino acid lysosomal membrane transport protein. The most frequent mutation is a 57-kb deletion detected in 60% to 70% of patients originating from northern Europe. Over 60 other *CTNS* mutations have now been reported worldwide. Prenatal diagnosis can be obtained by genetic analysis in families with a previous affected child, or by measuring 35S-labeled cystine incorporation into fibroblasts cultured from amniotic fluid or trophoblastic cell samples. An effective treatment for cystinosis is oral cysteamine, which lowers lysosomal cystine concentrations, thereby slowing the progression to renal failure and preventing non-renal complications. For efficient treatment, cysteamine should be given as early in life as possible, so early diagnosis of the disease is invaluable. We explored possibilities to include a genetic test in newborn screening programs, evaluating molecular methods. Specifically, from a newborn blood spot, the technique should be able to discriminate all the known *CTNS* mutations involved in infantile cystinosis and be able to differentiate carriers from patients. In addition, the technique should be rapid and amenable to high throughput. Two approaches are considered: the Luminex beads-based method (XMAP) and MALDI-TOF mass spectrometry from Sequenom (Iplex). Pilot experiments have been designed to show effectiveness of these techniques. Our cystinosis patient cohort is comprised of about 250 different DNA samples. Each sample was genotyped for the 57kb deletion and every non-homozygous deleted sample was sequenced for other mutations. We are using this large group of patients' DNA as templates for these techniques. So far, newborn screening tests based on molecular grounds are not yet available or implemented. Therefore, cystinosis will serve as a prototype disease for proof of principle for newborn screening on molecular grounds, after which it is likely that screening for other genetic diseases will follow.

2113/T

Beyond Genotype-phenotype Correlations In Lesch-Nyhan Disease. *R. Fu, S. Radhika, H.A. Jinnah.* Department of Neurology, Emory University, Atlanta, GA.

Lesch-Nyhan disease (LND) is a neurogenetic disorder of purine metabolism, which results from deficiency of the purine salvage enzyme, hypoxanthine-guanine phosphoribosyltransferase (HGprt). Clinically the disorder is characterized by overproduction of uric acid, motor disability, intellectual impairment, and self-injurious behavior. This enzyme has two functions, recycling both hypoxanthine and guanine into purine nucleotide pools. A mutation database for HGprt shows that the locations and types of mutations associated with human diseases are heterogeneous, with only limited genotype-phenotype correlations. However, prior studies with relatively small number of patient samples have shown a correlation between residual enzyme activity and clinical severity, but which of the two functions of HGprt may be most directly relevant to different aspects of the clinical phenotype remains uncertain. It is possible that loss of only hypoxanthine recycling or guanine recycling is responsible for the entire clinical phenotype, or for specific elements of the clinical phenotype. To explore a relationship between substrate recycling and clinical severity, 15 clinically relevant HGprt mutants were constructed in vitro and biochemically compared with normal HGprt. The mutants were selected based on clinical severity, recurrence among unrelated patients, and location of the mutation at the active site. All enzymes were engineered by site-directed mutagenesis with an amino-terminal his-tag, expressed and purified from *Escherichia coli*, and characterized in vitro with varying substrate concentrations to define k_{cat}, K_m and k_{cat}/K_m values for all substrates. The results show that each of these parameters are affected independently by different mutations and provide a solid framework to understand the correlation of substrate specificity with different aspects of the clinical phonotype. The results also provide an essential next step to a better understanding of pathogenesis that goes beyond simple genotype-phenotype correlations.

2114/T

Dilated cardiomyopathy associated with HMG-CoA Lyase deficiency in an adolescent: Does Carnitine supplementation prevent cardiomyopathy? *A. Saronwala[1], B.P. Abraham[2], C. Steigman[3], S.G. Kahler[1], E.A. Frazier[2], U. Dyamenahalli[2].* 1) Genetics (Pediatrics), University of Arkansas for Medical Sciences, Little Rock, AR; 2) Cardiology (Pediatrics), University of Arkansas for Medical Sciences, Little Rock, AR; 3) Pathology, University of Arkansas for Medical Sciences, Little Rock, AR.

3-Hydroxy-3-Methylglutaryl-CoA (HMG-CoA) lyase deficiency is a rare autosomal recessive inborn error of metabolism characterized by impaired leucine catabolism and ketogenesis. HMG-CoA lyase deficiency typically presents in the neonatal period and occasionally in early infancy or childhood with metabolic acidosis and hypo-ketotic hypoglycemia triggered by fasting or excessive protein intake. Cardiomyopathy has been reported in two patients, an infant and a 23 year old male. We report a patient with HMG-CoA lyase deficiency who was diagnosed with dilated cardiomyopathy in the adolescent period. At age 14 he presented with a two week history of cough and shortness of breath. He had frequent premature ventricular ectopic beats, moderate cardiomegaly and pulmonary edema, left ventricular hypertrophy and non-specific T-wave abnormality, and severely dilated left ventricle with severe reduction of left ventricular systolic function. Review of chest X-rays revealed slowly progressive cardiomegaly during the prior two years. He was hospitalized for intensive treatment, and then maintained on oral medications for congestive heart failure. After seven months of poor but stable cardiac function, he died suddenly during a hospitalization for metabolic decompensation. He was diagnosed with HMG-CoA lyase deficiency as an infant and was on a low-protein diet and L-carnitine supplement. This was given consistently during his first several years. He may not have been compliant in taking carnitine regularly during the several months before the onset of heart failure. All recorded plasma carnitine levels were normal. Autopsy revealed a firm small liver with a nutmeg-like pattern, small kidneys and brain, and marked cardiomegaly. Further studies are pending. Carnitine supplementation in HMG - CoA lyase deficiency is intended to replace carnitine lost as conjugates of hydroxymethylglutaric acid and related compounds, and to maintain a favorable free carnitine/acylcarnitine ratio. Carnitine is standard therapy in other organic acidemias, like propionic acidemia (PA). Cardiomyopathy has occurred several PA patients, many of whom have been on carnitine supplementation, and whose heart failure was not accompanied by systemic metabolic decompensation. Currently, there are no standard cardiac surveillance protocols for organic acidemias. Careful monitoring and prospective studies will be needed to understand the mechanisms involved.

2115/T

Mutation analysis of peroxisome biogenesis disorders (PBD) using targeted massively parallel sequencing. *S. Levesque[1], W. Yang Yik[2], P. Marquis[3], S. Steinberg[4], J. Hacia[2], K. Dewar[3], N. Braverman[1].* 1) Dept. of Human Genetics, Montreal Children's Hospital Research Institute, McGill University, Montreal, Quebec, Canada; 2) Keck School of Medicine, University of Southern California, California, USA; 3) Genome Quebec Innovation Center, McGill University, Montreal, Quebec, Canada; 4) Dept. of Neurogenetics and Neurology, Kennedy-Krieger Institute and the John Hopkins Medical Center, Baltimore, Maryland, USA.

Genetic heterogeneity in the PBD precludes the identification of mutations with reasonable cost using Sanger sequencing in diagnostic DNA labs. Currently, sequencing of selected exons of a subset of PEX genes (PEX gene screen) enables detection of both mutations in 54% of patients. Although complementation studies can identify the defective gene, they remain time-consuming and labor intensive. Massively parallel sequencing technologies can now provide accurate sequences at high throughput with low cost per base, and could permit rapid identification of mutations in PBD patients. **Methods:** In a pilot experiment, singleplex PCR was performed on one patient DNA sample with unknown mutations. 58 amplicons were designed to cover all exons and splice site junctions of 6 PEX genes implicated in >96% of PBD. PCR products were pooled in equimolar quantity, tailed with sequencing primers and a unique barcode by a PCR-based protocol, and finally sequenced using the Roche/454 GS FLX Titanium. To assess sensitivity and specificity for different types of mutations, 39 unique mutations were amplified from multiple patients, pooled and processed as above. NextGENe® software was used for SNP/indel discovery, using an allele frequency cut off of 15% and a minimum of 3 reads for positive calls. **Results:** 93.4% of the sequence reads mapped to the selected genes, and only 5% of amplicons failed to produce sequencing reads. A homozygous non-synonymous mutation was found in the single patient. Sequence analysis of the pooled sample (mean coverage of 343 reads/amplicon) yielded a total of 119 variations, of which 34 were the expected mutations. Sanger traces are currently being reviewed to assess the specificity. Sensitivity was dependent on the mutation type. The detection rate was: 16/17 (94%) for SNPs, 10/13 (77%) for indel of £3bp and 8/9 (89%) for indel >3bp. Large indels up to 91bp were successfully detected given the long GS FLX Titanium reads. **Conclusion:** Our preliminary results showed that mutations in PBD patients can be identified with good sensitivity, including large indels. Further optimisation of coverage is needed to increase sensitivity and an algorithm will be developed to prioritize mutation confirmation. The panel of genes is being increased to all PEX genes and peroxisome single enzymes defects with overlapping phenotypes. This will allow efficient molecular diagnosis of most PBD patients and enable mutations detection in PEX genes not yet implicated in PBD.

2116/T

Pilot Study of Newborn Screening for Adrenoleukodystrophy in Maryland. *G. Raymond[1], W. Hubbard[2], K. Gibbons[1], A. Gifford[1], R. Jones[1], A. Liu[1], A. Kline[3], C. Theda[1,4], P. Donohue[2], W. Golden[2], S. Panny[5], F. Gulamali-Majid[5], A. Moser[1].* 1) Dept Neurogenetics, Kennedy Krieger Inst, Baltimore, MD; 2) Johns Hopkins Hospital, Baltimore, MD; 3) Greater Baltimore Medical Center, Towson, MD; 4) Frederick Memorial Hospital, Frederick, MD; 5) Maryland Department of Health and Mental Hygiene, Baltimore, MD.

Peroxisomal disorders are a genetically diverse group of conditions characterized by biochemical disruption of organelle-specific metabolic pathways. One of the major peroxisomal pathways is beta oxidation of long chain fatty acids and the most common disease involving this pathway is X-linked adrenoleukodystrophy (ALD) resulting from mutations in the ABCD1 gene. ALD has an incidence of approximately 1:17,000 and is characterized by adrenal and neurologic manifestations. Over 90% of affected males will develop primary adrenal insufficiency and 35% of boys will develop inflammatory cerebral demyelination that will result in severe disability and death before the age of ten. Newborn screening through tandem mass spectroscopy allows the detection and intervention in many genetic disorders. We have recently developed a technique that allows diagnosis of peroxisomal disorders using LC-MS/MS from newborn blood spots on filter paper and have demonstrated that the detection of lysophosphatidyl choline (lysoPC) conjugated to very long chain fatty acids is highly accurate and sensitive. We proposed to study the specificity of the testing by evaluating 5000 samples from three hospitals in Maryland. Consent was obtained from the parents for additional testing of the sample. Newborn cards were collected and processed in the standard fashion and sent to the Maryland State laboratory where a punch was obtained and then run on tandem MS to detect the conjugated lysoPC fraction. A follow up protocol was developed for the confirmation of abnormalities found on testing. To date we have consented over 4000 individuals and analyzed 3200 samples without a false positive and to date no affected individuals. While it is presently not certain what effect newborn screening for ALD will have, it is expected that the accurate detection and monitoring of at risk individuals will improve their care and outcomes.

2117/T

Pharmacokinetics and pharmacodynamics of migalastat, a pharmacological chaperone of α-galactosidase A, in healthy volunteers. *D. Greene, A. Bragat, M. Adera, P. Boudes.* Amicus Therapeutics, Clinical Research, Cranbury, NJ 08512.

Background: Fabry disease is a lysosomal storage disorder caused by α-galactosidase A (α-Gal A) enzyme deficiency. 1 Deoxygalactonojirinmycin (DGJ, Amigal, migalastat hydrochloride, AT1001) is currently being evaluated as an oral therapy to treat Fabry disease in patients with responsive mutations in the gene that encodes the lysosomal enzyme α-Gal A. DGJ acts as a pharmacological chaperone for α-Gal A and has been shown to increase α-Gal A activity in vitro, in mouse models of Fabry disease and in Fabry patients. **Objective:** Characterize the pharmacokinetics of DGJ and evaluate the relationship between dose and the effect of DGJ on α-Gal A activity in healthy volunteers. **Methods:** Healthy volunteers were administered single oral doses of DGJ ranging from 25 to 2000 mg in the fasted state; single 100 mg oral doses of DGJ in the fasting state and immediately after a meal; and doses of 50 mg and 150 mg DGJ given twice daily (BID) for 7 days. DGJ concentrations in plasma and urine were measured by liquid chromatography-mass spectrometry. α-Gal A activity in WBC lysates from subjects on the BID regimen was measured using a fluorimetric assay that quantifies catalysis of a surrogate substrate, 4-methylumbelliferone. **Results:** DGJ was generally safe and well tolerated. Bioavailability was high (≥50%). Co-administration of DGJ with food resulted in a 40% reduction in bioavailability. Peak plasma concentrations were measured 2-3 hours after dosing. DGJ plasma levels increased proportionally with dose up to 1250 mg. DGJ was eliminated primarily unchanged by the kidney with an initial plasma half-life of approximately 4 hours. No accumulation was observed after seven days of BID dosing. Seven days of 50 mg and 150 mg twice-daily DGJ resulted in a dose-related increase in WBC α-Gal A activity of up to 1.9-fold of baseline. **Conclusion:** DGJ exhibits favorable pharmacokinetic properties. Renal elimination of DGJ is expected to result in elevated drug concentrations in the kidney, an organ significantly affected by Fabry disease. The DGJ-associated increases of α-Gal A activity in healthy volunteers is consistent with findings in the Fabry mouse model and in Fabry patients with responsive α-Gal A mutations. DGJ should be administered in the fasting state to optimize absorption of the drug. The safety, pharmacokinetic and pharmacodynamic data support the evaluation of DGJ as a potential oral agent for treating Fabry disease in patients with responsive mutations.

2118/T

Association of Methylenetetrahydrofolate reductase genotypes with diabetes mellitus. *K. Nakatani, K. Noma, K. Yasuda, J. Nishioka, T. Nobori.* Molec & Lab Med, Mie Univ Grad Sch Med, Tsu, Japan.

[Objective] Homocysteine (Hcy) is a sulfur-containing amino acid formed during the metabolism of methionine. Elevated levels of Hcy are leading to impaired endothelial through induction of the oxidative stress. The oxidative stress has also been shown to impair insulin action and secretion. Methylenetetrahydrofolate reductase (MTHFR) is an enzyme of to convert Hcy to methionine and its activity is closely related with Hcy levels. Single nucleotide polymorphism (SNP) of MTHFR 677C>T, which is located within the enzyme catalytic domain, has association with MTHFR activity and levels of Hcy. In this study, we investigated association between Hcy, glucose metabolism and MTHFR genotypes. Moreover, whether MTHFR genotypes could be a marker of impaired glucose metabolism. [Subjects and Methods] 451 Japanese volunteers (245 males and 206 females) were recruited in the hospital for medical check. After obtaining the written informed consent of each subject, anthropometry, 75-g oral glucose tolerance test (OGTT), blood pressure determination and biochemical examination were performed early in the morning after fasting overnight. SNP677 of MTHFR gene was genotyped by the single primer extension method. Insulin resistance was assessed using the homeostasis model assessment for insulin resistance (HOMA-R). [Results] (1) Plasma Hcy levels showed significant gender difference (male, female: 10.7±3.9 μmol/L, 8.6±2.0 μmol/L) and further analysis was performed within each gender. (2) Plasma Hcy levels were significantly associated with MTHFR 677TT genotype in both male and female, but no association with adiponectin levels which were associated with insulin resistance. male. (3)Of male subjects with each MTHFR genotypes, no differences were observed in OGTT. However, female subjects with MTHFR 677TT genotype showed significantly elevated plasma glucose after glucose loading in comparison with other genotypes. [Discussion]Insulin resistance was slightly influenced by plasma Hcy concentration but not by MTHFR genotypes. However, MTHFR genotypes were significantly associated with plasma Hcy in both female and male. Moreover, MTHFR genotypes were significantly associated with impaired glucose tolerance in female. Taken together, Hcy could affect glucose metabolism through impaired insulin secretion in female. In conclusion, MTHFR genotype could be a predictive marker of impaired glucose tolerance in female.

2119/T

Urinary Exosomes: Biomarkers for Kidney Disease. *D. Maynard[1], W. Westbroek[1], W. Gahl[1], M. Gunay-Aygun[1,2].* 1) Section on Human Biochemical Genetics, Medical Genetics Branch, NHGRI/NIH, Bethesda, MD; 2) Office of Rare Disease Research, Office of the Director, NIH, Bethesda, MD.

Oral-facial-digital-syndrome type 1 (OFD-1) is an X-linked, male lethal ciliopathy, characterized by malformations of the face, oral cavity and digits and polycystic kidney disease (PKD). Renal cysts in OFD-1 generally originate from the glomeruli; cysts in autosomal dominant polycystic kidney disease (ADPKD) develop from any part of the nephron. Polarity in kidney epithelial cells is essential to the integrity and function of the kidney, since insertion of specific transporters and other proteins into apical membranes lining the renal tubule lumen or basal domains adjacent to the interstitium is critical. Studies of ADPKD renal tubule epithilia demonstrate that defects in membrane polarity affect specific sets of proteins involved in sodium transport and in EGF signal transduction. OFD-1 renal cells have not been studied.

Exosomes are 50-90 nm vesicles containing discrete packets of cytosol and are released by many cell types. Exosome membranes have the same orientation as the plasma membrane. Study of urinary exosomes provides a noninvasive means for detection and analysis of protein-expression/trafficking changes in renal tubule cells. Exosomes from OFD-1 and other PKD patients may contain a different contingent of membrane and/or cytosolic proteins due to protein mis-targeting/polarity defects in renal epithelial cells. We isolated exosomes from the urines of OFD-1 and other ciliopathy patients with PKD and from controls. We removed Tamm-Horsfall protein from the exosome samples and demonstrated by electron microscopy that this technique does not alter their morphology. We used proteomics combined with mass spectrometry to analyze the protein content of the exosomes. Preliminary proteomic results from OFD-1 and control urine samples found known exosome marker proteins (CD63, CD81, and CD9) as well as VPS (TSG101, Alix, and VPS28) proteins involved in multivesicular body (MVB) targeting and biogenesis. Proteins associated with hypertension and PKD or other kidney diseases (aquaporin-2, polycystin-1, -2, podocin, and neprilysin) were also found. These findings establish urinary exosomes as a system that reflects the contingent of proteins present in OFD-1 cells, and that can be investigated in other kidney disorders.

2120/T

Novel therapies for glycogen storage disease type III. *B. Sun[1], K. Boyette[1], B.L. Thurberg[2], P.S. Kishnani[1].* 1) Pediatrics, Duke University, Durham, NC; 2) Genzyme Corporation, Framingham, MA.

Mutations in the glycogen debranching enzyme gene cause glycogen storage disease type III (GSD III), resulting in accumulation of abnormal cytoplasmic glycogen in liver and muscle. Currently, other than symptomatic management of hypoglycemia there is no effective treatment for this disease. In this study, we evaluated the feasibility of using two FDA-approved therapies for other diseases to treat GSD III. 1) Enzyme replacement therapy with recombinant human acid alpha-glucosidase (rhGAA) is an approved therapy for Pompe disease. Based on the role of lysosomal glycogen degradation in general glycogen metabolism, we hypothesize that enhanced GAA enzyme activity will lead to rapid lysosomal glycogen clearance, increase glycogen shuffling from cytoplasm into lysosomes, thus reduce overall cytoplasmic glycogen levels in GSD III. 2) Rapamycin is a specific inhibitor of the mammalian target of rapamycin (mTOR) with clinical applications. mTOR is a key regulator of cell growth, protein synthesis, and glucose and lipid metabolism by interacting with a number of signaling pathways. We hypothesize that inhibition of mTOR signaling by rapamycin will alleviate glycogen accumulation in GSD III by reducing glycogen synthesis and glucose uptake. Primary muscle cultures were used as a cellular model of GSD III to test our hypotheses. Myoblasts were isolated from two GSD IIIa patient muscle biopsies. Pathology of the muscle biopsies was examined by light and electronic microscopy to confirm abnormal glycogen accumulation. Differentiation of myoblasts into mature myotubes was induced in low-serum medium. Fully differentiated GSD III myotubes were treated for 48 hours with 100 μg of rhGAA or 30 nM rapamycin in the culture medium. GAA enzyme activity and glycogen content were analyzed biochemically in these cells. rhGAA reduced glycogen level by 48% and 17% respectively, in cells from the two patients. In contrast, rapamycin reduced glycogen content by 50% and 29% respectively, in the same two patient cells accompanied by increased GAA enzyme activity. We next profiled the expression of 84 key genes involved in glucose and glycogen metabolism pathways using RT-PCR array after rapamycin treatment. The differential expression of some genes was further confirmed by Western blot analysis. In summary, rhGAA and rapamycin significantly reduced glycogen accumulation in GSD III muscle cells. These approaches have great potential for immediate clinical application for GSD III.

2121/T

Novel quantitative virtual microscopy-based method to evaluate GL-3 inclusions in renal peritubular capillaries in patients with Fabry disease. *L. Barisoni[1], R. Colvin[2], C. Jennette[3], R. Gordon[4], J. Castelli[5], S. Sitaraman[5], P. Boudes[5].* 1) Department of Pathology, New York University School of Medicine, New York, NY; 2) Harvard Medical School, Massachusetts General Hospital, Boston, MA; 3) Department of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, NC; 4) Department of Pathology, Mount Sinai School of Medicine, New York, NY; 5) Amicus Therapeutics, Cranbury, NJ.

INTRODUCTION & OBJECTIVES: Renal failure is a major cause of morbidity and mortality in patients with Fabry disease (FD), an X-linked disorder characterized by accumulation of globotriaosylceramide (GL-3) in different tissues. Levels of GL-3 cytoplasmic inclusions in peritubular capillaries (PTCs) of renal biopsies are considered the most useful pathologic measure of therapeutic efficacy. Previous trials of enzyme replacement therapy used a semi-quantitative light microscopy (LM) approach to measure GL-3 inclusions in PTCs; however, limitations in quantifying low levels of GL-3 using this method were identified. Here we describe a novel virtual microscopy (VM) quantitative method to measure GL-3 inclusions in PTCs. METHODS: Renal biopsies were collected from 17 patients with variable FD severity enrolled in Phase 2 studies of AT1001, a pharmacological chaperone in clinical development for the treatment of FD. Biopsy samples were scanned at 100X magnification into whole slide digital images to enable virtual annotation of PTCs and scoring of GL-3 inclusions by three pathologists. For each digitally-imaged biopsy sample, a minimum of 50 PTCs were annotated by one pathologist. The number of GL-3 inclusions within the cytoplasm of each annotated PTC was recorded by the other two pathologists using identical duplicates of the annotated virtual images. The final score for each biopsy sample was calculated as the average number of GL-3 inclusions per PTC. Inter-reader variability was assessed using the method of Bland and Altman. RESULTS: Using the published, semi-quantitative LM method, two pathologists scored 4/17 and 7/17 pre-treatment samples as “0” (using a scale from 0 to 3+), making it difficult to assess the effect of therapeutic interventions. Using the new quantitative VM method, GL-3 inclusions were detected in all samples tested, and the average number of GL-3 inclusions per PTC was calculated. Matched-pairs analysis of scores showed no evidence of systematic bias and indicated low inter-reader variability. CONCLUSIONS: The VM-based quantitative scoring system improves sensitivity of measuring GL-3 inclusions in PTCs. Additional advantages include the ability to annotate images for review, allow multiple pathologists to score the same slides and inclusions from remote locations, and create an auditable archive of samples for clinical trials.

2122/W

Progressive hearing loss in a knock-in mouse model for γ-actin related deafness. *M.C. Drummond[1], M. Zhu[2], I.A. Belyantseva[3], K. Halsey[4], D.F. Dolan[4], S.A. Camper[5], K.H. Friderici[1,6].* 1) Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI; 2) Department of Genetics, Harvard Medical School, Boston, MA; 3) Section on Human Genetics, NIDCD, NIH, Rockville, MD; 4) Kresge Hearing Research Institute, University of Michigan, Ann Arbor, MI; 5) Department of Human Genetics, University of Michigan, Ann Arbor, MI; 6) Department of Pediatrics and Human Development, Michigan State University, East Lansing, MI.

Ten dominant missense mutations in γ-actin (*ACTG1*) have been reported as the cause of hearing loss in DFNA20 families. Although the mutations are located in different functional domains of γ-actin, the end result is a progressive form of non-syndromic sensorineural hearing loss beginning in the high frequencies with an onset in the second to third decade of life. This shared phenotype is indicative of a common functional deficit in mutant γ-actin protein function (Zhu *et al* 2003). To address questions regarding the effects of these mutations on the structure and function of the inner ear and whether these mutations cause hearing loss via a loss of function versus a dominant negative mode of action, we generated a knock-in mouse model for the p.P264L mutation. Mice harboring the p.P264L substitution of ACTG1 in both the heterozygous and homozygous state are born at the expected Mendelian ratio, are viable, and do not have noticeable vestibular deficits. Mice homozygous for the p.P264L allele (normal level of expression) exhibit a high frequency loss by 4-5 weeks and nearly complete hearing loss by 6-7 weeks of age. In the organ of Corti, the hearing loss manifests by a loss of inner and outer hair cells with a base to apical progression. Morphologically, the outer hair cell loss is preceded by degeneration of the two shorter rows of stereocilia. Mice homozygous for a p.P264L allele that expresses only ~20% of the mutant protein exhibit a moderate threshold shift at 6 weeks of age and a similar but less severe phenotype of stereocilia degeneration. Compared to the previously characterized Actg1-null mice which have adult-onset progressive deafness, reduced viability and muscular myopathy (Belyantseva *et al* 2009), the p.P264L mutation produces fewer pleiotropic effects and a profound deafness by 6-7 weeks of age. Taken together, our data suggest a dosage-dependent dominant negative mode of action for p.P264L.

2123/W

Comparison of hearing and vestibular deficits in the *Coch*$^{G88E/G88E}$ and the *Coch*$^{-/-}$ mouse models and correlation with DFNA9 hearing and balance disorder. *N.G. Robertson[1], S.M. Jones[2], S. Given[3], A.B.S. Giersch[1], C.C. Morton[1].* 1) Dept Ob/Gyn & Pathology, BWH & Harvard Medical School, Boston, MA; 2) Dept of Communication Sciences and Disorders, East Carolina University, Greenville, NC; 3) Brody School of Medicine, East Carolina University, Greenville, NC.

Two mouse models have been developed to study the late-onset, progressive, sensorineural hearing loss and vestibular dysfunction known as DFNA9. This disorder results from missense and in-frame deletion mutations in *COCH* (coagulation factor C homology), encoding cochlin, the most abundantly detected protein in the inner ear. We have performed hearing and vestibular analyses by auditory brainstem response (ABR) and vestibular-evoked potential (VsEP) testing of *Coch*$^{G88E/G88E}$ (knock-in) and *Coch*$^{-/-}$ (knock-out) mouse models. In both, at 21 months of age, ABR thresholds were substantially elevated, with all frequencies affected in the *Coch*$^{G88E/G88E}$, but only at the highest frequency in the *Coch*$^{-/-}$ mouse model. Four of 8 *Coch*$^{G88E/G88E}$ mice, and 9 of 11 *Coch*$^{-/-}$ mice had absent ABRs. Interestingly, homozygous *Coch*$^{-/-}$ mice, but not the heterozygotes showed elevation of ABR thresholds, revealing that haploinsufficiency of *Coch* does not appear to affect hearing. In contrast, the heterozygotes of the knock-in mouse model *(Coch*$^{G88E/+}$*)* show ABR threshold elevations similar to that of the homozygotes (*Coch*$^{G88E/G88E}$), an observation in agreement with the autosomal dominant mode of inheritance for DFNA9. Vestibular testing showed elevated VsEP thresholds in *Coch*$^{-/-}$ mice at 13 and 21 months, the two ages tested. For the *Coch*$^{G88E/G88E}$ mice, VsEP thresholds were increased as early as 7 months of age but not at 5 months. These results indicate that in both mouse models, vestibular function is compromised before cochlear function. Analysis and comparison of hearing and vestibular function in these two DFNA9 mouse models, where deficits occur at such an advanced age, provide insight into the pathology of DFNA9 and age-related hearing loss and vestibular dysfunction as well as an opportunity to investigate potential interventional therapies.

2124/W

Sox10 BAC transgenic mouse modeling a complex neurocristopathy, PCWH. *K. Inoue[1], N. Aoyagi-Inoue[1], Y. Itoh[1], Y. Inoue[2], Y. Matsuda[3], M. Inagaki[3], T. Inoue[2], Y. Goto[1], S. Kohsaka[5], C. Akazawa[4].* 1) Dept MR & BD Res, Natl Inst Neurosci, NCNP, Kodaira, Japan; 2) Dept Biochem & Cell Biol, Natl Inst Neurosci, NCNP, Kodaira, Japan; 3) Dept Developmental Disorders,Natl Inst Mental Health, NCNP, Kodaira, Japan; 4) Tokyo Med & Dent Univ, Tokyo, Japan; 5) Natl Inst Neurosci, NCNP, Kodaira, Japan.

SOX10 is a transcription factor that is essential for the neural crest development and the myelin formation both in the central and peripheral nerve systems (CNS and PNS). SOX10 mutations are associated with two distinct neurocristopathies in human, i.e., Waardenburg-Hirschsprung disease (WS4) and peripheral demyelinating neuropathy, central dysmyelinating leukodystrophy, Waardenburg syndrome and Hirschsprung disease (PCWH). PCWH shows more complex and severe neurological phenotype. Clinical and molecular findings suggested that WS4 is caused by haploinsufficiency of SOX10, while PCWH is predicted to result from SOX10 acting as either dominant-negative or gain-of-function allele. Although model animals are available for WS4, no animal models for PCWH are currently present. To determine the molecular mechanisms for PCWH in vivo, we generated BAC-transgenic mice (Tg) carrying a PCWH-causing mutant Sox10. In addition to the circling behavior, hypopigmented coat color, neurosensory deafness, and abnormal enlargement of colon, all of which are characteristics of WS4, the Tg mice also showed abnormal motor coordination, suggestive of neurological abnormalities. Microscopic examinations in the CNS and PNS showed relatively normal migration and differentiation of oligodendrocytes and Schwann cells, but apparently delayed myelination in Tg mice. These findings suggested that the mutant Sox10 Tg mice mimic phenotypes observed in human patients with PCWH and thus serve as a model for PCWH.

2125/W

Alterations in cognition, affect, and neuromotor function implicate roles for *GTF2I* and *GTF2IRD1* in the cognitive and behavioural profile of Williams-Beuren syndrome. *E. Lam[1], E.J. Young[1], L.R. Osborne[1,2].* 1) Institute of Medical Science, University of Toronto, Toronto, Ontario, Canada; 2) Departments of Medicine and Molecular Genetics, University of Toronto, Toronto, Ontario, Canada.

Williams-Beuren syndrome (WBS) is a neurodevelopmental disorder that is associated with mild to moderate intellectual disability (average IQ 55 to 60) and relatively preserved language capabilities but extremely poor visuospatial and math skills. People with WBS are described as over-friendly with a lack of many normal social boundaries, but they also have increased levels of anxiety and specific phobias. WBS is caused by the deletion of approximately 1.55 million nucleotides on a single copy of human chromosome 7q11.23, resulting in the loss of over 25 genes, however, the specific genes that cause the neurological symptoms have yet to be identified. Studies of individuals with atypical deletions of the WBS region suggest that GTF2IRD1 and GTF2I may be involved in many neurological features of the disorder. We previously generated *Gtf2ird1*$^{-/-}$ mice and showed that they have behavioural features similar to the lack of inhibition seen in people with WBS. We have now generated a *Gtf2i*$^{+/-}$ mouse model and a combined-*Gtf2ird1/Gtf2i*-deletion (*Gtf*$^{+/del}$) mouse model where the genomic sequence of both genes has been deleted, to examine the phenotypic effects of hemizygosity for these two genes, either alone or in combination. In a test of spatial learning and memory using the Morris water maze, no significant differences between wildtype and mutant mice were found. The novel object recognition task, a measure of entorhinal cortical function, revealed that *Gtf2i*$^{+/-}$ and *Gtf*$^{+/del}$ mice have impairments in long-term recognition memory. Thus, deficiency of GTF2I protein alters cortically-encoded object memory but not hippocampal spatial memory. Female *Gtf*$^{+/del}$ mice also showed deficits in associative fear conditioned learning and memory, implicating a dysfunction of the amygdala that is consistent with the reduced amygdala activation observed in WBS patients. In rotarod performance, which provides an index of cerebellar-dependent neuromotor function, both *Gtf2i*$^{+/-}$ and *Gtf*$^{+/del}$ animals exhibited a decreased latency to fall as well as deficits in motor learning acquisition. Collectively, these results suggest that hemizygosity for both *Gtf2i* and *Gtf2ird1* affect aspects of cognition and behaviour. It is likely that these genes both contribute to the cognitive, affective, and motor impairments observed in WBS individuals. Additional analyses will help to determine how these proteins effect their influence on cognition and behaviour in rodents.

2126/W

A second-generation mouse model to study cerebral cavernous malformations lesion development. *D.A. McDonald[1], R. Shenkar[2], C. Shi[2], A.L. Akers[1], R.A. Stockton[3], M. Kucherlapati[4], R. Kucherlapati[4], M.H. Ginsberg[3], I.A. Awad[2], D.A. Marchuk[1].* 1) Molecular Genetics & Microbiology, Duke University Medical Center, Durham, NC; 2) Department of Neurosurgery, University of Chicago Hospitals, Chicago, IL; 3) Department of Medicine, University of California, San Diego, La Jolla, CA; 4) Department of Genetics, Harvard Medical School, Boston, MA.

Cerebral cavernous malformations (CCMs) are vascular lesions affecting the central nervous system. Late-stage lesions in humans appear as dilated, multicavernous, blood-filled capillaries lacking structural support. Patients with the disease suffer from recurrent headaches, seizures and hemorrhagic stroke. CCM occurs either sporadically or in a familial, autosomal dominant manner. Inherited forms of CCM are due to heterozygous germline mutations in one of three genes: *CCM1/KRIT1*, *CCM2/MGC4607*, and *CCM3/PDCD10*. Previous studies in late-stage, surgically-resected human CCM lesions found genetic and molecular evidence for a two-hit (germline plus somatic) mutation mechanism underlying CCM pathogenesis. No clinical samples of early-stage human CCM lesions are available, so the mechanisms of lesion genesis and development remain unknown. In order to study CCM pathobiology, we have created a knockout allele of the murine *Ccm1* gene. Mice heterozygous for *Ccm1* do not develop CCM lesions. Based on the two-hit mechanism of pathogenesis, we hypothesized that increasing the somatic mutation rate in these heterozygous mice would increase the chance of somatic mutation of the remaining wild-type allele, and increase the penetrance of the model. The mismatch repair complex gene *Msh2* is required for repair of point mutations and small insertions/deletions, consistent with the types of somatic mutations identified in human CCM lesions. Thus, we crossed *Ccm1* heterozygotes into the mutant background lacking *Msh2*. The sensitized $Ccm1^{+/-}Msh2^{-/-}$ mice show CCM lesions with high penetrance both by MRI and histology. These lesions are not observed in littermates possessing the various control genotypes. Significantly, the CCM lesions identified in these mice range in size from early-stage, single caverns to large, late-stage, multicavernous lesions. This *in vivo* model of CCM will enable us to study the molecular and genetic events that occur in the earliest stages of CCM lesion formation. Future work will use this mouse model to investigate the specific mechanisms that cause lesion genesis and growth as well as possible therapeutic strategies.

2127/W

A zebrafish model of human fetal motoneuron disease. *H.O. Nousiainen[1,2], R.J. White[2], D.L. Stemple[2].* 1) Pub Hlth Genomics, Nat Inst Hlth & Welfare, Helsinki, Finland; 2) Wellcome Trust Sanger Institute, Hinxton, UK.

Motoneurons are affected in several neurological disorders with variable severity and age of onset. The most early-onset forms of motoneuron disease, LCCS (MIM 253310) and LAAHD (MIM 611890), manifest already in utero and are characterized by severe atrophy of spinal cord motoneurons and fetal immobility. Both syndromes are caused by mutations in *GLE1*, which encodes for a protein that is involved in mRNA export, translation initiation, and translation termination. Gle1 knockout (Gle1-/-) zebrafish present with necrosis in the central nervous system at 2 days post fertilization. Acridine orange staining shows significantly more dying cells in the brain and spinal cord of Gle1-/- zebrafish, than in age-matched wild type siblings. The distribution of mRNA within the cells of Gle1-/- zebrafish also differs from controls. Thus Gle1-/- zebrafish provide an excellent model for studying the molecular mechanisms underlying neurodegeneration, and can also provide valuable insights into the pathogenesis of human motoneuron disease.

2128/W

A Novel Systems Biology Pathway Approach Identifying Pathways with Both Genetic and Biological Support in the Etiology of Parkinson's Disease. *Y. Edwards[1], S. Khuri[2], G. Beecham[1], G. Bademci[2], D. Tekin[2], E.R. Martin[1], W.K. Scott[2], Z. Jiang[3], D. Mash[4], J. Ffrench-Mullen[5], M.A. Pericak-Vance[1], N. Tsinoremas[3], J.M. Vance[2].* 1) Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL; 2) Dr. John T. Macdonald Foundation Department of Human Genetics, University of Miami Miller School of Medicine, Miami, FL; 3) Center for Computational Science, University of Miami Miller School of Medicine, Miami, FL; 4) Department of Neurology, University of Miami Miller School of Medicine, Miami, FL; 5) Gene Logic (an Ocimum Biosolutions Company), Gaithersburg, MD.

Parkinson Disease (PD) is a complex neurodegenerative disease for which 6 genome-wide association studies (GWAS) and several gene expression studies have been performed. Despite these efforts, only variants in the microtubule-associated protein tau and α-synuclein genes have been consistently replicated. To improve the utility of these approaches, we applied a unique pathway analysis approach that utilized existing GWAS and gene expression data for PD. The top 5000 SNPs ($p<0.01$) from the GWAS described in Edwards et al (2010) were localized and the genes within a 20Kbp upstream and downstream window for each SNP were identified. Genes were only counted once, so linkage disequilibrium would not be a factor. The gene expression data from Papapetropoulos et al (2006) was sorted and for each of 7 patients and 4 controls one brain region was chosen to represent each of the 5 PD Braak stages. Rather than the traditional gene expression comparison of pooling one anatomical region between patients and controls, we identified genes that were significantly differentially expressed between each adjacent Braak region in each single individual. This adjusts for environmental variation and that all patients are not at the same stage of their disease. The average control expression profile per brain region was then subtracted from the corresponding brain region expression profile of each patient. GOstats was used to examine the pathways in KEGG. Over-represented pathways for the identified genes were calculated using a classical hyper-geometric statistical comparison of a query gene list against a reference gene list. A meta-analysis of the pathway results from GOstats was performed (based on the expression and GWAS results) using the Fisher's combined probability test. Melanogenesis (containing dopamine precursors) was the top overrepresented pathway in the GWAS analysis ($p=7.8E-5$) and axon guidance was the top pathway in the gene expression analysis ($p=7.0E-7$). 13 of the 205 KEGG pathways tested were overrepresented by both the GWAS and expression data; 4 of the top 10 pathways from each approach were identical. The top three pathways in meta-analysis were: axonal guidance ($p=1.4E-9$), focal adhesion ($p=4.2E-8$) and calcium signaling ($p=1.2E-7$). These pathways have both statistical and biological support to be important in PD and provide strong candidates for further evaluation of rare variants and potential therapeutic intervention for PD.

2129/W

Spatiotemporal expression of the NSCLP gene, Crispld2, in neural crest cells and pharyngeal arches during mouse development. *Q. Yuan, E. Swindell, J.T. Hecht.* Pediatrics, Univ Texas Med Sch, Houston, Houston, TX.

Cysteine-rich secretory protein LCCL domain containing 2 (CRISPLD2 in human; Crispld2/Lgl1 in mice) was first identified in mice as a factor that plays a role in early branching morphogenesis of the developing lung and kidney. This gene in human maps to 16q22-24, a chromosomal region identified by genome scan studies as potentially containing a causal gene for nonsyndromic cleft lip with/without palate (NSCLP). Interrogation of candidate genes within this region found an association between CRISPLD2 and NSCLP in our nonHispanic white and Hispanic families. Expression of Crispld2 was detected in the mandible, nasal and palatal regions of E13.5 - E17.5 mouse embryos, which spans critical stages of lip/palatal formation. Our results suggest that Crispld2 is involved in craniofacial development and may be a causal gene for NSCLP. To further examine the role of Crispld2 in craniofacial development, we performed whole mount in situ hybridization to investigate the expression of Crispld2 at early embryonic stages. Expression of Crispld2 was detected in neural crest cells of E9.0 mouse embryos at the midbrain/hindbrain junction, from which the first pharyngeal arch originates. In addition, expression of Crispld2 in the budding first and second pharyngeal arches was observed in E10.0 mouse embryos. These results provide evidence that Crispld2 is involved in the formation of the two arches that are destined to form the oral and nasal structures. This expression data demonstrates that Crispld2 plays an important role in orofacial development.

2130/W

The Human GATA4 G296S Mutation Disrupts Mouse Cardiac Morphogenesis. *C. Misra[1], N. Sachan[3], S. Koenig[1], C. McNally[3], D. Srivastava[4], V. Garg[1,2].* 1) Center for Cardiovascular and Pulmonary Research, Research Institute at Nationwide Children's Hospital, Columbus, OH; 2) Department of Pediatrics, Ohio State University; 3) Department of Pediatrics, University of Texas Southwestern Medical Center, Dallas, Texas; 4) Gladstone Institute of Cardiovascular Disease and Department of Pediatrics, University of California, San Francisco, San Francisco, California.

Cardiac septal defects (CSD) are the most frequent form of congenital heart disease in children, accounting for almost 50% of all cases. We previously reported a heterozygous G296S missense mutation of the transcription factor, GATA4, in a large family with CSD (Garg et al.,2003). In vitro studies demonstrated the mutant GATA4 protein has reduced DNA binding activity and does not interact with the cardiac transcription factor, TBX5. To investigate the molecular and in vivo deficits of this point mutation, we generated a mouse harbouring the same mutation, Gata4 G295S. Homozygous Gata4-null mice have previously been reported to have cardiac bifida and exhibit defects in ventral morphogenesis. These defects are proposed to be due to abnormal Gata4 function in the endoderm. However, mice homozygous for Gata4 G295S have normal ventral body patterning and develop until approximately embryonic day 9.5, after which normal Mendalian ratios are not observed. Homozygous Gata4 G295S embryos dispay a looped heart tube, a single ventricular chamber but have thin ventricular myocardium. Gata4 protein levels are unchanged in the homozygous Gata4 G295S mutant embryos when compared to wildtype littermates. The cardiac abnormalities and the absence of ventral body patterning defects suggest the Gata4 G295S mutant protein can sufficiently activate downstream targets of Gata4 in the endoderm but not mesoderm. Consistent with this, decreased expression of Gata4 target genes in the developing heart was found by RT-qPCR. Decreased cardiomyocyte proliferation was found in the homozygous mutant embryos consistent with the thin myocardium. The Gata4 G295S heterozygous embryos and adult mice are being analyzed for cell proliferation and structural cardiac defects and the data will be presented. In summary, the Gata4 G295S mutant allele functions as a hypomorph in the mesoderm, and disrupts proper cardiac morphogenesis. The phenotype may be due to inability of the Gata4 G295S protein to interact with TBX5 in the heart which has an effect on cardiomyocyte proliferation.

2131/W

Elucidation of Sensors and Effectors in Matrix Equilibrium Derives Novel Therapeutic Strategies for Scleroderma. *E. Gerber[1], D. Huso[2], B. Loeys[3], E. Davis[4], F. Wigley[5], H. Dietz[1, 6].* 1) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Molecular and Comparative Pathobiology, Johns Hopkins University, Baltimore, MD; 3) Center for Medical Genetics, Ghent University Hospital, Ghent 9000, Belgium; 4) Department of Anatomy and Cell Biology, McGill University, Montreal, Quebec, Canada H3A 2B2; 5) Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; 6) Howard Hughes Medical Institute, Chevy Chase, MD.

In the most common and severe presentation of scleroderma, systemic sclerosis (SSc), previously healthy individuals show acquired fibrosis of the skin and viscera associated with the production of autoantibodies. To gain a foothold in the pathogenesis of SSc, we studied a rare inherited form of scleroderma called stiff skin syndrome (SSS) that shows childhood onset of diffuse skin fibrosis. We showed that all patients with SSS have heterozygous missense mutations in the 4th latent transforming growth factor-β (TGFβ) binding protein-like domain of the extracellular matrix protein fibrillin-1. This domain harbors the only Arg-Gly-Asp (RGD) sequence in fibrillin-1 that mediates matrix-cell attachments via integrin bridging. Skin in SSS shows disorganized macroaggregates of fibrillin-1 that fail to contact neighboring cells at the dermal-epidermal junction. Concordant findings were observed in SSc patient skin. These data led to our hypothesis that cells sample the matrix using integrins, that fibrillin-1 is a critical informant regarding the status of the matrix, and that loss of integrin binding to fibrillin-1 results in a failure of communication, context-inappropriate matrix production, and fibrosis. In support of this concept, we now show that mice heterozygous for a SSS-associated mutation (W1570C) or one that substitutes RGE for RGD (leading to an obligate loss of integrin binding) both show rapidly progressive scleroderma, with dramatic thickening of the dermis and complete replacement of subcutaneous fat by collagen by 3 months of age. MicroRNA29 (miR29) emerged as a candidate modulator of matrix synthesis in scleroderma since it suppresses expression of multiple matrix proteins (e.g. types I and III collagens) and has been implicated in cardiac fibrosis. SSc fibroblasts show markedly reduced expression of miR29 and increased expression of miR29 target transcripts; both abnormalities are corrected upon treatment of cells with an integrin β1 activating antibody in a dose-dependent manner. Furthermore, animal models of SSS also show reduced miR29 expression in skin. These data are consistent with a model in which integrins act as sensor and miR29 as effector in the regulation of matrix equilibrium. Perturbation of this axis, as in SSS or SSc, would be sufficient to initiate profibrotic programs. Therapeutic strategies that activate integrins, including antibodies, soluble peptides and nutritional manipulations, are now being tested in SSS mice.

2132/W

Dissecting the role of PORCN in focal dermal hypoplasia or Goltz syndrome. *W. Liu, I. Van den Veyver.* Dept OB/GYN, Baylor Col Med, Houston, TX.

Focal Dermal Hypoplasia (FDH) is an X-linked dominant developmental disorder with typical defects of skin, eyes and skeleton caused by mutations in PORCN. PORCN orthologs in mouse and Drosophila (Porcupine) lipid-modify Wnt proteins, which is essential for their secretion. We therefore hypothesize that PORCN is essential for skin, limb and skeletal development by mediating Wnt signaling and are using two approaches to address this hypothesis. One is to generate and characterize a mouse model with an inactivating mutation in Porcn. In a chimera generated with a Porcn gene-trapped ES cell line, we observed typical skin and distal limb defects, and congenital anomalies of kidneys and internal genital organs, with true hermaphroditism that precluded reproduction. Chimeric phenotypes can be expected for this X-linked dominant mutation and genotyping of affected tissues confirmed the Porcn mutation. This line did not result in germline transmission and we therefore set out to generate a conditional Porcn-mutant mouse; germline transmission has been obtained. Our second approach is to investigate how PORCN overexpression and knockdown affect WNT-signaling in cultured cells. To investigate if PORCN regulates WNT protein secretion, we co-overexpressed WNT3A, Wnt5a or Wnt1 with wild-type (WT) and mutant PORCN in HEK293T cells and assessed the amount of WNT protein in the cells and culture media by Western Blot. Compared to controls, we observed less WNT3A and Wnt5a in cell-extracts and more WNT3A and Wnt5a in the media with overexpressed wild-type PORCN, but not with overexpressed PORCN containing a nonsense mutation, indicating that as expected human PORCN up-regulates WNT3A and Wnt5a secretion. Interestingly, compared to controls, we observed less Wnt1 in cells and less in media with WT PORCN overexpression, while PORCN mutations did not affect Wnt1 secretion. These results indicate that, PORCN affects WNT protein secretion in humans, but the contribution or requirement of this pathway for secretion may vary for individual WNT proteins. We will further study other WNTs with this system and we are also investigating by immunolocalization whether human PORCN knockdown or mutations result in retention of WNTs in the endoplasmic reticulum (ER) and in reduced β-catenin activation. These studies will allow us to study PORCN function, its role in FDH.

2133/W

***C. elegans bbs* mutants display a variety of behavioural and developmental endophenotypes.** *C.A. Mok[1], T. Shekhar[1], M. Zhen[2], E. Héon[1].* 1) Prog Gen & Genome Biol, Sick Kids Hosp, Toronto, ON, Canada; 2) Sumuel Lunenfeld Research Institute, Mount Sinai Hosp, Toronto, ON, Canada.

Ciliopathies are a growing class of pleiotropic and genetically heterogenous disorders where the causative proteins localize to the primary cilia. Bardet-Biedl syndrome (BBS) proteins localize to the base of the cilia and undergo intraflagellar transport. The loss of their activity leads to ciliary dysfunction. The functional conservation of BBS genes in *C. elegans* has made this a useful tool to decipher the biological aspects of BBS. Previous studies revealed that *C. elegans* BBS proteins are present in ciliated neurons and their loss causes neurosensory deficits, manifested by defects in chemotaxis and thermotaxis. We further characterized the *C. elegans bbs* mutant endophenotypes for shared functional, behavioural, and developmental defects. Herein we identified previously unreported *bbs* endophenotypes that include changes to body/cell size, developmental timing, and exploratory behaviour. We observed defects to body size in *bbs* mutants, quantifiable as early as the L4 larval stage, and as late as 72-hours into adulthood. These defects applied to body length and width in *bbs* mutants, with no visible defects to cell count. In examining developmental timing, *bbs* mutant strains exhibited a developmental delay in transitioning between L1 and L4 larval stages. Behaviourally, *bbs* mutants exhibited previously described chemotaxis defects as well as defects to exploratory behaviour. Although *bbs* endophenotypes are likely linked to ciliary dysfunction, the underlying defects to protein localization and trafficking, or signalling pathways remain to be elucidated. These endophenotypes can now be used to further study biological questions relating to this pathology as well as the validation of possible variants linked to known or novel human ciliopathy genes. Furthermore an investigation of these endophenotypes may identify specific or shared interactors or forms of regulation yet to be defined.

2134/W

Intrathecal TPP1 replacement in a canine model of LINCL. *B. Vuillemenot[1], M. Katz[2], J. Coates[2], S. Kanazono[2], P. Lobel[3], I. Sohar[3], S. Xu[3], P. Tiger[1], D. Kennedy[1], D. Musson[1], S. Keve[1], L. Tsuruda[1], C. O'Neill[1].* 1) BioMarin Pharmaceutical Inc., Novato, CA; 2) University of Missouri, Columbia, MO; 3) Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, NJ.

Late infantile neuronal ceroid lipofuscinosis (LINCL) is a lysosomal storage disorder caused by mutations in the gene encoding tripeptidyl peptidase1 (TPP1). LINCL patients display accumulation of lysosomal storage materials in the brain and other organs accompanied by CNS degeneration and neurological decline. The LINCL dachshund model is a spontaneously occurring mutant that lacks expression of TPP1 and recapitulates many aspects of the human disease. The objective of this study was to determine if intrathecal (IT) TPP1 enzyme replacement attenuates the accumulation of storage materials and disease progression observed in TPP1-/- dachshunds. An additional goal was to determine the CNS distribution of TPP1 activity after IT administration. TPP1 was administered to one TPP1-/- and one homozygous wild type (WT) animal. An additional TPP1-/- and WT animal each received vehicle. Four IT administrations via the cisterna magna of 32mg TPP1 formulated in 2.3mL of artificial cerebrospinal fluid (CSF) or vehicle were administered monthly from four to seven months of age. Retinal electrophysiology, cognitive testing, and neurologic and ophthalmic examinations were performed throughout the study to measure functional changes. Animals were euthanized 48 hours after the fourth administration. Tissues from different areas of the CNS were analyzed for TPP1 activity and autofluorescent storage materials. TPP1 activity was distributed at greater than WT levels in many areas of the CNS of the TPP1-/- animal administered TPP1, with higher concentrations in areas proximal to CSF flow, especially those downstream from the injection site. There was a decrease in autofluorescence in CNS tissues correlating with TPP1 activity level. Systemic TPP1 exposure, peaking at one hour post dose, was also observed. No improvement was observed in neurologic, cognitive, or ophthalmic function in this animal compared to a vehicle treated TPP1-/- control. These results demonstrate the biochemical activity of IT administered TPP1 in an animal model of LINCL. Further studies with this model are in progress to optimize the delivery route and dosing regimen to attenuate neurological decline.

2135/W

Conditional knockout mouse model for the *Fxr2* gene. *S.S. Pataskar[1], B.A. Oostra[2], D.L. Nelson[1].* 1) Molecular & Human Genetics, Baylor College of Medicine, Houston, TX.USA; 2) CBG Department Of Clinical Genetics, Erasmus University, Rotterdam, The Netherlands.

Fragile X Syndrome is a common form of intellectual disability caused by the absence of Fragile X Mental Retardation (FMR1) protein, FMRP. FXR1P and FXR2P (Fragile X Related Protein) are paralogs of FMRP. The three proteins share high sequence similarity, functional similarity and show overlapping tissue distribution. FXR2P is also highly expressed in brain and testis. FXR1P and FXR2P provide functional redundancy to FMR1P and may mask some phenotypic consequences of loss of function mutations in mice and humans. To unravel the biological mechanisms involved in the normal and abnormal functioning of Fragile X associated proteins, we are developing knockout mice lacking each of the genes and combinations including mutations that can be conditionally expressed via Cre/loxP system. A conditional knockout allele for the mouse *Fxr2* gene (cKO*Fxr2*) was created by engineering loxP sites flanking its exon 7. Heterozygous conditional cKO*Fxr2* mice were obtained by crossing cKO*Fxr2* mice with mice expressing neuron specific Cre recombinase (*Nse-cre*). Pups homozygous for the cKO*Fxr2* allele and also for *Nse-cre* transgene were analyzed for *Fxr2* expression. RT PCR analysis confirmed deletion of exon 7 in *Fxr2* transcript from brain tissues of these mice whereas no such ablation was observed in *Fxr2* transcript from testis. Immunohistochemical staining of brain tissue sections from these mice showed absence of FXR2P especially in hippocampus, thalamic and hypothalamic regions of the brain. A similar staining of tissue sections from testis showed presence of FXR2P in levels comparable to those in the testis of control cKO*Fxr2* mice without *Nse-cre*. We have also crossed cKO*Fxr2* mice with mice expressing *Hprt-Cre* recombinase which is expressed ubiquitously. RT PCR analysis confirmed deletion of exon 7 in *Fxr2* transcript from tissues like brain, liver, kidney and testis from mice homozygous for the cKO*Fxr2* allele and for *Hprt-Cre* transgene. We are in the process of crossing cKO*Fxr2* mice with those carrying cKO*Fxr1* and cKO*Fmr1* alleles. We expect to examine circadian rhythm activity patterns of these mice along with other behavioral phenotypes in animals where the *Fxr2* gene has been ablated in specific tissues individually and in combination with that of *Fxr1* and *Fmr1* genes.

2136/W

Identification of candidate regions for phenotype modifier gene in a new mouse model for Marfan syndrome. *G.R. Fernandes[1], B.L. Lima[1], S.M.G Massironi[2], L.V. Pereira[1].* 1) Departamento de Genética e Biologia Evolutiva, Instituto de Biociências ,USP; 2) Departamento de Imunlogia, Instituto de Ciências Biomédicas, USP .

Marfan Syndrome (MFS) is an autossomal dominant disorder associated with pleiotropic manifestations affecting the skeletal, ocular and cardiovascular systems. Despite presenting complete penetrance, MFS displays a wide clinical variability even within family members carrying the same mutation. Our group has developed a new mouse model, in which the Fbn-1 gene had the exons 19-24 replaced by a neomycin-resistance expression cassette flanked by LoxP sequences. Heterozygous mice developed some of the human phenotypes across the skeletal and cardiovascular systems, and lung manifestation (emphysema). Moreover, the onset of the phenotypes varies according to the isogenic background in which the mutation is present: in the 129/Sv strain manifestations are present as early as 3 months of age, while in the C57/BL6 animals will present the phenotypes only at 6 months of age. Therefore, this model is capable of reproduce the clinical aspects and variability observed in humans, and are an experimental system in which to identify modifiers genes of MFS. Here we report the initial results from the screening for modifier genes in both strains that control the MFS phenotypes. For this purpose, skeletal, lung and vascular phenotypes of 3-month-old heterozygous 129xC57 F2 mice were quantified, and the 10 most and less affected animals for each phenotype were genotyped along the genome with a 64 microssatelite set. Statistical analyses were made using the R/qtl package and the Minitab software. The analysis of the isogenic animals indicated that, independent of the onset and severity of disease, there is a significant phenotypic correlation, so a more severe skeletal phenotype is accompanied by a severe lung and cardiovascular phenotypes (0.70 £ρ£0.89). This correlation is not observed among the phenotypes of F2 mice (|ρ|£0.2), indicating that each system must be influenced by particular modifying factors. So far the screening has identified 4 suggestive (P<0.63) regions in chromosomes 3, 4, 6 and 14 controlling the skeletal phenotype; and one region on chromosome 18 that affects the aortic phenotype. Our results indicate the existence of at least 5 different modifiers genes controlling two distinct MFS phenotypes.

2137/W

A mutation in *Srebp2* leads to cataract formation in *lop13* mice. *K.M. Merath[1], R.R. Dubielzig[2], B. Chang[3], D.J. Sidjanin[1].* 1) Cell Biology, Medical College of Wisconsin, Wauwatosa , WI, 53226; 2) School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI , 53706; 3) The Jackson Laboratories, Bar Harbor, ME, 04609.

Cataracts are the leading cause of blindness worldwide and in order to develop novel therapies, the identification of the underlying molecular events participating in the development of cataracts is essential. The focus of this research is on the identification of the gene and associated molecular pathways that lead to autosomal recessive cataracts in the lens opacity 13 (*lop13*) mice. The *lop13* mutation arose spontaneously and the mice exhibit nuclear cataracts and fissures of the skin surrounding the eyes and the back of the neck. F2 mice were generated and genotyped and the linkage map established the *lop13* critical region as being between D15Mit118 and D15Mit33 on mouse chromosome 15. All 35 genes within the critical region were sequenced and the region was further narrowed to 520 Kb between *Csdc2* and Cyp2d9. A single C®T base pair substitution was identified in nucleotide 3112 of Sterol Regulatory Element Binding Protein 2 (*Srebp2*). This mutation results in the substitution of an evolutionarily conserved arginine to cysteine at amino acid 1038. To provide further genetic proof that the identified mutation was disease causing, mice were generated that carried a gene trapped *Srebp2* allele and these mice were then bred to the *lop13* mouse. The compound heterozygote mice exhibited cataracts and skin fissures, thus confirming that the disease causing mutation in the *lop13* mice is Srebp2^{Arg1038Cys}. Srebp2 plays an essential role in activating the transcription of several genes involved in the cholesterol biosynthesis pathway. Although *SREBP2* has been extensively studied, this gene has not been previously linked to cataract formation. Studies are currently in progress to evaluate the role of Srebp2^{Arg1038Cys} in maintaining the homeostasis of cholesterol within the lens.

2138/W

Lens opacity 11 (lop 11) is a loss of function mutation in Hsf4. *A. Ronchetti, B. Endres, D. Sidjanin.* Cell Biology, Neurobiology, and Anatomy, Medical College of Wisconsin, Milwaukee, WI.

Lens opacity locus 11 (*lop11*) is a spontaneous mouse mutant that exhibits autosomal recessive cataracts. In our previous study we identified an insertion of an early transposable element (ETn) in intron 9 of the *Hsf4* gene as responsible for the *lop11* mutation. The ETn insertion altered splicing of the *Hsf4* transcript following exon 9, resulting in a premature stop codon and a loss of exons 10-13. Although we were able to detect truncated $Hsf4^{lop11}$ protein in *lop11* lenses, the molecular consequences of the ETn insertion on the normal function of the Hsf4b protein and *lop11* cataract formation have not yet been investigated. We initially evaluated if *lop11* is a loss of function mutation due to the reduced protein stability of $Hsf4^{lop11}$. No difference between the stability of wt Hsf4 and $Hsf4^{lop11}$ proteins was identified. Western blot analysis showed presence of both the wt Hsf4 and $Hsf4^{lop11}$ in cytosolic and nuclear protein extracts. EMSA using nuclear extracts showed that $Hsf4^{lop11}$ abrogated binding to the heat shock element (HSE). Luciferase assay confirmed abolished ability of $Hsf4^{lop11}$ to bind to HSE. It has been previously shown that wt Hsf4 protein can bind to HSE only as a trimer. Native gel electrophoresis showed a lack of trimer formation for $Hsf4^{lop11}$. These findings suggest that in *lop11* a loss of the Hsf4 C-terminal end results in disrupted trimerization and abolished binding to HSE. A loss of Hsf4 function in the *lop11* lenses was further evaluated. Between P10-12 in *lop11* lenses the vacuole formation was noted between cortical fiber cells. Prior to P10 *lop11* lenses exhibited persistent nuclei in fiber cells. No differences were observed in the proliferation rate between wt and *lop11* lenses. These findings suggest that in lop11 a loss of Hsf4 function most likely results in a defect in fiber cell maturation and denucleation that ultimately leads to vacuole formation and cataracts.

2139/W

Animal modeling of TARP syndrome: Knocking down and knocking out *Rbm10*. *J.J. Johnston[1], K.S. Bishop[3], E.J. Spaulding[1], J.K. Teer[1, 2], P.F. Cherukuri[1, 2], N.F. Hansen[2], S.K. Loftus[1], K. Chong[4], J.C. Mullikin[2], L.G. Biesecker[1, 2].* 1) Genetic Disease Research Branch, NHGRI, Bethesda, MD; 2) NIH Intramural Sequencing Center, Bethesda, MD; 3) Zebrafish Core Facility, Genetics and Molecular Biology Branch, NHGRI, Bethesda, MD; 4) Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital, Toronto, ON, Canada.

Micrognathia, glossoptosis, and cleft palate comprise a common malformation, Robin sequence. It is a component of the TARP syndrome, which comprises Talipes equinovarus, Atrial septal defect, Robin sequence, and Persistent left superior vena cava. This disorder is X-linked with apparently 100% pre- or postnatal lethality in affected males. We have previously characterized two families with TARP, performed massively parallel sequencing of X chromosome exons, filtered the results, used a unique algorithm to characterize variants, and showed that TARP is caused by truncating mutations in *RBM10*, which encodes RNA binding motif 10. These are the only known human mutations in this gene and there are no recognized animal models of this disorder. We hypothesize that *RBM10* dysregulates other developmental genes that cause other forms of Robin sequence or clefting. To better define the role of *RBM10* in development, we proposed to use zebrafish as a model organism. We designed a total of 3 splice morpholinos targeting zebrafish *rbm10*. Fish were collected at 6-8 dpf for alcian blue staining of cartilage to identify abnormal jaw development. The first morpholino yielded a phenotype of mandible underdevelopment, consistent with the human phenotype. The second and third morpholinos did not yield a recognizable phenotype. Only 1 of the 3 morpholinos blocked splicing as shown with RT-PCR analysis from 3 dpf fish. We hypothesized that maternal transcripts may rescue the embryos and worked toward a translation blocking morpholino. As the 5' end of the zebrafish transcript was incomplete, we used 5'-RACE to identify alternative splicing with 2 alternative translation start sites (equally represented in cloned products). Due to the complexity of the 5' end of the transcript and the negative result from the splice morpholinos we concluded that the fish is not a useful model for TARP syndrome. Concurrently, we have identified two mouse *Rbm10* knockout ES cell lines (*Rbm10*$^{tm1a(KOMP)Wtsi}$, *Rbm10*$^{GT(CSI176)Byg}$), which we obtained and are working towards creating transgenic lines.

2140/W

Modeling Mental Retardation in Cornelia de Lange Syndrome (CdLS) with *Drosophila melanogaster*. *I. Krantz[1], J. Li[2], D. Xu[3].* 1) Dept Pediatrics/Human Gen, Children's Hosp Philadelphia, Philadelphia, PA; 2) School of Arts and Sciences, University of Pennsylvania, Philadelphia, PA; 3) Division of Human Genetics, The Children's Hospital of Philadelphia, Philadelphia, PA.

Cornelia de Lange Syndrome (CdLS) is a dominant genetically heterogeneous diagnosis characterized by a striking constellation of cognitive impairment, growth delay and birth defects. Approximately 65% of CdLS probands have an identifiable mutation in cohesin structural (*SMC1A*, *SMC3*) or regulatory (*NIPBL*) genes. Cohesin has been well characterized for its role in sister chromosome cohesion during mitosis, however we, and others, have described a non-canonical role for cohesin as a critical regulator of gene expression, that is most likely the mechanism by which it manifests its phenotypic effect in CdLS. Homozygous mutation of cohesin factors in *Drosophila* (and other organisms) is embryologically lethal, while heterozygous loss has minimal known phenotypic effect. Targeted homozygous mutation of cohesin genes or disruption of cohesin structure has been shown to cause failure of γ-neuron pruning, a post-mitotic event, during *Drosophila* brain development. It remains unclear if loss of one copy of these cohesin genes, more closely representing the gene dosage in CdLS probands, also causes any structural or functional brain differences in *Drosophila*. We investigated brain development in *Drosophila* heterozygous mutants of cohesin structural and regulatory genes and observed γ-neuron pruning defects in mushroom bodies during the pupal stage. γ-neurons failed to be pruned at 20 hours after pupae formation in approximately 30% of the *SMC1*, *Rad21*, and *Nipped-B* heterozygous mutant pupae. This indicates that heterozygous cohesin mutations do exert an effect on normal brain development in the fly, although the effect is not fully penetrating. Parallel genome-wide expression analysis of the brain of these mutant flies will provide molecular insight into the effects of haploinsufficiency of cohesin genes. These experiments will be very important in understanding the alterations of the transcriptome in the *Drosophila* brain and how that relates to the cognitive status resulting from haploinsufficiency. These and ongoing learning/memory behavior studies will help to validate if the heterozygous cohesin mutant flies resemble CdLS probands with respect to the cognitive phenotype and can serve as a valuable model to study the neurocognitive aspects of CdLS.

2141/W

The first OPA1 mouse model of Dominant Optic Atrophy "plus phenotype" reveals the pathophysiological mechanism of this mitochondrial disease. *E. Sarzi[1], A. Benyagoub[1], C. Prouteau[2], N. Gueguen[2], V. Rigau[3], C. Delettre[1], P. Brabet[1], C. Hamel[1], P. Reynier[2], G. Lenaers[1].* 1) INSERM U583, Institute for Neurosciences, Montpellier, France; 2) Département de Biochimie et Génétique, CHU Angers - Inserm U694, 4 rue Larrey, 49933 Angers Cedex 9, France; 3) Service d'Anatomopathologie, Hôpital Saint-Eloi, 34090 Montpellier.

Dominant Optic Atrophy (DOA) is an inherited mitochondrial disease mainly caused by mutations in the OPA1 gene, encoding a dynamin-related GTPase involved in mitochondrial fusion, cristae organization and control of apoptosis. DOA is characterized by visual failure marked by a loss of Retinal Ganglion Cells (RGCs) which axons form the optic nerve. Recently, thorough characterization of DOA patients previously thought to be non-syndromic revealed neuromuscular involvements. We have generated a new Opa1 mouse model carrying the c.2708delTTAG mutation in Exon27, the most frequent mutation found in patients with DOA (30% of all cases). Firstly, using molecular, electrophysiological (Electroretinogram (ERG) and Visual Evoked Potentials (VEP)) and immuno-histological studies, we characterized the visual function of this mouse. Secondly, we evaluated the pathophysiological mechanism in the retina and optic nerve by dosage of the respiratory chain complex activities and by observation of the mitochondrial network structures with fluorescent and electron microscopy. Lastly, we studied peripheral organs such as skeletal muscles looking for potential neuromuscular defects. We show that from 6 months, heterozygous Opa1 animals present altered visual function, with significant delay in the VEP latencies, indicative of impaired optic nerve activity. We also demonstrated a loss of the RGCs, hallmark of DOA. In addition, prior to the appearance of the visual defect, we detected an isolated complex IV deficiency of the mitochondrial respiratory chain specifically in retina, while this parameter remains normal in the optic nerve and brain. Mitochondrial distribution and structure are also affected early in RGC soma and around the lamina cribosa, where axons get myelinated. Moreover, we also found a complex IV deficiency in muscle confirmed by the presence of COX negative fibres characteristic of mitochondrial myopathies. For the first time, we have generated a mouse model of Dominant Optic Atrophy with a DOA "plus phenotype" showing a specific deficiency of the mitochondrial respiration in the retina and skeletal muscle. In addition to being a more appropriate model for studying the pathophysiology of DOA, this mouse model represents a precious tool for better understanding mitochondrial diseases.

2142/W

Pleiotropic Quantitative Trait Loci (QTL) Influence Phenotypes in the ATP8B1-Deficient Mouse. *L. Bull[1], U. Sanford[1], S. Shah[1], J. Robinson[1], C. Paulusma[2], J. Grenert[1,3], A. Knisely[4], R. Oude Elferink[2], S. Sen[1,5].* 1) Liver Center, University of California San Francisco, San Francisco, CA; 2) Tytgat Institute for Liver and Intestinal Research, Academic Medical Center, Amsterdam, The Netherlands; 3) Dept of Pathology, University of California San Francisco, San Francisco, CA; 4) Institute of Liver Studies, King's College Hospital, London, United Kingdom; 5) Department of Epidemiology and Biostatistics, University of California San Francisco, San Francisco, CA.

Background: ATP8B1-deficient (*Atp8b1*$^{-/-}$) mice serve as a model of FIC1/ATP8B1 deficiency in humans. Phenotypes in the *Atp8b1*$^{-/-}$ mouse are influenced by strain background. To map QTL modifying phenotypes in *Atp8b1*$^{-/-}$ mice, we performed genetic mapping studies using mice of C57Bl/6J (B6) and 129S4 backgrounds, and both backcross and intercross strategies. Methods: *Atp8b1*$^{-/-}$ mice (121 backcross, 245 intercross) were phenotyped: At age ≥3 months, mice were fed a diet supplemented with 0.5% cholate (a bile acid) for 6 days. Mice were then fasted for ≥4 hours, and serum, gallbladder bile, and liver and intestinal tissue collected at sacrifice. DNA samples were genotyped for 242 informative markers; serum collected before and after diet administration was assayed for cholesterol, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and bilirubin; and concentrations of cholesterol, phospholipid and bile salts in bile were measured. We have performed QTL analysis for 14 traits to date, using J/qtl. Pleiotropic QTL were defined as regions linked to ≥4 traits, including at least 1 trait that reached genomewide significance (established using 10,000 permutations), with remaining traits attaining pointwise significance. Results: Four genomic regions contain pleiotropic QTL. The phenotypes influenced vary between QTL, but in total encompass: initial and final serum cholesterol, ALP, AST, and ALT; final total serum bilirubin; rate of weight loss on cholate diet; liver: body weight ratio; and biliary phospholipid concentration. For 3 of the 4 pleiotropic QTL, serum cholesterol (initial or final) yielded the most significant evidence of linkage. B6 alleles were usually associated with greater phenotypic abnormalities than were 129 alleles. Conclusion: We have genetically mapped 4 pleiotropic modifier QTL of ATP8B1 deficiency in the mouse. The sequence variants underlying these loci likely influence processes central to manifestation of ATP81 deficiency. Their identification will enhance our understanding of the role of ATP8B1, and the human orthologs of genes at these loci may be modifier genes in human ATP8B1 deficiency.

2143/W

A53T-alpha-synuclein overexpression impairs dopamine signaling and striatal synaptic plasticity in old mice. *S. Gispert Sanchez[1], A. Kurz[1], K.L. Double[2], I. Lastres-Becker[1,10], A. Tozzi[3,9], M. Tantucci[3], V. Bockhart[4], M. Bonin[5], M. Garcia-Arencibia[6], J. Fernandez-Ruiz[6], M. Gerlach[7], U. Wüllner[8], H. Lüddens[4], P. Calabresi[3,9], G. Auburger[1].* 1) Dept Neurology, Frankfurt Univ, Frankfurt, Frankfurt, Germany; 2) Prince of Wales Medical Research Institute and the University of New South Wales, Randwick, Sydney, Australia; 3) Clinica Neurologica, Università di Perugia, Ospedale S. Maria della Misericordia, Via S. Andrea delle Fratte, Perugia, Italy; 4) Dept. Psychiatry and Psychotherapy, University Medical Center Mainz, Germany; 5) Dept. Med. Genetics, University of Tübingen, Tübingen, Germany; 6) Dept. of Biochemistry and Molecular Biology and "Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED)", Faculty of Medicine, Complutense University, Madrid, Spain; 7) Laboratory for Clinical Neurochemistry, Dept. Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, Bayerische Julius-Maximilian-Universität, Würzburg, Germany; 8) Dept. Neurology, Rheinische Friedrich Wilhelms Universität, Bonn, Germany; 9) Fondazione Santa Lucia I.R.C.C.S. -C.E.R.C., European Brain Research Institute, Roma, Italy; 10) Departamento de Bioquímica, Instituto de Investigaciones Biomédicas "Alberto Sols", and Centro de Investigación en Red en Enfermedades Neurodegenerativas (CIBERNED), Madrid, Spain.

Alpha-synuclein was implicated in Parkinson's disease when missense mutations in the alpha-synuclein gene were found in autosomal dominant Parkinson's disease and alpha-synuclein was shown to be a major constituent of protein aggregates in sporadic Parkinson's disease and other synucleinopathies. We have generated two mouse lines with overexpression of A53T-SNCA in nigrostriatal neurons which show a progressive reduction of spontaneous movement in absence of neurodegeneration. Now we studied striatal dysfunction to understand early disease mechanisms. Analysis of the neurotransmitter content demonstrated that dopamine (DA) levels correlated directly with the level of expression of SNCA, an observation also made in SNCA-deficient (knockout, KO) mice. However, the elevated DA levels in the striatum of old A53T-SNCA overexpressing mice are not transmitted appropriately since the DA degradation enzyme COMT showed reduced expression and DA receptors detected by immunoblots and autoradiography were upregulated. Extensive transcriptome studies via microarrays and quantitative real-time RT-PCR (qPCR) of altered transcript levels of the DA-inducible genes Atf2, Cb1, Homer1 and Pde7b indicated a progressive and genotype-dependent reduction in the postsynaptic DA response. As a functional consequence, long term depression (LTD) was absent in corticostriatal slices from old transgenic mice. In conclusion, we observe a dysfunction of dopaminergic neurotransmission and impaired synaptic plasticity in the A53T-SNCA overexpressing mice, reflecting early changes within the basal ganglia prior to frank neurodegeneration.

2144/W

A new mouse model for Marfan Syndrome present phenotypic variability associated to the genetic background, and to the overall levels of Fbn1 expression. *BL. Lima[1], E. Santos[1], G. Ribeiro[1], C. Merkel[1], M. Soukoyan[1], A. Kerkis[1], S. Massironi[2], J. Visintin[3], LV. Pereira[1].* 1) Departamento de Genética, Instituto de Biociências - USP, São Paulo, Brazil; 2) Departamento de Imunologia, Instituto de Ciências Biomédicas - USP, São Paulo, Brazil; 3) Departamento de Reprodução Animal, Faculdade de Veterinária e Zootecnia - USP, São Paulo, Brazil.

Marfan syndrome is an autosomal dominant disease of connective tissue caused by mutations in the fibrillin-1 encoding gene FBN1. Patients present cardiovascular, ocular and skeletal manifestations, and although being fully penetrant, MFS is characterized by a wide clinical variability both within and between families. Here we describe a new mouse model of MFS that recapitulates the clinical heterogeneity of the syndrome in humans. Heterozygotes for the mutant allele mgΔloxPneo, the same internal deletion of exons 19-24 of mgΔ mouse model, present defective microfibrillar deposition, emphysema, deterioration of aortic wall and kyphosis. However, the onset of the phenotypes is earlier in the 129/Sv than in C57BL/6 background, indicating the existence of genetic modifiers of MFS between these two mouse strains. In addition, we characterized a wide clinical variability within the 129/Sv congenic heterozygotes, indicating the influence of epigenetic factors in disease severity. Finally, we show a strong correlation between the overall Fbn1 expression and the severity of the phenotypes, i.e., mice with lower expression of Fbn1 gene tend to have more severe phenotypes in the three affected systems, supporting the hypothesis of the protective role of normal fibrillin-1 in MFS pathogenesis.

2145/W

Access to Mouse Models of Human Disease. *S. Rockwood, M. Sasner, C. Lutz, S. Murray, C. Heffner, L. Donahue.* Genetic Research Science, Jackson Lab, Bar Harbor, ME.

Access to model organisms is of paramount importance to advancing the research objectives of the bio-medical research community. To facilitate this access, the Mouse Repository at The Jackson Laboratory has served as a centralized resource that distributes and preserves high health status mouse models of human disease for over a half century. Hundreds of new strains are added each year to one of the largest collections of characterized mouse strains available. Advances in recent years have lead to increasing numbers of models in several notable categories. **Micro RNA mutants.** Mouse mutants involving microRNAs represent a rapidly growing subset of the Repository. The bic/miR-155 knockout mice can be used to assess the role of miR-155 and its target genes (including cytokines, chemokines, and transcription factors) in homeostasis and regulation of the immune system. miR-17~92 cluster overexpression is associated with human lymphomas and other cancers, autoimmune defects, and altered expression of tumor suppressor and pro-apoptotic genes. **Alzheimer & Parkinson's Models.** Specific sub-repositories have been formed for both disease areas, with some aged models available. **Cre-expressing models.** Equally important are the Cre recombinase-expressing strains that are used in conjunction with floxed mutants. Because most existing Cre strains have not been fully characterized and 'ectopic' expression of Cre recombinase can confound analysis, we have undertaken a project to characterize Cre recombinase activity in embryonic and adult mouse tissues. Results are displayes in a database including images and expression patterns annotated with terms from the Mouse Anatomical Dictionary. **Tool Strains.** These strains allow the detection of recombinase activity, permit tissue specific expression of fluorescent proteins, make possible tissue specific ablation and make accessible recent advances in iPS technology. Although each mouse model may differ in its scientific application, each shares similar operational aspects in that each is part of the Repository's quality assurance program. The Repository maintains a searchable online resource (www.jaxmice.jax.org/query). Researchers wishing to have strains considered for inclusion in the Repository may submit their strains to: (www.jax.org/grc/index.html). This project has been supported by NCRR (RR09781, RR11083, RR16049), NIA, HHMI, The Ellison Medical Foundation and from several private charitable foundations.

2146/W

A VCP R155H Knock-In Mouse Model exhibits progressive muscle, bone and brain pathology typical of human IBMPFD Syndrome. *M. Badadani[1], A. Nalbandian[1], J. Vesa[1], G. Watts[2], D. Eric[1], V. Caiozzo[3], D. Wallace[1], V. Kimonis[1].* 1) Pediatrics, Univ California at Irvine, Irvine, CA; 2) School of Medicine, Health Policy and Practice, University of East Anglia, Norwich, Norfolk, UK; 3) Department of Orthopedic Surgery, University of California, Irvine, CA, USA.

Dominant mutations in the valosin containing protein (VCP) gene cause inclusion body myopathy associated with Paget's disease of bone and frontotemporal dementia (IBMPFD) is characterized by progressive muscle weakness, dysfunction in bone remodeling, and frontotemporal dementia. VCP plays an essential role in the endoplasmic reticulum associated degradation of proteins by binding to ubiquitinated substrates and transferring them to the 26S proteasomes of the ubiquitin proteasome system. To elucidate the pathological mechanisms underlying the disease progression of IBMPFD, we generated a knock-in mouse model with the R155H mutation and analyzed the progression of disease up to 15 months of age. Muscle strength measurements demonstrated progressive muscle weakness in mutant mice starting before the age of 6 months and reaching the 17.9% decrease by 15 months of age. Motor performance, analyzed by the rotarod analysis showed progressive decline beginning at 3 months of age and reaching 22% decline by the age of 15 months. Histological analyses of mutant muscle performed by hematoxylin and eosin staining, and electron microscopy showed vacuolization of myofibrils, centrally located nuclei, and disorganized muscle fibers. Immunohistochemical analyses of the quadriceps muscle tissues from mutant mice showed accumulation of TDP-43- and ubiquitin-positive inclusion bodies in 4% of quadriceps myofibrils. LC3 staining of muscle sections showed increased number of autophagosomes, and increased apoptosis was demonstrated by elevated Caspase-3 activity (2.8-fold increase) and increased number of TUNEL-positive nuclei in mutant quadriceps. X-ray microtomography (uCT) images show radiolucency of distal femurs and proximal tibiae in knock-in mice and uCT morphometrics shows decreased trabecular pattern and increased cortical wall thickness. Bone histology and bone marrow derived macrophage cultures in these mice revealed increased osteoclastogenesis observed by TRAP staining suggestive of Paget bone disease. Moreover homozygous mice die by 21 days and show increased number of vacuoles and central nuclei within quadriceps, and dysregulation of cardiac myofibers and hepatocytes. In conclusion, the R155H knock-in generated mouse model replicates human disease, and therefore, can be used to clarify the mechanisms causing IBMPFD and develop novel therapies for this important disease.

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Mutations in *RP1* cause dominant retinitis pigmentosa via a dominant-negative mechanism. *E.A. Pierce[1], A. Saveliev[1], R.W.J. Collin[2], I. van den Born[3], Q. Liu[1].* 1) FM Kriby Center for Ophthalmology, Univ Pennsylvania School of Medicine, Philadelphia, PA; 2) Department of Human Genetics and Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands; 3) The Rotterdam Eye Hospital, Rotterdam, The Netherlands.

The inherited retinal degeneration retinitis pigmentosa (RP) is a common cause of vision loss. Mutations in the retinitis pigmentosa 1 (*RP1*) gene are a common cause of autosomal dominant RP (adRP), and have also been found to cause recessive RP. The RP1 protein is a photoreceptor-specific microtuble-associated protein (MAP) that is required for normal organization membrane discs in the light-sensitive photoreceptor sensory cilia (PSCs; also called outer segments) of rod and cone cells. It is not currently understood how mutations in *RP1* lead to photoreceptor degeneration. **Methods and Results:** To ask if haploinsufficiency of *RP1* can cause adRP, we evaluated the retinal function of the parents of an RP patient who is homozygous for a c.686delC (p.Pro229GlnfsX35) mutation in exon 3 of *RP1*, which is predicted to be a null allele. Both parents, who are heterozygous for the mutation, were found to have normal retinal function, excluding haploinsufficiency as a cause of RP1 disease. To distinguish between dominant-negative and gain-of-function mechanisms of dominant RP1 disease, we generated and characterized gene targeted *Rp1*-Q662X point mutation knockin mice that mimic the common p.R677X mutation in human RP1, as well as the other pathologic truncating *RP1* alleles identified to date. The *Rp1*-Q662X mice express a truncated version of the Rp1 protein, and experience photoreceptor degeneration. We next characterized lines of *Rp1*-Q662X mice supplemented with tagged Rp1 wild-type transgenes, and found that normal levels of transgenic wild-type Rp1 protein delayed or prevented degeneration in homozygous *Rp1*-Q662X mice, excluding gain-of function as the causative mechanism. **Conclusions:** These results indicate that truncating mutations in *RP1* cause adRP via a dominant-negative mechanism, while null *RP1* alleles cause recessive disease. Furthermore, our data suggest that gene augmentation therapy could be beneficial for dominant RP1 disease, and demonstrate the importance of investigating the mechanisms underlying other forms of adRP.

2148/W

Blind-sterile 2* is a hypomorphic mouse mutation in *Alkylglycerone phosphate synthase (Agps). *R. Liegel[1], B. Chang[2], D.J. Sidjanin[1].* 1) Cell Biol, Med College Wisconsin, Milwaukee, WI; 2) The Jackson Laboratories, Bar Harbor, ME.

Blind sterile locus 2 (bs2) is a novel autosomal recessive spontaneous mouse mutation resulting in a phenotype of congenital cataracts as well as male-specific sterility. Clinically, *bs2* homozygote mice exhibit bilateral nuclear cataracts and microphthalmia. Histological evaluation of eye sections reveals that *bs2* mice have severely disrupted lens epithelial and fibroblast cells resulting in mature cataracts. Histological evaluation of testes show that *bs2* testes are smaller than WT controls, do not contain mature sperm, and contain large multinucleate cells in the seminiforous tubules. Initial linkage analysis mapped *bs2* to mouse chromosome 2, approximately 45cM from the centromere. Fine mapping narrowed the region containing the locus to a 3.1Mb region containing 19 genes. Sequencing of *alkylglycerone-phosphate synthase (Agps*), a gene within this region, revealed a G to A substitution at the +5 position of intron 14. This mutation, located within the splice-donor site, results in aberrant splicing of Agps mRNA and formation of two alternatively-spliced transcripts in the *bs2* mouse. Agps encodes a ubiquitously expressed peroxisomal protein which catalyzes an early step in the synthesis of ether lipids. We present evidence that *bs2* mice have significantly decreased levels of ether lipids. Human mutations in Agps result in rhizomelic chondrodysplasia punctata type 3 (RCDP3), a disease for which *bs2* is the only animal model. The hypomorphic *bs2* mouse represents a useful model for investigation of the tissue specificity of ether lipid requirements which will be particularly valuable for elucidating the mechanism of cataract formation resulting from ether lipid depletion.

2149/W

Podocyte-specific expression of wildtype or mutant Trpc6 in mice is sufficient to cause glomerular disease. *C.P. Canales[1,2], P. Krall[2,3], P. Kairath[2,3], P. Carmona-Mora[1,2], J.D. Carpio[4,5], S.A. Mezzano[5], J. Li[6], C. Wei[6], J. Reiser[6], J.I. Young[1,2], K. Walz[1,2].* 1) John P. Hussman Institute for Human Genomics, Miami, FL; 2) Centro de Estudios Científicos, CECS, Valdivia, Chile; 3) Universidad Austral de Chile, Valdivia, Chile; 4) Institute of Anatomy, Histology and Pathology , Valdivia, Chile; 5) Nephrology Laboratory, School of Medicine, Universidad Austral, Valdivia, Chile; 6) Division of Nephrology and Hypertension, Leonard Miller School of Medicine, University of Miami.

Focal and Segmental Glomerulosclereosis (FSGS) is a major cause of end stage renal disease and is increasing in frequency. TRPC6 (Transient receptor potential channel 6) is a six transmembrane domains protein which function is related with the calcium transport towards the cytoplasm through a pore located between the transmembrane domains 5 and 6. Mutations in the TRPC6 gene have been associated with familiar forms of FSGS affecting children and adults. In addition, acquired glomerular diseases are associated with an increase in TRPC6 expression levels. However, the exact role of TRPC6 in the pathogenesis of FSGS remains to be elucidated. In this work we have generated and characterizated three different transgenic mouse lines with podocyte-specific overexpression of the wild type and two previously described mutant forms of Trpc6 (P111Q and E896K) that were related to FSGS. Consistent with the human phenotype a significantly increased but non-nephrotic range of albuminuria was detectable in some transgenics. The histological analysis of the transgenic lines demonstrated that they developed a kidney disease similar to human FSGS with significant increase of the mesangial matrix, perihilar hyalinosis and glomerular collapse. Moreover, differences in the presence of glomerular lesions were found between the wild type mice and mice expressing the wild type Trpc6 as well as the mutant forms of the channel. Finally, electron microscopy analysis of glomerulus of transgenic mice showed extensive podocytes foot process effacement. Taken together, our results indicates that overexpression of Trpc6 (wild type or mutated) in podocytes is sufficient to cause a kidney disease consistent with FSGS. This data contribute to reinforce the central role of podocytes in the etiology of FSGS and these mice constitute an important new model to study future therapies and outcomes of this complex disease.

2150/W

Function of the Williams Syndrome Transcription Factor Baz1b. *G. Didelot, A. Reymond.* Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland.

One example of recurrent chromosomal rearrangements is the heterozygous ~1.5 Mb microdeletion associated with the Williams-Beuren syndrome (WBS). WBS is a neurodevelopmental disorder characterized by mental retardation with unique cognitive and personality profile, distinctive facial features, supravalvular aortic stenosis, short stature, connective tissue anomalies, hypertension, infantile hypercalcemia, dental and kidney abnormalities, premature aging of the skin, impaired glucose tolerance and silent diabetes. This syndrome results from hemizygosity of 25 genes, five of which encode transcription factors. We focused on one of these regulatory molecules, the Williams Syndrome Transcription Factor BAZ1B, because we hypothesized that even modest perturbations in his dosage might substantially alter the expression of target genes, thus profoundly affecting the phenotype of patients. BAZ1B was biochemically identified as a major component of two distinct chromatin-remodeling complex: WINAC, a SWI/SNF-type complex, and WICH, a ISWI-type complex. To address the physiological and molecular role of BAZ1B in vivo, we ablated the expression of its ortholog, Baz1b, in mice using a transposable element insertion in its 5'UTR region. All Baz1b-/- pups die just before birth from a cardiac defect. Fetuses with this genotype appear, however, healthy and alive at E18.5 suggesting a defect in the last steps of their development. No residual expression of Baz1b transcripts is found in the Baz1b-/- fetuses, while it is considerably reduced in the hemizygous Baz1b+/- models. We will present a detailed description of the cardiac anomalies of these animals. To identify the target genes of the Williams Syndrome Transcription Factor BAZ1B and thus possibly pinpoint the genes, which when dysregulated are potentially playing a role in the WBS phenotype and/or the Baz1b-/- cardiac defect, we performed ChIP-sequencing using two different anti-BAZ1B antibodies in lymphoblastoid cell lines from control individuals and WBS patients.

2151/W

Medaka Model for Fibrodysplasia Ossificans Progressiva. *A. Shimizu[1,2], N. Shimizu[2].* 1) Department of Molecular Biology, Keio University School of Medicine, Tokyo, Japan; 2) Advanced Research Center for Genome Super Power, Keio University, Tsukuba, Japan.

Fibrodysplasia ossificans progressiva (FOP) is a very rare genetic disease (1 in 2 millions) with a symptom of ectopic ossification (bone formation) in the skeletal muscle, tendon, ligament and articulation, and inherited in an autosomal dominant manner. Mutations in the ACVR1 (activin A receptor, type I) /ALK2 gene on the chromosome 2q24.1 was considered responsible for this disease. The ACVR1 is a member of ALK gene family (ALK1-ALK7), producing a 57-kDa transmembrane receptor kinase for a ligand BMP4, of which certain missense mutation results in uncontrolled signaling for bone formation. Previous report demonstrated that intramuscular injection of a viral vector carrying dominant mutant of Alk2 caused local bone dysplasia in the muscle of rats, but it was insufficient to use as FOP model for detailed analysis of pathogenesis. Here, we report a novel FOP model using small fish "medaka". In this study, we first confirmed the presence of all members of ALK family in the medaka genome and performed detailed analysis of medaka Alk2 gene in terms of the genome structure, tissue and developmental expression, cDNA cloning and in vitro mutagenesis. We produced a transgenic medaka that over-expressed a mutant Alk2 gene in the skeletal muscles and exhibited significantly reduced skeletal muscle formation. However, the ossification was not observed. Therefore, we changed a strategy to produce more intricate transgenic medaka by employing the Cre-loxP system, Desmin gene promoter for specific expression in muscle progenitor cells and hsp70 gene promoter for stringent expression of Cre upon heat shock induction. Moreover, ligand BMP4 was used in place of receptor Alk2. We produced two types of transgenic medaka that carry the construct DES::loxP-GFP-loxP-BMP4 and hsp::Cre-Cherry, respectively. By crossing these two types of transgenic medaka, we were able to produce a novel transgenic medaka, in which expression of relevant genes can be under the control of time and space during development or local heat shock induction. We will present the interesting phenotypes as a medaka model of FOP.

2152/W

Genetic Association Study between *ABCG5*, *ABCG8* genes and Cholelithiasis. *H. Yang[1], S. Shih[2,4], K. Hu[2,4], H. Chan[1], T. Chang[1], C. Hung[2], H. Wang[2], M. Lin[1], Y. Lee[1,3,5].* 1) Medical Research Dept, Mackay Memorial Hospital, Taipei, Taiwan; 2) Internal Medicine Dept, Mackay Memorial Hospital, Taipei, Taiwan; 3) Pediatrics Dept, Mackay Memorial Hospital, Taipei, Taiwan; 4) Mackay Medicine, Nursing and Management College, Taipei, Taiwan; 5) Pediatrics Dept, Taipei Medical University, Taipei, Taiwan.

Cholelithiasis prevalence was increased in Taiwan in decades. It is thought to be a complex process caused by genetic and environmental factors. Composition of biliary bile and genes involved in biliary bile regulation are highly associated with cholelithiasis. Adenosine tripohsphate-Binding Cassette (ABC) transporters are known to involved in biliary bile excretion and transport. The ABCG5 and ABCG8 belong to G subfamily of ABC transporter. Both of them have been demonstrated in association with cholelithiasis in Caucasian via genome-wide association scan. The aim of this study is to find if *ABCG5* and *ABCG8* genes polymorphisms associate with cholelithiasis in Taiwanese population. We enrolled 375 patients with cholelithiasis and 379 gallstone-free controls in our study. Polymorphisms of *ABCG5 E604Q* (rs6720173), *ABCG8 D19H* (rs11887534), *C54Y* (rs4148211) and *T400K* (rs4148217) were performed by TaqMan SNP genotyping assays. The frequencies of genotype, allele, carrier and haplotype were calculated directly and analyzed by Haploview 4.1 program. Our data showed no significant associations between cholelithiasis and specific *ABCG5/8* SNPs. This suggests that *ABCG5/8* genes do not confer increased susceptibility to cholelithiasis.

2153/W

In-vitro treatment with pravastatin and dexamethasone normalizes abnormal deposition of elastic fibers in dermal fibroblasts derived from patients with Restrictive Dermopathy - Possible therapeutic implications. *A. Hinek[1], B.H.Y. Chung[2,3], R. Teitelbaum[3], P. Shannon[4], D. Chitayat[2,3].* 1) Heart Centre, Hosp Sick Children, Toronto, Canada; 2) Division of Clinical and Metabolic Genetics, Hosp Sick Children, Toronto, Canada; 3) The Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital, Toronto, Canada; 4) Department of Pathology and Laboratory Medicine, UoT, Toronto, Ontario, Canada.

Restrictive dermopathy (RD) is a lethal genodermatosis caused by mutations in lamin A or ZMPSTE24. We hypothesize that potential treatments used in another laminopathy, Hutchinson-Gilford progeria (HGPS), may also produce therapeutic effects in RD. We report the effect of statin and bisphosphonate on the fibroblasts and the surrounding extracellular matrix (ECM) obtained from a fetus with RD. CASE REPORT: The couple was 1st cousins of Pakistani origin with unremarkable family history. They had 2 pregnancies resulting in intra-uterine death at 27-28 weeks, both preceded by decreased fetal movement and oligohydramnios. In the 2nd pregnancy, autopsy revealed classical features of RD and their 1st pregnancy was retrospectively diagnosed with the same condition. IN-VITRO STUDIES: Sequencing of ZMPSTE24 gene showed homozygous p.Glu237Stop mutations in the 2nd fetus. Cultured skin fibroblasts showed abnormal lamin accumulation in the rim of the nuclei. Immuno-histochemistry showed abnormal assembly of elastin fibers due to defective deposition of fibrillin-1. While the RT-PCR showed normal expression of fibrillin-1 mRNA, Western blotting demonstrated peculiar accumulation of pro-fibrillin-1 and proportional deficiency in mature fibrillin-1. This indicates a defective processing of profibrillin-1 to fibrillin-1 which may prevent its assembly into normal microfibrils. Importantly, we found that pravastatin (100μM), but not pamidronate (100μM), ameliorated defective cleavage of pro-fibrillin-1 and improved deposition of microfibrils. Combination of pravastatin and dexamethasone (20μM) lead to further normalization of faulty elastic fibers deposition in the RD fibroblasts. CONCLUSION: Defective nuclear envelope architecture is associated with various phenotypes in laminopathies. There are in-vitro and in-vivo evidence that suggests inhibitors of fernesylation and geranylgeranylation are promising treatment for HGPS. However, for atypical progeria and mandibuloacral dysplasia, data available are so far not supportive. Our finding suggests that ZMPSTE24 deficiency may (indirectly) lead to defective profibrillin-1 processing and prevent the normal assembly of elastic fibers. This may explain the abnormal skin phenotype seen in RD. Our findings also highlight the potential of pravastatin and dexamethasone in alleviating RD phenotype. Further research is required to uncover the mechanism resulting in the correction of the abnormal elastogenesis in RD.

2154/W

Homozygosity Mapping and Linkage Studies in Saudi Arabian Patients with Hereditary Deafness. *F. Imtiaz[1], M. Al-Owain[2], K. Ramzan[1], G. Bin-Khamis[3], R. Allam[1], A. Al-Mostafa[1], S. Al-Hazza[4], K. Taibah[5].* 1) Genetics, KFSH & RC, Riyadh, Saudi Arabia; 2) Medical Genetics, KFSH & RC, Saudi Arabia; 3) Otolaryngology, KFSH & RC, Saudi Arabia; 4) Opthalmology, KFSH & RC, Riyadh, Saudi Arabia; 5) ENTMedical Centre, Riyadh, Saudi Arabia.

Deafness is the most common sensory deficit in human populations (1:1000 child births) with both genetic (50%) and environmental (50%) etiologies. Hearing impairment is clinically and genetically heterogeneous. It can be non-syndromic or can be associated with other symptoms (e.g. Usher or Pendred syndrome). It has been estimated that at least 300 human protein-coding genes are involved in the hearing process. Recessively inherited diseases are more prevalent in populations where consanguineous marriages are common, like Saudi Arabia. We are currently conducting a study to define the genetics of deafness in this population. Families with profound congenital deafness and an autosomal recessive mode of inheritance are a powerful resource for genetic linkage studies of recessively inherited deafness. Over 120 such families have been enrolled from different cities of Saudi Arabia. Affected individuals are initially screened for mutations in *GJB2* (*DFNB1*) by direct sequencing. According to our results, unlike various other populations, *DFNB1* is not a prevalent cause of deafness in the Saudi population. After performing homozygosity mapping and/or linkage analysis to identify candidate loci/genes in families where *DFNB1* has been excluded, mutations in numerous deafness-causing genes (*ATP6V1B1*, *LHFPL5*, *MARVELD2*, *MYO6*, *MYO7A*, *MYO15A*, *OTOF*, *SLC26A4*, *TMC1*, *TMPRSS3*, *USH1G*, and *WHRN*) have been identified. To conclude, a different set of genes are more common in Saudi Arabia as compared to other populations thereby complicating the identification of deafness loci/genes in this population. The benefit of this study will provide knowledge and awareness through carrier screening and genetic counseling; overall having a major impact upon early intervention and prevention of hereditary deafness.

2155/W

Identification of a novel locus on Chromosome 11 in a large family with IMAGe Syndrome. *V.A. Arboleda[1], H. Lee[1], D. Braslavsky[2], S.F. Nelson[1], I. Bergada[2], E. Vilain[1].* 1) Department of Human Genetics, University of California, Los Angeles; 2) Division of Endocrinology, Hospital de Niños, Buenos Aires, Argentina.

IMAGe Syndrome (Intrauterine growth restriction(IUGR), Metaphyseal dysplasia, Adrenal hypoplasia congenita, and Genital anomalies) is a multisystem developmental disorder with life-threatening consequences. The severity of each of the symptoms is variable, with some cases diagnosed in the neonatal period due to adrenal crisis while others only come to attention in early childhood with developmental delay, and discovery of the skeletal phenotype and/or endocrine anomalies, The genital anomalies -cryptorchidism and hypospadias- are only present in 46, XY neonates. Although initially described in isolated cases, the IMAGe syndrome was recently identified in a large five-generation family from Argentina. Within this pedigree, there were seven affected individuals who were studied here, while ten members of the family died in the neonatal period, presumably affected by the same condition. Saliva was collected from seven affected and one unaffected and genomic DNAs from these samples were analyzed on the Affymetrix 250K SNP arrays. Familial relationships were confirmed by examining the extent of sharing between every possible pair of samples, and no large blocks of homozygosity were detected in any of the samples, indicating that consanguinity was not likely to have contributed to the disorder. The samples were analyzed using a custom script that detects Identical-by-descent (IBD) regions (B. Merriman, in preparation). One region on chromosome 11 was identified as IBD among all seven affected individuals and not with the one unaffected. The region is 17.1Mb in size, containing 590 UCSC known genes. The equivalent LOD score for this linkage signal is 5.4. This region on chromosome 11 is the first locus identified in association with the IMAGe syndrome. We cross analyzed this small region using the UCLA Gene Expression Tool (UGET) to identify potential candidates that are co-expressed with known genes important in cartilage and adrenal development for sequence analysis. Identifying the genetic loci responsible for this severe and often fatal condition of the newborn will allow clinicians to better understand, diagnose and, ultimately, treat patients with IMAGe syndrome.

2156/W

An interactor of SPG11 and SPG15 also accounts for hereditary spastic paraplegia (SPG48). *M. Slabicki[1], E. Mundwiller[2], M. Theis[1], D.B. Krastev[1], S. Samsonov[3], M. Junqueira[1], M. Paszkowski-Rogacz[1], J. Teyra[3], A.K. Heninger[1], I. Poser[1], F. Prieur[4], J. Truchetto[2], C. Confavreux[5], C. Marelli[2], A. Durr[2], J.P. Camdessanche[4], A. Brice[2], A. Shevchenko[1], M.T. Pisabarro[3], F. Buchholz[1], G. Stevanin[2].* 1) Max Planck Institute for Molecular Cell Biology and Genetics, Dresden, Germany; 2) CR-icm, INSERM / UPMC UMR_S975 - NEB, Paris, France; 3) Structural Bioinformatics, BIOTEC TU Dresden, Germany; 4) Hôpital Nord, Saint Etienne, France; 5) Hôpital Neurologique, Lyon, France.

Hereditary spastic paraplegias (HSP) are rare neurodegenerative disorders caused by the degeneration of the cortico-spinal tracts leading to lower-limb spasticity. HSP is a highly heterogeneous condition with at least 46 loci identified so far. A potential interaction of two of the responsible genes, SPG11 and SPG15, has been suggested on the basis of similar neurological symptoms and common expression profiles in adult rat brain. Using proteomic studies, we have identified a protein partner of spatacsin and spastizin, the protein products of the SPG11 and SPG15 genes. We investigated if any unexplained HSP cases could be linked to mutations in this SPG11/15 partner. Direct sequencing of this gene in 166 unrelated HSP patients, including 38 and 64 cases with a recessive or dominant inheritance pattern and 64 sporadic cases identified 7 known and 15 new variants, respectively. Most of these variants were not considered causative, because they did not affect protein sequence, were not predicted to alter correct splicing or were also found frequently in controls. More interestingly, we found a homozygous mutation in two French siblings (FSP-083), which was not detected in 156 Caucasian and 242 North-African control chromosomes. In these patients, a complex indel generated a frameshift and a premature stop codon. Both affected patients presented with progressive spastic paraplegia associated with urinary incontinence since age 50 and 49, respectively. Cerebral MRI was normal but spinal hyperintensities at C3-C4 and C7 were observed in one. Both parents died at the age of 72 and 77, respectively, of non neurological causes. They originated from two neighboring villages but there was no known consanguinity. However, the analysis of three close microsatellite markers and the loss of heterozygosity (LOH) search using CYTO_12 (Illumina) microarrays confirmed that the region is homozygous in both affected patients. In conclusion, we identified mutations in 2 HSP patients in a novel and rare HSP-associated gene, SPG48.

2157/W

Determining the impact of *GALNT12* germline mutations on hereditary colorectal cancer. *E. Clarke, R. Green, J. Green, P. Parfrey, M. Simms, K. Mahoney, B. Younghusband, M. Woods.* Discipline of Genetics, Memorial University, St. John's, Newfoundland, Canada.

Approximately 35% of all colorectal cancer can be attributed to familial risk. The Canadian province of Newfoundland and Labrador (NL) has the second highest incidence rate of colorectal cancer (CRC) in North America. Previous mutation analysis investigating the NL CRC population has revealed that the high frequency of hereditary CRC in NL is likely attributed to novel susceptibility genes. Recently, Guda et al. identified somatic and germline disease causing variants in *GALNT12* among CRC patients. These results suggest that *GALNT12* may be a novel gene associated with CRC susceptibility. *GALNT12* encodes an enzyme that is involved in glycosylation and is highly expressed in the human colon. The purpose of this study was to investigate the impact of *GALNT12* germline mutations in NL CRC families referred to the genetics clinic. We sequenced *GALNT12* in 130 CRC patients to investigate if variants within this gene segregate with the disease. Probands included in the study have a high family incidence of CRC and were referred to the Provincial Medical Genetics Program. Twenty percent (20%) of our cohort fulfills the high risk Amsterdam I criteria while an additional 65% of the families fulfill the Revised Bethesda criteria. Approximately 8% of the cohort harbours a mutation in one of the known genes associated with CRC. We identified three different variants in the *GALNT12* gene - c.907G>A (3 probands), c.781G>A (2 probands) and c.1187A>G. Functional testing by Guda et al. has shown that the c.907G>A variant reduces the enzymatic activity of the protein to 37% of wild-type (WT). These tests also revealed that c.781G>A decreases the enzymatic activity to 85% of WT and therefore this variant is considered neutral. The third variant identified, c.1187A>G, has not been previously reported and the effect this variant has on enzymatic activity is currently unknown; however, PolyPhen, SIFT and Panther predict this variant to be pathogenic. Two families showed segregation of c.907G>A with the disease. Both of these families fulfill the Revised Bethesda criteria. In total, we identified three CRC probands that harbour a *GALNT12* mutation and another proband with a possible causal variant. None of the probands identified with a *GALNT12* variant harboured any other mutations that occur within the known genes associated with CRC, namely *APC,MUTYH, MLH1, MSH2, MSH6* and *PMS2*. This study further supports the association between *GALNT12* and the development of hereditary CRC.

2158/W

The rs854560 variant of the PON1 predisposes individuals to harbouring low high density lipoprotein levels. *B. Baz[1], M. Vigilla[1], O. Alboudary[1], P. Muiya[1], E. Andres[1], M. Alshahid[2], S. Majid[1], N. Dzimiri[1].* 1) Department of Genetics, Reseach Centre, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia; 2) King Faisal Heart Institute. King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia.

The involvement of paraoxonase 1 (PON1) gene polymorphism in dyslipidaemic disorders and early onset of coronary artery disease (CAD) remains poorly studied. In a preliminary genome-wide linkage study on early onset CAD involving two families (22 individuals) with predominant heterozygous familial hypercholesterolaemia, we identified several loci, among others, on chromosome 7 which harbours the PON1 gene, using the Affymetrix Gene Chip 250 sty1 mapping array. The present study pursued a population-based association analysis for 5 selected PON1 variants rs854560, rs662, rs854552, rs3917577 and rs3735590 in 1013 cases harbouring low high density lipoprotein (lHDL) levels versus 2035 normolipidaemic controls using the Applied Biosystems real-time PCR procedure. Among these variants, rs854560 [Odds ratio(95% Confidence Interval = 1.13(1.02-1.23); p=0.015] was associated with lHDL levels. Thereby, the autosomal recessive mode of inheritance [1.28(1.10-1.51); p=0.002] appeared to be responsible to this causative association. None of the variants was related with CAD per se. However, the recessive mode for rs3917577 showed a causative relationship [1.23(1.03-1.47); p=0.023] with the disease, but no confounding effect of gender, age, myocardial infarction, type 2 diabetes mellitus, or obesity was observed. The results indicate that PON1 is a susceptibility gene for harbouring lHDL, and the presence of the rs3917577 variant increases the risk of acquiring CAD among dyslipidaemic individuals.

2159/W

Prader-Willi syndrome with normal intellect: A forme fruste phenotype caused by deletion of the HBII-85 small nucleolar RNA cluster. *S. Demczuk[1,2], G.E. Graham[2], N. Carson[2].* 1) Dept of Laboratory Medicine, Saskatoon Health Region, Saskatoon, SK; 2) Dept of Genetics, Children's Hospital of Eastern Ontario, Ottawa, ON.

Prader-Willi syndrome (PWS) is caused by paternal deficiency of genes in the 15q11.2 chromosomal region either through deletion, imprinting defect or maternal uniparental disomy. Our patient is a 36year-old woman who came to our attention after her first-born child with Down syndrome was referred to "rule out Prader-Willi syndrome". The patient provided the intriguing history that she had been diagnosed with Prader-Willi syndrome in the mid-1980s and that she had a paternal cousin with typical PWS. She recalled being told that her future children would be at risk of having PWS. We were able to obtain copies of consultations from three clinical geneticists dating back to this period. One clinician had made the diagnosis of PWS; the other two did not feel that the patient had enough features to make this diagnosis. At the time a 550 band karyotype was normal; FISH or methylation investigations of the 15q11.2 region were not performed. The patient had a measured IQ of 87 in childhood and was treated for hypogonadotrophic hypogonadism in adolescence. Her current findings include normal stature, moderate obesity without dietary restriction, the characteristic facial features and straight ulnar borders of the hands. She meets at least 5 of the seven major diagnostic criteria for PWS. Molecular testing for PWS by methylation sensitive multiplex ligation-dependant probe amplification (MRC Holland) showed a deletion of maximum 363.8 kb involving the HBII-85 C/D box small nucleolar (sno)RNA cluster. Most C/D box snoRNAs are involved in 2'-O-ribose methylation of ribosomal RNAs. There are two clusters of C/D box snoRNAs in 15q11.2: HBII-52 and HBII-85. They are paternally imprinted, highly expressed in brain and contained within introns of the SNRPN gene. Their targets and functions are unknown. Two reported families with a paternally inherited microdeletion of the entire HBII-52 cluster did not manifest features of PWS, so a crucial role of the HBII-85 C/D box snoRNA cluster in the pathogenesis of PWS is suggested by the report of a patient with a microdeletion encompassing the HBII-85 cluster, the HBII-438A and a portion of the HBII-52 cluster of snoRNAs. However, unlike our patient, this individual has a classical cognitive phenotype with mild intellectual disability. Our data further supports a critical role for the HBII-85 snoRNA cluster in the causation of PWS and demonstrates that deletion of this cluster is compatible with a normal intellect.

2160/W

SNP Genotyping Supporting CTT Deletion in NFIC Gene Associated with RDD. *J. Dong[1], A.C. Acevedo[2], M.G. Martinez[3], H. Riveria[3], L. ÖZER[4], YX. Wu[1], M. MacDougall[1].* 1) Orthodontics, University of Alabama at Birmingham, Birmingham, AL; 2) 2Dental Anomalies Clinic, University Hospital of Brasilia, Department of Dentistry, Faculty of Health Science, University of Brasilia, Brasilia, Brazil; 3) Central University of Venezuela, Ciudad Universitaria, Caracas, Venezuela; 4) Ankara University Faculty of Dentistry Department of Pedodontics 06500 Besevler Ankara Turkey.

Radicular dentin dysplasia (RDD) is a rare disease with an estimated prevalence of 1/100,000 in general population. RDD patients present with short roots that lead to premature loss of both primary and permanent dentitions. The genetic cause of the condition is unknown. Previously, our laboratory have identified a c*+8-10delCTT in the 3' terminal untranslated region of human NFIC gene in a RDD family. The deletion was found in both alleles of the proband but only a single deleted allele in his unaffected sister and parents who are first cousins. To confirm if the deletion is a disease causing mutation in RDD, four additional RDD families from 4 different countries (Untied States, Brazil, Venezuela and Turkey) were added to this panel. NFIC gene mutational analysis was performed by PCR and sequencing using NFIC gene specific primers and SNPs genotyping was carried out by sequencing and TaqMan assay. SNPs distributed around 130kb segment of the NFIC gene on chromosome 19 were genotyped in the five RDD families as well as in unrelated controls. Our results showed that the homozygous deletion was seen in the probands from three out of the five RDD families, the heterozygous carriers for the deletion allele are cosegregated in their unaffected parents and siblings. The frequency of the c*+8-10delCTT allele was 1.13% (4/354) in Caucasians, and 0% in Hispanics (0/72) and African American (0/80) unrelated control chromosomes. Furthermore, we constructed the haplotypes based on the genotyping data and pedigree information. Haplotype analysis showed that the patients carrying the homozygous deletion have two identical haplotypes, where both of their biological parents and their siblings who are heterozygous healthy carriers for the mutation do share one affected haplotype, but the patients from other two RDD families who do not carry the CTT deletion present different haplotypes. Our studies indicated that the NFIC c*+8-10delCTT allele is likely associated with the autosomal recessive RDD and suggested that the RDD is a genetically heterogeneous disorder. This study will allow us to understanding tooth root formation and mechanisms underlying abnormal root development. The study is supported by IOHR/ UAB-SOD.

2161/W

Mutations in MYBPC2 cause Distal Arthrogryposis type 2B (DA2B). *H. Gildersleeve, M. McMillin, A.E. Beck, M.J. Bamshad.* Dept Pediatrics, Univ Washington, Seattle, WA.

The Distal Arthrogryposis (DA) syndromes are a group of autosomal dominant disorders characterized by multiple congenital contractures of the digits, wrists, and ankles. We have defined at least 10 different DA types (DA1-DA10) based on clinical criteria, and over the past decade we and others have discovered mutations in TNNI2, TPM2, TNNT3, MYH3, MYH8, MYH2, MYH13, and, most recently, MYBPC1 that explain about half of all DA cases. The proteins encoded by these genes are perhaps both qualitatively and quantitatively the most important molecules in skeletal muscle, as they bring about the production of force. As part of an ongoing effort to screen candidate genes that encode components of the contractile complex for mutations that cause DA, we sequenced MYBPC2 in 70 families with DA2B or Sheldon-Hall syndrome (OMIM#601680). MYBPC2 is an ortholog of MYBPC1 that encodes a myosin regulatory protein expressed in fast-twitch myofibers. In 2 of 70 families, we found frameshift mutations that segregate with affected individuals in each family and that were not found in 200 control chromosomes matched by ancestry. Both mutations are single base pair deletions, c.1995delT in exon 18 and c.2808delC in exon 24, which are predicted to lead to premature protein truncation. This finding suggests that haploinsufficiency of myosin binding protein C2 results in congenital contractures. These results bring to nine the total number of genes that are known to underlie DA syndromes. While mutations in MYBPC2 appear to be an infrequent cause of DA2B, understanding the mechanism by which contractile force production is perturbed by MYBPC2 mutations could provide important insights about general mechanisms that underlie heritable congenital contractures.

2162/W

Loss of PAK3 kinase activity underlies a novel form of X-linked neuroichthyosis. *P. Magini[1], T. Pippucci[1], S. Coppola[2], E. Stellacci[2], R. Zuntini[1], S. Miccoli[1], E. Pompilii[1], C. Graziano[1], G. Cenacchi[3], I. Neri[4], C. Garone[5], D.M. Cordelli[5], V. Marchiani[5], R. Bergamaschi[6], G. Neri[7], O. Zuffardi[8], L. Mazzanti[9], A. Patrizi[4], E. Franzoni[5], G. Romeo[1], M. Tartaglia[2], M. Seri[1].* 1) Genetica Medica, Università di Bologna, Bologna, Italy; 2) Dipartimento di Biologia Cellulare e Neuroscienze, Istituto Superiore di Sanità, Roma, Italy; 3) Anatomia e Istologia Patologica, Università di Bologna, Bologna, Italy; 4) Dermatologia, Università di Bologna, Bologna, Italy; 5) Neuropsichiatria Infantile e Disturbi Comportamento Alimentare, Univerità di Bologna, Bologna, Italy; 6) Pronto Soccorso Pediatrico e Pediatria d'Urgenza, Università di Bologna, Bologna, Italy; 7) Istituto di Genetica Medica, Università Cattolica del Sacro Cuore, Roma, Italy; 8) Genetica Medica, Università di Pavia, Pavia, Italy; 9) Ambulatorio di Auxologia, Sindromologia e Malattie Rare, Università di Bologna, Bologna, Italy.

The term 'neuroichthyosis' defines a clinically and genetically heterogeneous group of syndromes in which ichthyosis can be found in association with various neurological disorders, including spasticity, epilepsy, polyneuritis and pyramidal tract signs. Here we report the identification of a gene potentially responsible for a novel X-linked form of syndromic neuroichthyosis characterized by ichthyosis, agenesia or hypoplasia of corpus callosum, microcephalia, seizures, mental retardation and spastic tetraparesis. Linkage analysis performed on a three-generation family transmitting the trait revealed a critical region of nearly 25 Mb in Xq21.33q24 including about 250 genes. Based on the clinical overlap with cardio-facio-cutaneous syndrome, the scanning of the entire coding sequence of PAK3, which encodes a serine/threonine kinase with an important role in the cytoskeleton organization and linked to RAS signaling, allowed the identification of a G-to-T missense change at position c.1167 (K389N) co-segregating with the trait in the family. The mutation affected a highly conserved residue within the kinase domain of the protein, and was not observed in more than 450 control chromosomes X, strongly arguing against the possibility that the variant was a disease-unrelated polymorphism. PAK3 had been previously reported to be mutated in isolated mental retardation. Functional characterization of the PAK3 K389N mutant documented impaired kinase activity in vitro, similarly to what observed for some of the mutants associated with non-syndromic mental retardation. Confocal laser scanning microscopy analysis indicated a cytoplasmatic localization, as observed for the wild type protein. These data suggest a causative role of the PAK3 K389N mutation in the pathogenesis of this novel form of X-linked neuroichthyosis. While the possibility that another gene within the critical region might contribute to the trait, worsening the clinical picture caused by loss of PAK3 catalytic activity, can not ruled out, the relevant expression of PAK3 in keratynocytes at both mRNA and protein levels would suggest an implication of this gene also in the ichthyosis occurrence, possibly resulting from kinase activity-independent function of the protein.

2163/W

Molecular Analysis of *SEC23IP* as a Potential Candidate Waardenburg Syndrome Gene. *T.A. Maher[1], G. Zhou[1], J.M. Milunsky[1,2,3].* 1) Center for Human Genetics; 2) Department of Pediatrics; 3) Genetics and Genomics, Boston University School of Medicine, Boston, MA.

Waardenburg syndrome (WS) is a genetically heterogeneous congenital neurocristopathy. It is an auditory-pigmentary syndrome characterized by pigmentary abnormalities of the hair, including a white forelock, premature graying, pigmentary changes of the iris, such as heterochromia irides, and congenital sensorineural hearing loss. There is quite marked expressivity of the syndrome both within and between families. Waardenburg syndrome has been classified into 4 main phenotypes. WS type 1 is distinguished by the presence of dystopia canthorum. WS type 2 is distinguished from type 1 by the absence of dystopia canthorum and is further divided into 5 subtypes. WS type 3 has dystopia canthorum and upper limb abnormalities. WS type 4, also known as Waardenburg-Shah syndrome, has the additional feature of Hirschsprung disease and is also divided into 3 subtypes. WS1 and WS3 are caused by mutations in the *PAX3* gene, WS2A by mutations in *MITF*, WS2D by mutations in *SNAI2*, WS2E by mutations in *SOX10*. WS2B and 2C have been mapped to 1p21-p13.3 and 8p23 respectively. WS4A is caused by mutations in the *EDNRB* gene, WS4B by mutations in *EDN3* and WS4C by mutations in *SOX10*. The detection rate for mutations in the 4 main phenotypes is quite variable and very limited in some phenotypes thus indicating one or more undiscovered genes are involved in WS. Over the past 20 years there have also been at least 8 other genes associated with WS. Our clinical molecular diagnostics laboratory currently sequences *PAX3*, *MITF*, *SOX10*, *EDNRB* and *EDN3* and also performs deletion/duplication (MLPA) analysis for the above genes. Recently, a publication by McGary et al, performing an analysis of orthologous genes to look at alternative models for human disorders, indicated *SEC23IP* may be involved in causing WS. They observed a plant-human phenolog relating negative gravitropism in Arabidopsis to WS in humans. This finding was confirmed by *SEC23IP* expression in the neural crest cells of Xenopus embryos. We sought to determine if the *SEC23IP* gene was responsible for the majority of WS2 and/or a minority of WS1 in patients who previously had negative clinical molecular testing. We performed *SEC23IP* sequencing of the 18 coding exons on a small cohort of 9 WS2 and 6 WS1 patients. No mutations were identified in this cohort. Further molecular studies of a larger cohort are necessary to evaluate if mutations in this gene are causative of a minority of WS.

2164/W

Autosomal recessive spastic paraplegia with thin corpus callosum among Arabs, clinical and genetic findings. *S. Majid[1], H. Murad[2], B. Baz[1], R. Al Amr[1], S. Al Yamani[2], S. Al-Wadaee[1], B. Meyer[1], S. Bohlega[2].* 1) Functional Genomics, Dept. of Genetics, King Faisal Specialist Hosp, Riyadh, Saudi Arabia; 2) Department of Neurosciences, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia.

Spastic paraplegia represents a group of heterogeneous neurodegenerative disorders. One of the most common and clinically distinct forms of familial spastic paraplegia is autosomal recessive hereditary spastic paraplegia with thin corpus callosum (ARHSP-TCC). Mutations in spatacsin gene (KIAA 1840) have been recently identified as a frequent cause of ARHSP-TCC. All the mutations described in the spatacsin. This far results in loss of function. Three unrelated Saudi Arabian families were studied with ARHSP-TCC. Ten affected individuals were studied. Clinical presentations include early gait disturbance at early age (2-14 years), rapid progression of spastic paraplegia with mild to moderate cognitive impairment and evidence of peripheral neuropathy in two families. Brain MRI showed thin corpus callosum accompanied by perventricular white matter changes and cortical atrophy. Linkage analysis localized the gene to SPG11 (chr. 15). Sequencing revealed three mutations. The first is an insertion of 23 bp (L1268L fsX), the second is a 1 bp deletion (S1923R fsX), and the third is a substitution leading to a stop codon (R651X). All three mutations lead to premature truncations within the coding region of the SPG11 gene encoding spatacsin. The identification of these mutations confirms further a causative link between spatacsin gene and ARHSP-TCC in these families.

2165/W

Homozygous duplication at 10p11.21 in a boy with clinical manifestations of Lin-Gettig syndrome. *J.F. Mazzeu[1,2], F. Zhang[3,4], C.M. Carvalho[3], A.C. Krepischi[1,5], S. Yatsenko[3], E. Pardono[1], P.A. Otto[1], C. Rosenberg[1], J. Lupski[3,6,7], A.M. Vianna-Morgante[1].* 1) Departmento de Genética e Biologia Evolutiva, Universidade de São Paulo, São Paulo, Brazil; 2) Departmento de Genética e Morfologia, Universidade de Brasília, Brasilia, Brazil; 3) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA; 4) State Key Laboratory of Genetic Engineering and MOE Key Laboratory of Contemporary Anthropology, School of Life Sciences and Institutes of Biomedical Sciences, Fudan University, Shanghai, China; 5) Hospital AC Camargo, São Paulo, Brazil; 6) Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA; 7) Texas Children's Hospital, Houston, TX, USA.

Lin-Gettig syndrome is an extremely rare disorder previously reported in only three patients presenting with craniosynostosis, agenesis of the corpus callosum, severe mental retardation, peculiar facies, camptodactyly and hypogonadism. Here we report a boy with clinical manifestations of Lin-Gettig syndrome, born to consanguineous parents. He presented with severe developmental delay, bilateral deafness, hoarse cry, short stature, craniostenosis of sagittal suture, plagiocephaly, microcephaly, dolicocephaly, bitemporal depression, facial asymmetry, apparent hypertelorism, downslanting palpebral fissures, ptosis, strabismus, blue sclerae, long philtrum, micrognathia, highly arched palate, submucosal cleft, low-set ears, hyperfolded ear-helix, short neck, pterygium coli, mamilar hypertelorism, shawl scrotum, cryptorchidism, café-au-lait spots, congenital hip-dislocation, bilateral single palmar crease, camptodactyly, and posterior protrusion of calcaneus. Magnetic resonance imaging documented agenesis of the corpus callosum with enlarged lateral ventricles. He had an atrial sept defect. Since the etiology of Lin-Gettig syndrome is unknown, we tested the patient for microimbalances by array-CGH using an Agilent 244K platform. Two homozygous duplications 300 kb apart at 10p11.21 were detected: a 250 kb duplication including the 5'end of *PARD3* gene, and a 160 kb duplication encompassing *CREM* and the 3'end of *CCNY*. Both parents were found to be heterozygous for each of the two duplications. Although present in four copies in the patient, the *CREM* gene is less likely to contribute to his clinical phenotype since its main role is in spermiogenesis, and both parents carry three copies of this gene. On the other hand, disruption of *PARD3* and/or *CCNY* might be causative of the phenotype, acting as recessive mutations. *CCNY* is involved in the control of cell division, in the regulation of cyclin-dependent kinases. *PARD3* is involved in asymmetrical cell division and polarized growth, and knock-out mice for *Pard3* present with embryonic lethality, abnormal heart development, growth retardation, prominent telencephalic vesicles, short tail and edema. The complexity of these malformations is comparable to that of our patient's clinical signs, supporting homozygous *PARD3* disruption as potentially contributing to the phenotype.

2166/W

Association of LOC339766 with High Myopia in Caucasian Family Cohorts. *R. Metlapally[1,2], K. Tran-Viet[2], D. Abbott[2], F. Hawthorne[2], F. Malecaze[3], P. Calvas[3], D. Mackey[4], T. Rosenberg[5], S. Paget[3], J. Guggenheim[6], Y. Liu[2], R. Allingham[1], M. Hauser[2], Y. Li[2,7], T. Young[1,2].* 1) Duke Eye Center, Durham, NC, USA; 2) Duke Center for Human Genetics, Durham, NC, USA; 3) Toulouse University Hospital, Toulouse, France; 4) Lions Eye Institute, Centre for Ophthalmology and Visual Science, University of Western Australia, Perth, Australia; 5) Gordon Norrie Centre, National Eye Clinic, Hellerup, Denmark; 6) School of Optometry and Vision Sciences, Cardiff University, Cardiff Wales, United Kingdom; 7) Department of Biostatistics and Bioinformatics, Duke University Medical Center. Durham, NC, USA.

Introduction: We previously reported a high-grade myopia locus on chromosome 2q37 which has been independently replicated in other studies. To identify the causative variant/s, we employed a multi-pronged approach comprised of intra-locus densely spaced SNP family-based association analyses to refine the interval, contracted interval candidate gene identification, SNP variant discovery by case/control cohort candidate gene sequencing, and subsequent genotyping and association analysis in a full high-grade myopia family dataset for assessing myopia susceptibility. **Methods:** Illumina GoldenGate genotyping was performed on the original 4.56 Mb interval using 145 SNPs with an average spacing of 31.5 kb in a cohort of 146 multiplex high-grade myopia families. Family-based pedigree disequilibrium test (PDT) and the association in the presence of linkage (APL) test were used for determining association of markers with myopia phenotype stratified by severity. The region incorporating the LOC339766 gene showed significant association. To identify potential risk-associated sequence variants, sequencing was performed on coding exons in this gene in an independent select case (< -6.00 diopters (D) spherical equivalent (SE)) and control (> +0.50 D SE) dataset. Genotyping and association analyses were performed on variants identified and tagging SNPs using Taqman™ allelic discrimination assays in Caucasian Duke and International cohorts. Expression profiling studies were performed using an RNA panel of systemic tissues and retinal tissue. **Results:** PDT analyses from the GoldenGate assay data revealed significant association of several SNPs with high-grade myopia status involving the LOC339766 gene (p<0.01). Genotyping results (with variants identified and tagging SNPs) revealed significant association of the LOC339766 gene polymorphisms with myopia. The Duke cohort showed association with SNPs rs10203853 and rs17868346 (p <0.01, min p=0.004) and the International cohort showed association with SNPs rs17863814 and rs28900694 (p <0.01, min p=1x10-4). The results were consistent after correcting for multiple testing. LOC339766 was expressed in several systemic tissues and the retina. **Conclusions:** LOC339766 gene variants are associated with myopia in Caucasians. The 2q37 locus may play an important role in autosomal dominant juvenile-onset myopia development. Studies to further characterize this hypothetical gene are underway.

2167/W

Missense mutations in *TCF8* cause late-onset Fuchs Corneal Dystrophy and interact with *FCD4* on chromosome 9p. *S.A. Riazuddin[1,2], N. Zaghloul[1], A. Al-Saif[1], L. Davey[1], B. Diplas[1], D. Meadows[2], A. Eghrari[2], M. Minear[3], Y. Li[3], G. Klintworth[4], N. Afshari[4], S. Gregory[3], J. Gottsch[2], N. Katsanis[1,5].* 1) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore,MD 21205, USA; 2) Center for Corneal Genetics, Cornea and External Disease Service,Wilmer Eye Institute, Johns Hopkins Hospital, Baltimore,MD 21205, USA; 3) Center for Human Genetics, Duke University Medical Center, Durham, NC 27710, USA; 4) Department of Ophthalmology, Duke University Medical Center, Durham, NC 27710, USA; 5) Center for Human Disease Modeling, Department of Cell Biology, Duke University, Durham, NC 27710, USA.

Fuchs corneal dystrophy (FCD) is a degenerative genetic disorder of the corneal endothelium that represents one of the most common causes of corneal transplantation in the United States. Despite its high prevalence (4% over the age of 40), the underlying genetic basis of FCD is largely unknown. Here we report missense mutations in *TCF8*, a transcription factor whose haploinsufficiency causes posterior polymorphous corneal dystrophy (PPCD), in a cohort of late-onset FCD patients. In contrast to PPCD-causing mutations, all of which are null, FCD-associated mutations encode rare missense changes suggested to cause loss of function by an *in vi*vo complementation assay. Importantly, segregation of a recurring p.Q840P mutation in a large, multigenerational FCD pedigree showed this allele to be sufficient but not necessary for pathogenesis. Execution of a genome-wide scan conditioned for the presence of the Q840P allele identified an additional late-onset FCD locus on chromosome 9p, whereas haplotype analysis indicated that the presence of the TCF8 allele and the disease haplotype on 9p leads to a severe FCD manifestation with poor prognosis. Our data suggest that PPCD and FCD are allelic variants of the same disease continuum and that genetic interaction between genes that cause corneal dystrophies can modulate the expressivity of the phenotype.

2168/W

Altered mTOR-dependent Signaling and Differential mGluRs expression patterns in Fragile X Syndrome. *F. Tassone[1,2], C. Hoeffer[3,4], E. Klann[3], H. Wong[3], E.I. Sanchez[1], M. Careaga[2,5], P. Ashwood[2,5], R.J Hagerman[2,6].* 1) Department of Biochemistry and Molecular Medicine, University of California, School of Medicine, Davis, CA, USA; 2) M.I.N.D. Institute, University of California Davis Medical Center, Sacramento, CA, USA; 3) Center of Neural Science, New York University, NY , USA; 4) Smilow Neuroscience Institute, New York School of Medicine, NY, USA; 5) Medical Microbiology and Immunology, University of California at Davis, CA, USA; 6) Department of Pediatrics, University of California, School of Medicine, Davis, CA, USA.

Multiple studies have revealed the important role played by the mTOR (mammalian target of rapamycin) signaling pathway in learning and memory. All components of the mTOR pathway, which is involved in protein synthesis-dependent phase of synaptic strengthening, are present in dendrites suggesting a role for mTOR in the regulation of local translation through phosphorylation of its downstream targets. Thus, we have investigated whether altered mTOR signaling is present in subjects with fragile X syndrome (FXS), as the fragile X mental retardation protein, FMRP, absent in FXS subjects, plays an important role in translational repression. Our preliminary findings indicate that translation control mediated by the mTOR pathway is compromised in FXS. mTOR signaling cascade controls initiation of cap-dependent translation and is under control of metabotrobic Glutamate Receptors (mGluR). Group I mGluRs have been implicated in the pathogenesis of FXS. It is believed that the Fragile X Mental Retardation Protein (FMRP), which is absent in FXS, normally serves as a brake to the signaling pathway involving these receptors. Looking at the many mGluRs are functionally important in the periphery and are widely expressed on peripheral blood mononuclear cells. Our preliminary results show an altered cellular expression and signaling of mGluRs on PBMCs resulting in a differentially altered immune response following immune activation in the presence of mGluR agonists. The observed increases in translational signaling suggest excessive basal translation in FMRP-deficient cells, and this activity may contribute to the cognitive and behavioral deficits observed in subjects with FXS. The dysregulation of this system leads to down regulation of neurotransmitter receptors and possibly to abnormal neurodevelopment. Finally, the differentiation of mGluRs expression patterns and their role in the immune response in children with FXS will help differentiate therapeutic targets for future research.

2169/W

Genetic Mapping of Hereditary Vitreoretinopathy in a Three-Generation Family. *K. Tran-Viet[1], D. Abbott[1], E. Burner[1,3], C. Johnson[2], M. Johnson[2], W. Call[1], B. Zhao[1], R. Metlapally[1,3], Y. Li[1], T. Young[1,3].* 1) Duke Ctr Human Gen, Duke Med Ctr, Durham, NC; 2) Retina Consultants, Ltd, Fargo, ND; 3) Duke University Eye Center, Durham, NC.

Introduction: Wagner syndrome (WS) (OMIM 143200) is an autosomal dominant (AD) vitreoretinopathy characterized by vitreous fibrillary condensations, chorioretinal dystrophy, lattice degeneration, and high myopia, with a predisposition of developing cataracts and retinal detachment. WS maps to chromosome 5q13-14 with reported mutations in the CSPG2 gene. We studied a large three-generation Caucasian family with presumed WS, with either an AD inheritance model with reduced penetrance or an autosomal recessive (AR) model. No CSPG2 sequence variations were identified. A whole genome linkage study was performed to identify a novel locus. **Methods:** Genomic DNA samples derived from venous blood or saliva were collected from 38 family members (9 affected). Linkage mapping of genomic DNA was performed using the Illumina Infinium HumanLinkage-24 Panel (5913 SNPs). Genotype data were checked for Mendelian inconsistencies. Affected-only AD and AR linkage models were run using FASTLINK/HOMOG and MERLIN for two-point and multipoint analyses, respectively. Multipoint linkage was analyzed by MERLIN using two sub-pedigrees. Full pedigree multipoint analysis for suggestive linkage regions (LOD >1.5) was performed using SIMWALK. **Results:** AD model parametric multipoint analysis demonstrated HLOD scores > 2.00 at chromosome 12q12-12q14.1, with a peak HLOD score of 2.27 (rs1107654). Multipoint analysis with SIMWALK replicated a peak in this region (peak LOD = 1.975). FASTLINK two-point analysis demonstrated several clustered chromosome 12q SNPs with HLOD > 1.0, with a peak HLOD score of 1.23 on rs10875671. Parametric multipoint AR model analysis demonstrated HLOD scores of > 1.80 at chromosomal regions 7q21.3-7q31.1 (rs714438), 8p12-8p11.21 (rs1522845), 8q12.1-8q13.3 (rs884839), 9q21.31-9q22.2 (rs1359168), and 14q31.3-14q32.32 (rs942190). SIMWALK analysis of MERLIN multipoint results replicated all regions. Two-point linkage analysis only confirmed the 9q and 14q regions. **Conclusion:** We report a linkage peak at chromosome 12q12-12q14.1 [13.23 cM] associated with vitreoretinopathy in an AD model. Novel loci at chromosomes 9q [17.88 cM] and 14q [36.61 cM] were determined using a recessive model. Chromosome 12q12-12q14.1 is a known locus of the COL2A1 gene associated with Stickler syndrome. Interval refinement and candidate gene screening studies are underway.

2170/W

Novel FRMD7 gene mutation in a large multigenerational Indian family with X-linked congenital Nystagmus. *R. Uppala[1,5], R. Singh[2], S.K. Nath[3], M. Naveed[4], U. Ratnamala[1].* 1) Cancer Centre, Creighton Univ, Omaha, NE, USA; 2) Dr. P.L. Desai Eye Center, Navrangpura, Ahmedabad-380009, India; 3) Arthritis and Immunology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK USA; 4) Center for Arab Genomic studies (CAGS), Dubai, United Arab Emirates; 5) Green Cross Blood Bank, Ahmedabad, India.

Congenital nystagmus (NYS) is characterized by bilateral, and uncontrollable, involuntary movement of the eyes; its frequency is ~1:1500 live births. It is one of the most common neuro ophthamalological disorders among live births, and also reduces visual acuity. Nystagmus is transmitted in X-linked dominant, X-linked recessive and autosomal dominant forms with high penetrance and variable expressivity. Four NYS genomic locations (NYS1, NYS2, NYS3 and NYS4), mapped to Xq26.2, 6p12, 7p11.2 and 13q31-q22 respectively using family-based linkage analysis have been documented. Very recently, ~30 mutations of the FRMD7 gene, which maps to Xq26.2 region, have been found in NYS patients, suggesting that this gene plays an important role in the NYS development. We report a novel mutation of the FRMD7 in a large eleven-generation Indian pedigree with an isolated non-syndromic NYS in which 62 members affected by NYS with different phenotypic expression including asymmetric pendular eye movements with unidirectional jerky NYS and head nodding. The missense mutation, c.A917G, predicts a substitution of Arg for Gln at codon 305 (Q305R) within exon 10 of FRMD7. This mutation was found to be at a highly conserved residue within the FERM adjacent (FA) domain, hypothesized to have a regulatory function, based on similarity to other protein kinase substrates. A detailed data of the present family and mutation spectrum of FRMD7 gene will be presented.

2171/W

X-chromosome-specific array-CGH analysis indicates new genes, other than the *L1CAM* gene, involved in L1 syndrome. *Y.J Vos, K.K van Dijk-Bos, C.S. van der Werf, H. Alkema, R.M.W. Hofstra, K. Kok.* Department of Genetics, University Medical Centre Groningen, the Netherlands.

The L1 syndrome is an X-linked recessive disorder with a prevalence of one in every 30,000 newborn males. It comprises four neurological syndromes, all associated with mutations in the *L1CAM* gene. These syndromes are: HSAS (X-linked Hydrocephalus with Stenosis of the Aqueduct of Sylvius), MASA (Mental retardation, Aphasia, Spastic paraplegia and Adducted thumbs), SPG1 (X-linked complicated hereditary spastic paraplegia type 1) and X-linked ACC (Agenesis of Corpus Callosum).

Because only 20% of the patients clinically suspected of having L1 syndrome carry a mutation in the *L1CAM* gene, other genes might be involved. An X-chromosome specific 48K-oligonucleotide array was used to detect deletions that could include genes potentially involved in L1 syndrome. We have focused on the X-chromosome, since 95% of all L1 syndrome patients are males and family cases mostly show an X-linked recessive inheritance. By screening 60 selected patients (without a mutation in the *L1CAM* gene), 10 candidate deletions were identified containing 11 candidate genes, *DGAT2L6*, *POU3F4*, *GDI1*, *ASMTL*, *ATP6AP1*, *MID1*, *FRMPD4*, *MAP3K15*, *TGIF2LX*, *DDX53* and *TMEM27*. Based on additional criteria five genes were selected as being the best candidates, e.g. *POU3F4*, *DGAT2L6*, *GDI1*, *DDX53* and *TGIF2LX*. Sequence analyses of these five genes in 95 L1 syndrome patients showed in only one gene (the *GDI1* gene), in addition to the deletion, one probably pathogenic mutation, c.153-3C>T. From the low deletion and mutation frequency found, we conclude that at least the five selected genes do not appear to be major players in L1 syndrome.

Not finding a frequently involved second L1 syndrome gene might be caused by the selection of the candidate genes (maybe, one or more of the remaining six genes do harbor more mutations) or by the initial screening method searching for micro deletions (>2kb). By using this method, genes that only have point mutations or small deletions could have been missed. To overcome both we plan X chromosome-specific exome resequencing for this cohort.

2172/W

Heterozygous Germline Mutations in the CBL Tumor-Suppressor Gene Cause a Noonan Syndrome-like Phenotype. *S. Martinelli[1], A. De Luca[2], E. Stellacci[1], C. Rossi[3], S. Checquolo[4], F. Lepri[2], V. Caputo[1], M. Silvano[1], F. Buscherini[3], F. Consoli[2], G. Ferrara[4], M.C. Digilio[5], M.L. Cavaliere[6], A. van Hagen[7], G. Zampino[8], I. van der Burgt[9], G.B. Ferrero[10], L. Mazzanti[11], I. Screpanti[4], H.G. Yntema[9], W.M. Nillesen[9], R. Savarirayan[12], M. Zenker[13], B. Dallapiccola[5], B.D. Gelb[14], M. Tartaglia[1].* 1) Dipartimento di Ematologia, Oncologia e Medicina Molecolare, Istituto Superiore di Sanità, Rome, 00161, Italy; 2) Ospedale "Casa Sollievo della Sofferenza" IRCCS, San Giovanni Rotondo, 71013, Italy; 3) U.O. Genetica Medica, Policlinico S. Orsola-Malpighi, Bologna, 40138, Italy; 4) Dipartimento di Medicina Sperimentale, Università "La Sapienza", Rome, 00161, Italy; 5) Ospedale Bambino Gesù, Rome, 00165, Italy; 6) U.O. Genetica Medica, Ospedale "A. Cardarelli", Naples, 80131, Italy; 7) Department of Clinical Genetics and Human Genetics, VU University Medical Center, Amsterdam 1007, The Netherlands; 8) Istituto di Clinica Pediatrica, Università Cattolica del Sacro Cuore, Rome, 00168, Italy; 9) Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, 6500, The Netherlands; 10) Dipartimento di Pediatria, Università di Torino, Turin 10126, Italy; 11) Dipartimento di Pediatria, Università di Bologna, Bologna 40138, Italy; 12) Genetic Health Services Victoria and Murdoch Children's Research Institute, Royal Children's Hospital, Parkville, 3052, Melbourne, Australia; 13) Institute of Human Genetics, University Hospital, Magdeburg, 39120, Germany; 14) Child Health and Development Institute, Mount Sinai School of Medicine, New York, NY, 10029, USA.

RAS signaling plays a key role in controlling appropriate cell responses to extracellular stimuli, and participates in early and late developmental processes. While enhanced flow through this pathway had been established as a major contributor to oncogenesis, recent discoveries have revealed that aberrant RAS activation causes a group of clinically related developmental disorders characterized by facial dysmorphism, a wide spectrum of cardiac disease, reduced growth, variable cognitive deficits, ectodermal and musculoskeletal anomalies, and increased risk for certain malignancies. Here, we report that heterozygous germline mutations in CBL, a tumor-suppressor gene encoding for a multivalent adaptor protein with E3 ubiquitin ligase activity mutated in myeloid malignancies, can underlie a phenotype with clinical features fitting or partially overlapping Noonan syndrome (NS), the most common condition of this disease family. Independent CBL mutations were identified in two sporadic cases and two families from among 365 unrelated subjects with NS or suggestive features and negative for mutations in previously identified disease genes. Phenotypic heterogeneity and variable expressivity were documented. Mutations were missense changes altering evolutionary conserved residues located in the RING finger domain or the linker connecting this domain to the N-terminal tyrosine kinase binding domain, a known mutational hot-spot in myeloid malignancies. Mutations were shown to affect CBL-mediated receptor ubiquitylation and dysregulate signal flow through RAS. These findings document that germline mutations in CBL alter development to cause a clinically variable condition that resembles NS and that possibly predisposes to malignancies.

2173/W

TRIM50 Williams Beuren Syndrome gene, a novel component of aggresomes, interacts with p62. *C. Fusco[1], M. Egorov[2], M. Monti[3], L. Micale[1], B. Augello[1], E.V. D'Addetta[1], F. Cozzolino[3], M.G. Turturo[1], A. Calcagni[1], M.N. Loviglio[1], P. Pucci[3], R.S. Polishchuk[2], G. Merla[1].* 1) Laboratory of Medical Genetics, IRCCS Casa Sollievo Della Sofferenza Hospital, San Giovanni Rotondo, Italy; 2) Telethon Institute of Genetics and Medicine, Via P. Castellino 111, 80131 Naples, Italy; 3) CEINGE Advanced Biotechnology and Department of Organic Chemistry and Biochemistry, Federico II University, Napoli, Italy.

We recently showed that TRIM50 encodes a cytoplasmic E3-ubiquitin ligase. TRIM50 is hemizygous in the Williams Beuren syndrome, a developmental genomic disorder, caused by a 1.5 Mb deletion at 7q11.23 including about 28 genes. We have further characterized TRIM50 by performing fluorescence and electronic microscopy and proteomic approaches. Our results show that TRIM50 forms cytoplasmic bodies highly mobile, labile and dynamic. TRIM50 bodies, in response to MG132 proteasomal inhibition, by a microtubule dependent transport move towards to and colocalize to aggresomes by strengthen its interaction with HDAC6, a well-characterized aggresomes component. Further we provide experimental evidences that TRIM50 interacts and colocalize with p62/SQSTM1, a multifunctional adaptor protein involved in various cellular processes including autophagic clearance of aggregation-prone polyubiquitinated proteins. Together the data presented show that TRIM50 protein forms a multimeric protein complex with both HDAC6 and p62. The proposed model suggests that TRIM50 might mediate the ubiquitination of yet unknown substrates for the Proteasome-dependent degradation under basal cellular conditions. Under proteasome inhibition, TRIM50 through HDAC6-microtubules association participates to and likely directs the storage of polyubiquitinated misfolded protein to aggresomes. Then, in concert with p62, TRIM50 delivers them to autophagy for ultimate degradation. Finally this work point out that the haploinsufficiency of TRIM50 might play a role in the determination of Williams Beuren syndrome phenotypes through the accumulation of its yet unknown substrates.

2174/W

BBS Mutational Analysis in a Multi-Ethnic Cohort: A Strategic Approach. *G. Billingsley[1], C. Deveault[1], E. Héon[1,2].* 1) Programme of Genetics & Genome Biology; 2) Department of Ophthalmology and Vision Sciences, The Hospital for Sick Children, Toronto, Ontario, Canada.

Bardet-Biedl syndrome (BBS, OMIM 209900) is a rare autosomal recessive, genetically heterogeneous disorder for which 14 BBS genes have been identified. This genetic heterogeneity, with the size of the genes involved, challenged the cost effectiveness of mutational analysis of BBS, which influenced the various approaches used to find mutations. We present our approach to mutational analysis of our multi-ethnic cohort of 83 BBS families (104 cases) for BBS1 -12. Our results suggest that strategies for efficient and cost-effective screening are possible and should take in consideration the type of population studied. We have identified two BBS disease alleles in 75% of our families. In our study the most commonly mutated genes were BBS10 (30%), BBS1 (23%), BBS2 (17.5%) and BBS12 (16%). Together these 4 genes account for 61% (51/83families) of the total mutational load. No mutations were found for BBS3 or BBS11. 68% of the identified principle mutation alleles were novel (46/68) and were found only in one family. The presence of these "private" alleles creates a challenge for rapid screening. Recurrent mutations (BBS1-M390R, BBS10 - C91LfsX5, BBS2-Y24X) were mostly of European ancestry except for one (BBS10: K243IfsX15 - found in South African Black). 33 of our patients had homozygous BBS mutations. 39% of these (13/33) reported consanguinity and another 22% (7/33) were from geographic isolates. Initial screening for recurrent mutations in BBS1 (M390R - 13% of our cohort) and BBS2 (Y24X - 2.5%) by PCR-restriction enzyme, followed by sequencing of BBS10 and BBS12 (1-2 exons) can potentially detect 45-50% of the BBS patient mutations. Subsequent sequencing of BBS1 would bring this to at least 60% detection. The use of dedicated microarrays for assaying previously reported BBS mutations (e.g. Asper Ophthalmics) would have identified only 37% of our cohort, mostly in BBS1 and BBS10. Analysis of consanguineous or large isolated families using homozygosity mapping (STRP/SNP) and sequencing of the related BBS genes was successful in identifying mutations in BBS5 and BBS8, genes that are minor contributors to BBS. For a mixed population, preliminary screening of recurrent mutations, sequencing of BBS10 and BBS12 and homozygosity mapping of consanguineous families can greatly facilitate the detection of disease causing BBS mutations. For a predominantly Caucasian population, the sequencing of BBS1 could be included earlier.

2175/W

Genetic screening of LCA in Belgium: predominance of *CEP290* and identification of potential modifier alleles in *AHI1* of *CEP290*-related phenotypes. *F. Coppieters[1], I. Casteels[2], F. Meire[3], S. De Jaegere[1], S. Hooghe[1], N. Van Regemorter[4], H. Van Esch[5], H. Kroes[6], J. Vandewalle[7], T. de Ravel[5], B.P. Leroy[1, 8], E. De Baere[1].* 1) Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium; 2) Department of Ophthalmology, Leuven University Hospitals, Leuven, Belgium; 3) Hôpital Des Enfants Reine Fabiola, Brussels, Belgium; 4) Centre de Génétique de Bruxelles, Free University of Brussels, Brussels, Belgium; 5) Center for Human Genetics, Leuven University Hospitals, Leuven, Belgium; 6) Department of Medical Genetics, University Medical Center Utrecht, Utrecht, The Netherlands; 7) Department of Pediatrics, Ghent University Hospital, Ghent, Belgium; 8) Department of Ophthalmology, Ghent University Hospital, Ghent, Belgium.

Leber Congenital Amaurosis (LCA), the earliest form of all inherited retinal dystrophies, is genetically heterogeneous, with 14 genes accounting for 70% of patients. Here, 91 LCA probands underwent LCA chip analysis and subsequent sequencing of 6 genes (*CEP290*, *CRB1*, *RPE65*, *GUCY2D*, *AIPL1*, and *CRX*), revealing mutations in 69% of the cohort, with major involvement of *CEP290* (30%). In addition, 11 patients with early-onset retinal dystrophy (EORD) and 13 patients with Senior-Loken syndrome (SLS), LCA-Joubert syndrome (LCA-JS) or cerebello-oculo-renal syndrome (CORS) were included. Exhaustive re-inspection of the overall phenotypes in our LCA cohort revealed novel insights mainly regarding the *CEP290*-related phenotype. The *AHI1* gene was screened as a candidate modifier gene in three patients with the same *CEP290* genotype but different neurological involvement. Interestingly, a heterozygous novel *AHI1* mutation, p.Asn811Lys, was found in the most severely affected patient. Moreover, *AHI1* screening in five other patients with *CEP290*-related disease and neurological involvement revealed a second novel missense variant, p.His758Pro, in one LCA patient with mild mental retardation and autism. These two *AHI1* mutations might thus represent neurological modifiers of *CEP290*-related disease.

2176/W

Homozygosity mapping for Bardet-Biedl Syndrome genes reveals *SDCCAG8* as a novel BBS gene and expands the phenotypic ciliopathy spectrum. *H. Dollfus[1,3], X. Bei[2], E. Schaefer[3], A. Zaloszyc[3], Y. Perdomo[3], K. Aliferis[4], A. Toutain[5], M. Gerard[6], L. Perrin[6], M. Fischbach[7], JL. Mandel[8], V. Marion[3], N. Katsanis[9], C. Stoetzel[3].* 1) Service de Genetique Medicale, Hopitaux Universitaires de Strasbourg, Strasbourg, France; 2) Developmental and Stem Cell Biology Center for Human Disease Modeling 456 Nanaline Duke Building Duke University Medical Center Durham, NC 27710; 3) Laboratoire de Génétique Médicale, Equipe Avenir-Inserm, Faculté de Médecine de Strasbourg, Université de Strasbourg, Strasbourg, France; 4) Centre de Référence pour les Affections Rares en Génétique Ophtalmologique (CARGO) et Service de Génétique Médicale, Hôpitaux Universitaires de Strasbourg, Strasbourg, France; 5) Service de génétique, Pôle Biologie, CHRU de Tours - Hôpital Bretonneau, Tours, France; 6) Unité fonctionnelle de génétique clinique, Département de Génétique, CHU de Paris - Hôpital Robert Debré, Paris, France; 7) Service de pédiatrie 1, CHU Hôpital de Hautepierre, Strasbourg, France; 8) Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), CNRS, INSERM, Université de Strasbourg, Illkirch, and College de France, France; 9) Center for Human Disease Modeling Duke University 466A Nanaline Duke Building Box 3709 Duke University Medical Center Durham, NC 27710.

Whereas more then 75% of families presenting with Bardet-Biedl syndrome (BBS), an emblematic ciliopathy, are known to carry mutations in the known BBS genes, identifying novel genes remains a challenge as large consanguineous informative families are rare. Homozygosity mapping (6.0 Affymetrix SNP chip) was performed on 22 consanguineous BBS families. One large consanguineous gipsy family showed a unique segment of homozygosity on chromosome 1 (240-242.38 Mb) spanning 5 candidate genes (PLD5, CEP170, SDCCAG8, AKT3, ZNF238) that showed an unremarkable DNA sequence of their coding exons. RNA isolated from fibroblast cell lines, derived from 4 affected members, revealed a deep intronic mutation (c.740+356C>T) predicted to cause the loss of an ESE site showed by in silico, Western Blot and Q-PCR analysis. Further exploration revealed two additional BBS families that both showed a very small homozygosity region (less then 2Mb) matching the chromosomal region of interest which carried mutations in SDCCAG8 (p.K227X/K227X for the first family and p.R374X/R374X for the second). Analysis of another major BBS cohort revealed two other families with SDCCAG8 compound heterozygote mutations respectively: Y232X/R247fsX250 and T482fsX493/D543fsX566. Herein, we show that SDCCAG8 is a novel BBS gene as all the patients showed constantly 4 among the following major diagnostic features for BBS: obesity, retinal degeneration, kidney failure, hypogonadism and mild mental retardation. Strikingly, the patients did not show polydactyly (a very frequent but not obligatory BBS feature) as compared to our phenotype-genotype data on more than 300 BBS families. This is the first genotype-phenotype correlation that could be an indicator for priority screening of SDCCAG8 after classical screening of the common BBS mutations. Meanwhile, independently another group identified SDCCAG8 (NPHP10) as a major Senior-Loken gene using a powerful exon capture strategy (Otto et al, submitted). Therefore, the spectrum of SDCCAG8 mutation includes at least two ciliopathy phenotypes: SLS and BBS. Overall, SDCCAG8 mutations account for a small mutational load in BBS families (about 1%) but discloses interesting phenotypic characteristics important for genetic counselling and for deciphering the biological involvement of the BBS genes in their multiple clinical manifestations.

2177/W

Somatic/gonadal mosaicism explains reduced penetrance in a syndromic form of ectrodactyly, including eye abnormalities, suspected through array-based comparative genomic hybridization. *S. Raskin[1,2], A. Bonalumi[1], J. Souza[1], F. Faucz[1], V. Sotomaior[1], S. Alliman[3], B. Dupont[4], F. Bartel[4], R. Rodriguez[4], C. Schwartz[4], C. Skinner[4].* 1) Pontificia Universidade Catolica do Paraná, Curitiba, Parana, Brazil; 2) Centro De aconselhamento e Laboratorio Genetika, Curitiba, Parana, Brazil; 3) Signature Genomics, Spokane, Washington, U.S.A; 4) Greenwood Genetic Center, Greenwood, South Carolina, U.S.A.

Background:Split-hand/foot malformation (SHFM), or ectrodactyly, is characterized by underdeveloped or absent central digital rays, clefts of the hands and feet, and variable syndactyly of the remaining digits. SHFM occurs as both an isolated finding and a component of many syndromes. SHFM is a heterogeneous condition caused by abnormalities at one of multiple loci. These include SHFM1 (SHFM1 at 7q21-q22), SHFM2 (Xq26), SHFM3 (FBXW4/ DACTYLIN at 10q24), SHFM4 (TP63 at 3q27), and SHFM5 (DLX1 and DLX 2 at 2q31). Where most SHFM conditions are caused by deletions or sequence mutations, SHFM3 is unique in that it is caused by submicroscopic tandem chromosome duplications of FBXW4/ DACTYLIN. Methods and Results: In order to show that array-based comparative genomic hybridization (array CGH) should be considered an essential aspect of the genetic analysis of patients with SHFM, we report on a family with two brothers who have ectrodactyly. Interestingly, both also have ocular abnormalities. Their sister and both parents are healthy with no signs of ectrodactyly. DNA of all five family members were analyzed using a commercial oligonucleotide-based DNA microarray. The two affected brothers were found to have a small duplication of approximately 539 kb at 10q24.32. This duplication includes LBX1, BTRC, POLL, and FBXW4/ DACTYLIN genes. Using qPCR we confirmed that the patients' sister and father do not have the microduplication, but we found the mother to be a carrier of a mosaic duplication, estimated at 20%. Conclusions: In this family, two children were affected with ectrodactyly and were found to have a duplication over the SHFM3 locus. The mother carries a mosaic duplication as a constitutional change. Therefore, we demonstrate that somatic/gonadal mosaicism is a mechanism that gives rise to SHFM. We also suggest that ocular abnormalities may be part of the clinical description of SHFM3.

2178/W

Autozygosity mapping to uncover new genes causing spondylocostal dysostosis. *D.B. Sparrow[1,2], D. Sillence[3], M.A. Wouters[4,5], S.L. Dunwoodie[1,2].* 1) Developmental Biology, Victor Chang Cardiac Research Institute, Sydney, NSW, Australia; 2) St Vincents Clinical School, Faculty of Medicine, University of New South Wales, Sydney, NSW, Australia; 3) Discipline of Genetic Medicine, The Children's Hospital at Westmead, Clinical School, University of Sydney, Sydney, NSW, Australia; 4) Structural and Computational Biology Division, Victor Chang Cardiac Research Institute, Sydney, NSW, Australia; 5) School of Medical Sciences, Faculty of Medicine, University of New South Wales, Sydney, NSW, Australia.

Abnormal vertebral segmentation (AVS) occurs with an incidence of 2 in 1000 live births. 90% of cases involve one or two vertebrae, causing a non-progressive spinal curvature termed congenital scoliosis (CS). 10% of cases are more severe, with malformation of multiple blocks of vertebrae, such as in the disorder spondylocostal dysostosis (SCD). AVS arises during embryonic development by a disruption of somitogenesis (the process of formation of the somites, the precursors to all skeletal muscle of the body and the axial skeleton). The Notch signalling pathway is a key regulator of somitogenesis, and it is therefore not surprising that the developmental defects in SCD can be caused by disruptions to components of the Notch signalling pathway. We have identified causative mutations in four genes associated with this pathway: DLL3, MESP2, LFNG and HES7. However, the genetic lesion has been identified in only 30% of SCD cases, suggesting that additional genes are associated with SCD. To uncover these genes, we are using autozygosity screening in consanguineous SCD-affected families, allowing rapid identification of chromosomal regions that are identical-by-descent in affected individuals. Chromosomal regions identified in this way are being screened for genes involved in somitogenesis and/or Notch signalling, and candidate genes screened for sequence variation. Since not all variation is necessarily causative of the congenital abnormalities, missense mutations present in affected individuals are being screened by a variety of functional assays to determine their effects on protein function.

2179/W

Non-syndromic bilateral and unilateral optic nerve aplasia associated with microdeletion of 10q23.33q23.33: first familial case and potential role of CYP26A1 and CYP26C1 genes in optic nerve development. *E. De Baere.* Ctr Med Gen Ghent, Ghent Univ Hosp, Ghent, Belgium.

Optic nerve aplasia (ONA, MIM #165550) is a very rare unilateral or bilateral condition that leads to blindness in the affected eye, and is usually associated with other ocular abnormalities. Although bilateral ONA often occurs in association with severe congenital anomalies of the brain, non-syndromic sporadic cases with bilateral ONA have been described. So far no autosomal dominant non-syndromic ONA has been reported. The genetic basis of this condition remains largely unknown, as no other developmental genes than PAX6 are known to be implicated in sporadic bilateral ONA. Here we report an autosomal dominant form of non-syndromic ONA in a Belgian pedigree, with non-penetrance in the first generation (I:1), unilateral microphthalmia and ONA in the second generation (II:1) and bilateral ONA in two sibs of the third generation (III:1; III:2). The affected individuals underwent an extensive ophthalmological, endocrinological and neurological evaluation, including neuro-imaging of the visual pathways. The latter uncovered unilateral ONA in the microphthalmic eye of II:1. Genomewide copy number screening revealed a 249-363 kb microdeletion of chromosome 10q23.33q23.33 in all affected individuals (II:1, III:1; III:2) and in unaffected I:1, containing three genes EXOC6, CYP26A1 and CYP26C1. The latter two encode retinoic acid degrading enzymes. This is the first study reporting an autosomal dominant form of non-syndromic ONA. The diagnostic value of neuro-imaging in uncovering ONA in microphthalmic patients is demonstrated. Our findings implicate the CYP26A1 and CYP26C1 genes as potential novel candidate genes for non-syndromic ONA.

2180/W

Genome-wide SNP Genotyping Identifies the *Stereocilin* (STRC) Gene as a Major Contributor to Pediatric Bilateral Sensorineural Hearing Loss. *L. Francey[1], Y. Sun[1], H. Kadesch[1], D. Clark[1], H. Hakonarson[1,2], N. Spinner[3], L. Conlin[3], I. Krantz[1].* 1) The Division of Human Genetics; 2) The Center for Applied Genomics; 3) and The Department of Pathology, The Children's Hospital of Philadelphia, Philadelphia, PA.

Hearing loss is the most prevalent sensory perception deficit in humans, affecting 1/500 newborns. Hearing loss can be syndromic or nonsyndromic and is genetically heterogeneous. Nearly 80% of inherited nonsyndromic bilateral sensorineural hearing loss (NBSNHL) is autosomal recessive. Although many causal genes have been identified, most do not significantly contribute etiologically, excepting *GJB2*, that accounts for nearly 50% of all recessive SNHL. More than 60% of all children with a NBSNHL do not have an identifiable genetic cause. To identify genetic contributors, we genotyped 560 *GJB2* negative pediatric probands with NBSNHL. Assaying for copy number variants (CNVs) we identified 4,136 deletion CNV's which were filtered by a CNV ranking program, PECONPI, that generated a ranked list of 184 CNVs including over 285 unique candidate genes. 3/560 probands and 1 affected sibling were found to have a homozygous deletion of Chr15q15.3 that includes the *Stereocilin* (*STRC*) gene, an additional 3 probands and 1 affected sibling were found to have a heterozygous deletion. In a control population, 9/1,193 were found to have a heterozygous deletion. Mutations in *STRC* have been rarely reported as a cause of recessive SNHL. 'Deafness Infertility Syndrome (DIS)' was described in 3 unrelated consanguineous families cosegregating SNHL, male infertility and a homozygous, contiguous gene deletion of Chr15q15.3, encompassing *STRC* and *CATSPER2*. After identifying the Chr15q15.3 deletion CNV, sequencing of the *STRC* gene was undertaken in probands with the CNV as well as a CNV negative cohort of 48 probands and 3 siblings with mild-moderate NBSNHL and 40 probands with severe-profound NBSNHL who were all *GJB2* negative. Interpretation of sequencing results has been complicated due to a tandem duplication of Chr15q15.3 and the existence of a *STRC* pseudogene that is 99.6% homologous to the *STRC* coding region. We identified 15 mild-moderate BSNHL probands with homozygous or compound heterozygous alterations of the *STRC* gene. The heterozygous deletion CNV probands were subsequently found to have a deleterious variant on the trans allele of *STRC*. In our subcohort of 48 mild-moderate probands without the CNV, 9 were found to have biallelic alterations in *STRC*, representing a minimal prevalence of 18% among mild-moderate NBSNHL probands. The severe-profound NBSNHL cohort was not found to have homozygous or compound heterozygous mutations.

2181/W

A review of novel NKX2.1/TITF1 mutations in a Molecular Diagnostics Laboratory. *S.M. Kirwin, K.M.B. Vinette, I.L. Gonzalez, V.L. Funanage.* Biomedical Research/Molecular Diagnostics Lab, Nemours/duPont Hospital for Children, Wilmington, DE.

Background/Objective: The transcription factor NKX2.1/TITF1 (thyroid transcription factor 1) belongs to the homeodomain containing transcription factor family and is located on chromosome 14q13. Haploinsufficiency of the gene product has been linked to both benign hereditary chorea (BHC) as well as the more recently described brain-thyroid-lung disease. The objectives of the present work: 1) detection of NKX2.1 mutations in patients suspected to have BHC, congenital hypothyroidism, interstitial lung disease or a combination of these symptoms; 2) analysis of mutations in related family members, and 3) report of unpublished mutations found in our laboratory. Methods: Sequencing of the NKX2.1 gene in samples submitted for molecular confirmation in suspected cases of BHC, choreoathetosis, thyroid dysfunction, or respiratory distress was performed. When sequencing analysis proved negative for mutations, an assay developed in our lab for copy number/dosage analysis allowed us to determine whether a deletion of one or more exons had occurred. Results: We have sequenced 112 probands and 14 related family members. We have detected 24 cases (21% of probands), which were positive for 22 different mutations. Three patients were deleted for one copy of NKX2.1; 10 exhibited frameshift changes resulting in an alteration of the stop codon; 4 carried a missense mutation; 6 were found to have a nonsense mutation, and 1 family was found to carry a splice site change. Thus far no mutations associated with known phenotypes have been detected in exon 1 of NKX2.1. We believe that a mutational hot-spot exists in the homeodomain within exon 3, as a large number of mutations occur in this region. Amongst family members tested, 78% of family members were found to carry the proband's mutation. As not all parents have been available or considered for genetic testing, we were able to confirm a de novo origin for the proband's mutation in only one case. One particular patient and a similarly affected parent were found to carry a synonymous change (S291S) of unknown pathogenicity, although this serine codon is highly conserved in 4 classes of vertebrates. Conclusion: Our findings reveal the critical role of DNA sequencing and copy number analysis of the NKX2.1 gene in patients exhibiting respiratory distress, elevated TSH and/or movement disorders.

2182/W

Identification of a novel duplication of chromosome 12q14 and the *TBK1* gene in normal tension glaucoma patients. *J.H. Fingert[1], J.L. Stone[2], A.L. Robin[3], B. Roos[1], L.K. Davis[4], T.E. Scheetz[1], W.L.M. Alward[1], Y.H. Kwon[4], T.H. Wassink[4], V.C. Sheffield[5,6], E.M Stone[1,6].* 1) Ophthalmology and Visual Science, University of Iowa, Iowa City, IA; 2) Glaucoma Specialists, Baltimore, MD; 3) Ophthalmology and International Health, Johns Hopkins University, Baltimore, MD; 4) Psychiatry, University of Iowa, Iowa City, IA; 5) Pediatrics, University of Iowa, Iowa City, IA; 6) Howard Hughes Medical Institute, Iowa City, IA.

Purpose: Normal tension glaucoma (NTG) is a significant cause of vision loss in the United States. We studied a large pedigree to identify the gene that causes NTG in its members and to better characterize this gene. **Methods:** An African American NTG pedigree was studied with linkage and copy number variation (CNV) analysis using microarrays of SNPs. We tested a cohort of 400 glaucoma patients (74 of which had NTG) and 500 control subjects for a CNV that was found in the NTG pedigree. We tested expression of genes encompassed by this CNV in human retina using RT-PCR. The effect of the CNV on expression of encompassed genes was tested using RNA collected from pedigree member skin cells using expression microarrays. **Results:** Ten members of the pedigree had an autosomal dominant form of NTG. Linkage analysis mapped the gene that causes glaucoma in the pedigree to a 17 Mbp region of chromosome 12 (Max NPL score = 19.83). Further examination of SNP markers within the linked region identified a duplication that spanned 700 Kbp. This duplication was not detected in a cohort of 500 control subjects nor was it previously reported in publicly available databases. Furthermore, two unique overlapping duplications were detected in two (2.7%) of 74 normal tension glaucoma subjects. The duplication spanned four genes (*XPOT*, *TBK1*, *RASSF3*, and *GNS*). RT-PCR experiments showed that all four genes are expressed in human retina. Microarray studies showed that expression of *TBK1* in fibroblasts was increased by 1.55-fold in family members with the duplication when compared to family members without the duplication. Expression levels of other genes in the duplication were not altered to the same degree. **Conclusions:** Linkage and CNV analysis of a large pedigree identified an NTG locus with a 700 Kbp duplication on chromosome 12. Duplication of one of the encompassed genes (*TBK1*) is the likely cause of NTG in our pedigree. The expression of *TBK1* is increased by the duplication and *TBK1* is already known to interact with another NTG gene (optineurin). Prior studies of TBK1 find that it is a kinase that regulates the expression of genes in the NFkB pathway, some of which are involved in apoptosis. The duplication of *TBK1* may lead to NTG via dysregulation of such pathways. These data suggest that duplication of *TBK1* is the cause of NTG in our pedigree and perhaps up to 2.7% of NTG cases. However, functional studies and animal model studies will be necessary for definitive proof.

2183/W

Genetics of isolated microspherophakia: identification of a novel locus. *A. Kumar[1], M.R. Duvvari[1], J. Shetty[2], G. Murthy[3], S.H. Blanton[4].* 1) MRDG, Indian Institute of Science, Bangalore, Karnataka, India; 2) Bangalore West Lions Superspeciality Eye Hospital and Cornea Grafting Center, Bangalore, Karnataka, India; 3) Prabha Eye Clinic and Research Centre, Bangalore, Karnataka, India; 4) Miami Institute of Human Genomics, University of Miami Miller School of Medicine, Miami, USA.

Microspherophakia (OMIM 251750) is a rare autosomal recessive (AR) congenital disorder characterized by small spherical lens. It may present as an isolated finding or occur together with a variety of features in one of several syndromic disorders such as autosomal dominant (AD) Marfan syndrome, AD (OMIM# 608328) and AR (OMIM# 277600) forms of Weill-Marchesani syndrome (WMS), AD glaucoma-lens ectopia-microspherophakia-stiffness-shortness syndrome (GEMSS, OMIM 137765), AD microspherophakia with hernia (OMIM 157150), and AD microspherophakia-metaphyseal dysplasia (OMIM 157151). Marfan syndrome (MFS) and AD WMS are both caused by mutations in the FBN1 (fibrillin-1) gene. Mutations in the ADAMTS10 gene cause AR WMS. The gene for isolated microspherophakia (MSP) has not been identified so far. The purpose of this study was to map and identify the gene for MSP in five consanguineous Indian families. We have performed a whole-genome linkage scan in one of the five families and identified a locus for the MSP on chromosome 14q24.1-q32.12 between markers D14S588 and D14S1050. The remaining four families did not map to this locus, suggesting genetic heterogeneity. We are currently evaluating candidate genes in the region. The detailed results of our linkage scan and candidate gene analysis will be presented and discussed.

2184/W

Linkage of Fuchs endothelial corneal dystrophy (FECD) to a novel locus on chromosome 17q21.2-22. *M.A. Minear[1], N.A. Afshari[2], J. Rimmler[1], B. Zhao[1], E. Balajonda[2], G.K. Klintworth[2], S.G. Gregory[1], Y.-J. Li[1].* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) Department of Ophthalmology, Duke University Medical Center, Durham, NC.

Fuchs endothelial corneal dystrophy (FECD) is a late-onset disorder and a leading indication for corneal transplants in the USA. To identify novel genetic loci that contribute to FECD, we ascertained a large multiplex Caucasian family in which FECD appears to segregate as an autosomal dominant trait. 38 family members were recruited: 17 were affected with FECD (grade 2 guttae or above, as defined by the Krachmer scale), 9 were unaffected, 7 had an unknown FECD status (due to young age or insufficient exam data), and 5 were spouses. Genome-wide SNP genotyping was performed using the Illumina GoldenGate Linkage Panel IV (5858 SNPs) or the Infinium HumanLinkage-12 platform (6090 SNPs). Within the most significant linkage peaks, SNPs that did not overlap between the two linkage chips were genotyped using Taqman allelic discrimination assays (Applied Biosystems, Inc.). Family relationships were confirmed with RELPAIR. Quality control measures excluded SNPs that deviated from Hardy-Weinberg equilibrium or had genotype call rates <95%, and excluded samples with call rates <95%. Two-point parametric (dominant and recessive models) and non-parametric linkage analyses were performed using FASTLINK and MERLIN, respectively. Multipoint parametric and nonparametric linkage analyses were performed using MERLIN on three sub-pedigrees to meet the bit size criteria of the program. Regions of interest were followed up with SIMWALK using the full pedigree. Markers in high linkage disequilibrium ($r^2 \geq 0.16$) were excluded to reduce type I error inflation due to missing parental genotype data. Two-point LOD scores > 1.0 were noted for 19 SNPs on 11 chromosomes with FASTLINK, and for 21 SNPs on 11 chromosomes with MERLIN. Only chromosome 17q21.2-q22 (13 cM) showed overlapping evidence of linkage between multiple statistical approaches. The highest LOD score under the MERLIN dominant multipoint model was 2.36 at rs962272, and 14 flanking SNPs had LOD scores above 2.0; with the Merlin nonparametric multipoint model, rs962272 had a LOD score of 1.21 and the same 14 flanking SNPs had LOD scores above 1.0. The SIMWALK multipoint model showed supporting evidence within the same region (peak LOD = 1.39 at rs736604, slightly proximal to rs962272). Further work in this locus is underway to identify a minimal haplotype sharing region to narrow the width of the linkage peak. Our findings suggest that a novel locus for Mendelian FECD exists on chromosome 17.

2185/W

Identification of a chromosomal locus associated with recessive dysfunction of sodium homeostasis in a Bedouin family. *R. Parvari[1,2], E. Muhammad[1], N. Leventhal[3], V. Chalifa-Caspi[2], J.C. Beck[4], V.C. Sheffield[4], E. Hershkovitz[3].* 1) Dept Gen & Virology, Ben Gurion Univ, Beer Sheva, Israel; 2) National Institute of Biotechnology in the Negev, Ben Gurion University of the Negev, Beer Sheva, Israel; 3) Pediatric Endocrinology & Metabolism unit, Soroka Medical Center, Beer Sheva 84101 and Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva , Israel; 4) Department of Pediatrics - Division of Medical Genetics, Howard Hughes Medical Institute, University of Iowa, Iowa City, IA USA.

Genetic diseases resulting in excessive salt loss from sweat glands include aldosterone insensitivity syndrome of multi-system pseudohypoaldosteronism type I (PHA) and cystic fibrosis that result from mutations in genes encoding epithelial Na+ channel (ENaC) subunits and transmembrane conductance regulator (CFTR) respectively. In three nuclear consanguineous families we identified a novel autosomal recessive dysfunction of sodium homeostasis, characterized by salt wasting in sweat glands in infancy that is responsive to supplementary sodium. After linkage to the ENaC and CFTR genes were excluded, genome wide linkage analysis using the Affymetrix GeneChip mapping 250K array was performed. One homozygous region larger than 16 cM and 11.9 Mb was identified on chromosome 15q21.3-q23 and corroborated after testing all family members for microsatellite markers in the regions. The lod scores for linkage to this region were 2.81 and 3.65 for two point and multiple points, respectively. This finding establish the contribution of a novel gene in the regulation of NaCl uptake in sweat secretions.

2186/W

Homozygosity Mapping and Mutation Profile of the *MYO7A* Gene in Saudi patients with Usher Syndrome. *K. Ramzan[1], F. Imtiaz[1], M. Al-Owain[2], G. Bin-Khamis[3], R. Allam[1], A. Al-Mostafa[1], S. Al-Hazza[4], K. Taibah[5].* 1) Genetics, KFSHRC, Riyadh, Saudi Arabia; 2) Medical Genetics, KFSHRC, Riyadh, Saudi Arabia; 3) Otolarynology, KFSHRC, Riyadh, Saudi Arabia; 4) Opthalmology, KFSHRC, Riyadh, Saudi Arabia; 5) ENT Medical Centre, KFSHRC, Riyadh, Saudi Arabia.

Usher syndrome deafness-blindness disorder, is a clinically and genetically heterogeneous disorder. Three clinical subtypes of Usher syndrome have been identified, with mutations in different set of genes. Usher syndrome type 1 (USH1) is the most severe form of Usher syndrome. The *MYO7A* gene is responsible for *USH1B*, the most common subtype. Homozygosity mapping is a robust approach that is highly suited for genetically heterogeneous autosomal recessive disorders in populations where consanguinity is prevalent. For genotyping, DNA samples were processed on Gene Chip Human Mapping 250K Arrays (Affymetrix, Santa Clara, CA) following the instructions provided by the manufacturer. Homozygosity mapping was carried out using CNAG software that uses SNP genotypes generated by a DM algorithm for the detection of copy number changes and blocks of homozygosity. For affected individuals where the homozygosity blocks were identified on chromosome 11q13.5 harboring MYO7A, direct sequencing was performed for the entire coding and flanking intronic regions. Because of the high frequency of consanguinity in our patients this approach allowed us to quickly identify eleven *MYO7A* mutations in fourteen families with Usher Syndrome. Though many different mutations in this gene have been identified worldwide, the purpose of this study was to document the *MYO7A* variants present in the Saudi population.

2187/W

A new locus for otosclerosis, OTSC10, maps to chromosome 1q41-44. *I. Schrauwen[1], N. Weegerink[2], E. Fransen[1], C. Claes[1], K. Vanderstraeten[1], M. Ealy[3], R. Pennings[2], C.W.R.J. Cremers[2], P. Huyghen[2], R.J.H. Smith[3], H.P.M. Kunst[2], G. Van Camp[1].* 1) Department of Medical Genetics, University of Antwerp, Belgium; 2) Department of Otorhinolaryngology, Donder's Centrum for Brain, Cognition and Behaviour, Radboud University Nijmegen Medical Centre, The Netherlands; 3) Molecular Otolaryngology Research Laboratories, Department of Otolaryngology, University of Iowa, USA.

Otosclerosis is a common form of hearing loss characterized by a disordered bone remodeling in the otic capsule. The abnormal bone remodeling can result in conductive hearing loss due to fixation of the stapes footplate, but sensorineural hearing loss can also be present. Although its etiology remains unknown, otosclerosis can be considered a complex disease with rare monogenic forms. Linkage analysis in large families segregating autosomal dominant otosclerosis has led to the identification of 7 loci (OTSC1-5, 7-8). However, none of the corresponding genes has been cloned. In the OTSC2 region however, indications have been found that TCRB is the causative gene in the region. In this study a new large Dutch otosclerosis family with autosomal dominant inheritance is investigated. After exclusion of the known loci, a genome scan was performed using the HumanCytoSNP-12 BeadChips (Illumina Inc, San Diego, USA). Linkage analysis using Simwalk version 2.91 localized the gene on chr1q41-44 with a maximum LOD score of 3.3. This locus, named OTSC10, measures 26.1Mb and contains 306 genes/gene predictions. This new gene localization confirms the strong genetic heterogeneity of otosclerosis, as almost every new large family still maps to a different locus. As no mutation for monogenic otosclerosis has been identified yet, this represents another opportunity to identify the first one.

2188/W

An exome strategy to identify the genetic basis of nonsyndromic mental retardation in large consanguineous Arab families. *S. Kantarci[1,2], L. Al-Gazali[3], P. Tonellato[1,2], M. Boguski[1,2], N. Bissar-Tadmouri[4].* 1) Department of Pathology, Beth Israel Deaconess Medical Center, Boston, MA; 2) Harvard Medical School, Boston, MA; 3) Department of Pediatrics, Faculty of Medicine and Health Sciences, UAEU, Al-Ain, United Arab Emirates; 4) Department of Basic Medical Sciences, University Of Sharjah, College of Medicine, Sharjah, United Arab Emirates.

Mental retardation (MR; intellectual disability) affects approximately 1-3% of the general population. Genetic causes are found in approximately two-thirds of cases. Non-syndromic mental retardation (NSMR) is isolated MR without major physical abnormalities, dysmorphism, or neurological abnormalities. An autosomal recessive mode of inheritance may responsible for about one-fourth of all cases with NSMR. Identification of loci/genes responsible for NSMR is difficult due to broad genetic heterogeneity, small family sizes in Western populations, and the absence of clinical criteria for grouping the families for linkage analysis. To our knowledge, 13 autosomal recessive NSMR loci (MRT1-MRT13) have been mapped. While numerous X-linked genes responsible for NSMR have been identified, only six autosomal genes (PRSS12, CRBN, CC2D1A, GRIK2, TUSC3, and TRAPPC9) are known to cause NSMR. A traditionally successful approach for the identification of novel autosomal recessive NSMR genes is through studying populations with high rates of consanguineous marriage or by studying isolated populations with a strong founder effect. In the world, the highest rates of consanguineous marriages are present among Arab countries ranging around 20-50% of all marriages, and specifically first cousin marriages which may reach 25-30% of all marriages. Therefore, rates of rare autosomal recessive diseases are higher in these populations compared to Western populations. We have recruited three consanguineous Arab families with at least three affected siblings who have prior normal standard karyotype and Fragile-X molecular testing. Clinical history, three-generation pedigree, dysmorphologic and neurologic examinations were obtained from each family. We use SOLID whole exome resequencing approach to identify novel genes associated with autosomal recessive NSMR. We are in the process of analyzing data of ~40Mb genome size (exomes). Knowledge about the underlying genetic basis of NSMR can result in more accurate genetic counseling (particularly prenatal genetic counseling), improved understanding of fundamental mechanisms, and hope of specific therapeutic interventions that address the specific genomic pathology involved. Furthermore, the knowledge that is likely to be gained from this study will improve clinical delineation of NSMR.

2189/W

Identification of mutations in Meckel Syndrome using Exome Sequencing. *J. Tallila[1], A. Daly[1], M. Kestilä[2], R. Salonen[3], I. Barroso[1], A. Palotie[1], L. Peltonen[1,4].* 1) Department of Human Genetics, Wellcome Trust Sanger Institute, Hinxton, United Kingdom; 2) National Institute of Health and Welfare, Public Health Genomics Unit, Helsinki, Finland; 3) Department of Medical Genetics, Vaestoliitto, Helsinki, Finland; 4) Institute for Molecular Medicine, Finland (FIMM), Helsinki, Finland.

Meckel syndrome (MKS, [MIM 249000]) is an autosomal recessive, early lethal disorder characterized by a combination of severe malformations. Minimum diagnostic criteria are cystic dysplasia of the kidneys with fibrotic changes in the liver and occipital encephalocele or some other central nervous system malformation. Identification of the underlying disease causing genes has shown that MKS is an allelic disorder with several other syndromes with overlapping phenotypes, e.g. Joubert syndrome. In the Finnish population 90% of the MKS cases are caused by homozygous mutations in either *MKS1* or *CC2D2A* (MKS6) genes: a 29 bp intronic deletion in the *MKS1* gene (c.1483-7_35del) and a C>T substitution in the coding region of *CC2D2A* (c.1762C>T), respectively. Both of these mutations result in abnormal splicing. In addition, systematic sequence analysis of all known MKS genes showed that two Finnish MKS cases were compound heterozygotes: one for mutations in *MKS1*; and one for mutations in *CEP290* (MKS4). In six typical unrelated Finnish MKS cases the underlying mutation still remains unknown. To solve the molecular background of MKS in these families we used exome sequencing, which has proven to be a powerful, efficient strategy for identifying genes underlying rare Mendelian disorders. We sequenced the "GENCODE exome" consisting of a 47.9Mb target in one sibling pair and four unrelated affected fetuses. After Agilent solution-based hybrid capture the enriched targets were sequenced using an Illumina GAII sequencer. An average of 5Gb of sequence was generated per affected individual as paired-end, either 54 or 76-bp reads, with a mean depth of »100-fold. The sequence was aligned using the MACH program and variants were called using SAMtools. The sequencing data was filtered against variants found in 1000 Genomes and dbSNP data. As MKS is a recessive disease, we monitored for genes with two novel potential pathogenic variants (non-synonymous and splice site), which also should be shared by the siblings. All the cases were found to share at least two or more potentially pathogenic variants in 10 transcripts. We are currently conducting follow-up studies and validating the detected variants.

2190/W

Association Study of Primary Open Angle Glaucoma (POAG) with SNP Markers on 2p16 Region. *R. Sharafieh[1,2], J. Aragon-Martin[1,2], A.H. Child[2], M. Sarfarazi[1].* 1) Molecular Ophthalmic Genetics Laboratory, University of Connecticut Health Center, Farmington, CT. USA; 2) Dept. of Cardiac and Vascular Sciences, St. George's, University of London, London, England UK.

Purpose: A combination of linkage mapping and gene association studies have been used to establish an association between SNP markers and a group of Afro-Caribbean POAG subjects from Barbados. The two highly associated SNPs of rs12994401 and rs1533428 are located within the *AK127244* gene and map to the upper boundary of the POAG locus of *GLC1H* on 2p16. We aimed to reproduce this study in an unrelated group of British Caucasian POAG subjects, and further screen our previously linked POAG families for possible mutations in the *AK127244* gene. **Methods**: A total of 692 Caucasian subjects (277 POAG, 71 LTG and 344 normal controls) were genotyped for 3 SNP markers on 2p16.3 region. An additional group of 34 African subjects were also genotyped for the same SNPs. Genotypic and allelic association studies were carried out by standard Chi-squared tests. Seven familial probands were also sequenced for the *AK127244* gene using an ABI-3100 Gene Analyzer instrument. **Results**: No genetic association was identified for POAG, LTG or their combined group with 3 SNP markers of rs1533428 (Allelic p-values of 0.368, 0.715 and 0.378), rs11686436 (0.334, 0.511 and 0.298) or rs12994401 (0.447, 0.715 and 0.620), respectively. Likewise, haplotype association tests between each two set of SNP markers for the pooled group of glaucoma cases (POAG and LTG) was not statistically significant. Only the T&T haplotype permutation for the rs1533428/rs11686436 combination was marginally significant (p=0.013). However, this test proved to be insignificant after correction for multiple testing. Comparison of allelic frequencies in 34 African cases with the published data did not show any significance for the 3 SNP markers that we tested. Since these 3 SNP markers are located within the *AK127244* gene, we further sequenced this gene in 7 familial probands from the *GLC1H* locus. Of the 41 SNP markers observed, 18 were non-coding and the remaining showed no mutations in the affected subjects. **Conclusions**: The reported genetic association of rs1533428 and rs12994401 in the Barbados cohort of Afro-Caribbean POAG cases could not be replicated in our group of 348 British Caucasian POAG and LTG subjects. Moreover, the *AK127244* gene, which harbors these SNP markers, showed no mutations in our *GLC1H*-linked POAG families. In conclusion, the reported genetic association may either have a minute role in the non-African POAG populations or be limited to the Afro-Caribbean population of Barbados.

2191/W

Mutations in TTC37 cause tricohepatoenteric syndrome (phenotypic diarrhoea of infancy). *D. Chitayat[1], J.L. Hartley[2,3], N.C. Zachos[4], B. Dawood[5], M. Donowitz[4], J. Forman[6], R.J. Pollitt[7], N.V. Morgan[3], L. Tee[3], P. Gissen[3], W.H.A. Kahr[8], A.S. Knisely[9], S. Watson[5], I.W. Booth[10], S. Protheroe[11], M.S. Murphy[10], E. de Vries[12], D.A. Kelly[2], E.R. Maher[3].* 1) Prenatal Diag & Med Gen, Mount Sinai Hosp, Toronto, ON, Canada; 2) Liver Unit, Birmingham Children's Hospital, Birmingham, UK; 3) Medical and Molecular genetics, Birmingham University, UK; 4) Department of Medicine Division of Gastroenterology and Hepatology Hopkins Centre for Epithelial Disorders Johns Hopkins University School of Medicine Baltimore, MD 21205; 5) Centre for Cardiovascular Sciences Institute for Biomedical Research College of Medical and Dental Sciences University of Birmingham Birmingham B15 2TT; 6) Institut Pasteur Unite de Bioinformatique Strucurale, Paris 75015, France; 7) Clinical Chemistry Department The Children's Hospital Sheffield S10 2TH UK; 8) University of Toronto, Program in Cell Biology, Research Institute of the Hospital for Sick Children, Toronto, ON, Canada; 9) Institute of Liver Studies / Histopathology King's College Hospital Denmark Hill London SE5 9RS UK; 10) The Medical School University of Birmingham Edgbaston Birmingham B15 2TT; 11) Department of Gastroenterology and Nutrition Birmingham Children's Hospital Steelhouse Lane Birmingham B4 6NH; 12) Department of Paediatrics Jeroen Bosch Hospital PO Box 90153 5200ME s-Hertogenbosch The Netherlands.

Trichohepatoenteric syndrome (THES) is characterised by life-threatening diarrhoea, immunodeficiency, liver disease, trichorrhexis nodosa, facial dysmorphism, hypopigmentation and cardiac defects. We further characterised the phenotype and elucidated the molecular basis of THES. Methods: Twelve patients with classical THES from 11 families had detailed phenotyping. Autozygosity mapping was undertaken in consanguineous families using 250k single nucleotide polymorphism (SNP) arrays and linked regions were evaluated using microsatellite markers which confirmed one region from which candidate genes were analysed. The effect of mutations on protein production and/or localisation in affected patient liver and intestinal epithelial cells was characterised by immunohistochemistry. Results: Previously unrecognised platelet alpha granule abnormalities were identified. The THES locus was mapped to 5q14.3 - 5q21.2. Sequencing of candidate genes demonstrated mutations in TTC37, encoding the uncharacterised tetratricopeptide repeat protein- thespin, which bioinformatic analysis suggests may be involved in protein-protein interactions or act as a chaperone. Preliminary studies of enterocyte brush-border ion transporter proteins showed reduced expression or mislocalisation in all THES patients. Conclusion: THES is caused by mutations in TTC37. TTC37 mutations have a multisystem effect which may be due to abnormal stability and / or intracellular localisation of TTC37 target proteins.

2192/W

Combined genome-wide SNP genotyping and exome sequencing for disease-gene mapping in a single family. *L. Potocki[1,2], O.A. Shchelochkov[7], M.N. Bainbridge[4], S.A. Yatsenko[1], D.M. Muzny[4], D.A. Scott[1], S. Ben-Shachar[1], R.A. Lewis[1,2,3,5,6], R.A. Gibbs[1,4], J.W. Belmont[1,6], L.M. Franco[1,3].* 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Texas Children's Hospital, Houston, TX; 3) Department of Medicine, Baylor College of Medicine, Houston, TX; 4) Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX; 5) Department of Ophthalmology, Baylor College of Medicine, Houston, TX; 6) Department of Pediatrics, Baylor College of Medicine, Houston, TX; 7) Division of Genetics, Department of Pediatrics, University of Iowa Hospitals and Clinics, Iowa City, IA.

We describe the cases of two siblings with a similar phenotype of severe developmental delay, microphthalmia, blepharophimosis, optic nerve hypoplasia with absent retinal vessels, and progressive bone marrow failure. Chromosome analysis revealed a 46,XY complement in one sibling and a 46,XX complement in the other. Sex reversal, with female external genitalia and gonadal dysgenesis, was observed in the child with the 46,XY chromosome complement. Copy number analysis performed with an Agilent 244,000-oligonucleotide microarray revealed no abnormalities. SNP genotyping on both siblings and their parents, performed with Illumina 610-Quad BeadChips, revealed extended regions of homozygosity in each affected individual. Under the assumption of an autosomal recessive disorder, we searched the genotyping data for regions that were homozygous in both affected offspring and heterozygous in each parent. These regions comprised approximately 30 megabases. We then sequenced the entire exome of one affected sibling with NimbleGen capture and SOLiD sequencing. The regions of interest compiled from the SNP genotyping data were used as a filter to reduce the number of candidate mutations identified by exome sequencing. Our results illustrate the potential and the limitations of this combined approach to identify disease genes when only a small number of affected individuals from the same family are available for analysis.

2193/W

Genomic Deletion of a Single Isoform of MBD5 at 2q23.1 is Associated with Microcephaly, Intellectual disability, and Autistic Behaviors. *L. Zhang[1,3], S. Debrosse[3], R. Pyatt[4,5], D. Thrush[4,5], A. Parikh[2,3].* 1) Dept Medicine, Case Western Univ SOM, Cleveland, OH; 2) Dept Pediatrics, Case Western Univ SOM, Cleveland, OH; 3) Dept Human Genetics, Case Western Univ SOM, Cleveland, OH; 4) Nationwide Children's Hospital, Columbus, OH; 5) Dept Pathology, Ohio State Univ, Columbus, OH.

The 2q23.1 microdeletion syndrome has been described in 17 patients to date and the size of underlying genomic deletions vary considerably. Clinical features of this syndrome include severe mental retardation (MR), short stature, microcphaly, epilepsy and coarse facies. Genomic deletion of *methyl CpG-binding domain protein 5 (MBD5)* gene including all of its six isoforms has been implicated to be responsible for the core features of this syndrome. Here we describe a patient with a clinical phenotype including microcephaly, hypotonia, psychomotor retardation, intellectual disability, autistic behavior, chronic constipation, polydipsia, hyperphagia and subtle dysmorphism but no seizures to date. Oligonucleotide microarray study revealed a single microdeletion of ~210 kb at 2q23.1 in this patient. Neither of the biological parents were found to have this deletion. Interestingly, this *de novo* deletion involves the 5'-UTR of only one of the six predicted isoforms of *MBD5* gene and does not overlap with previously identified minimal critical region encompassing the coding region that is shared among all *MBD5* isoforms. MBD5 is a member of the methyl CpG-binding domain protein family including MECP2 which is deficient in Rett syndrome. MBD5 contains a PWWP domain, which has been shown to bind methylated lysines of histones. Previous studies have shown that *MBD5* is expressed in the mammalian brain and in particular the deleted isoform was cloned by 5'-RACE from a human brain cDNA library. This isoform has multiple non-coding 5' exons, and a proximal promoter region 450kb upstream from the proximal promoter region of all the other isoforms. Although the final protein product is predicted to be the same as another major isoform, this isoform allows the protein to be expressed under regulations that are different from the others, which may be crucial in the developing brain. Although the precise function of MBD5 has not been characterized, it is a promising candidate to advance our understanding of cognition and autistic behaviors.

2194/W

Identification of a novel gene associated with recessive high myopia. *O.S. Birk[1], S. Mordechai[1], A. Pasanen[3], R. Ofir[1], K. El Amour[2], J. Levy[4], N. Belfair[4], T. Lifshitz[4], G. Narkis[1,2], K. Elbedour[2], J. Myllyharju[3], L. Gradstein[4].* 1) The Morris Kahn Laboratory of Human Genetics at the National Institute for Biotechnology in the Negev, Beer-Sheva, Israel; 2) Institute of Genetics at Soroka Medical Center,Ben-Gurion University, Beer-Sheva, Israel; 3) University of Oulu, Finland; 4) Department of ophthalmology at Soroka Medical Center,Ben-Gurion University, Beer-Sheva, Israel.

Autosomal recessive high myopia was diagnosed in Bedouin Israeli consanguineous kindred. Some affected individuals had also variable expressivity of early onset cataract, peripheral vitreo-retinal degeneration, and secondary loss of sight due to severe retinal detachments. Through genome-wide linkage analysis, the disease-associated gene was mapped to ~1.7 Mb on chromosome 3q28 (maximum LOD score 11.5). Sequencing of the entire coding regions and intron-exon boundaries of the 6 genes within the defined locus, identified a single mutation in one of the genes within the locus. In vitro studies of the mutated vs. native protein have been done to demonstrate the functional effect of the mutation. This is the first identification of a gene whose mutation is associated with autosomal recessive pathological myopia.

2195/W

Pelizaeus-Merzbacher-Like disease caused by a homozygous mutation in AIMP1/P43. *M. Feinstein[1], B. Marcus[1], I. Noyman[2], H. Flusser[2], Z. Shorer[2], I. Shelef[4], I. Cohen[1], S. Khateeb[1], S. Sivan[1], O.S. Birk[1,3].* 1) The Morris Kahn Laboratory of Human Genetics at the National Institute of Biotechnology in the Negev, Ben-Gurion University of the Negev, Beer Sheva, Israel; 2) Division of Pediatrics at Soroka Medical Center, Beer Sheva, Israel; 3) Genetics Institute at Soroka Medical Center, Beer Sheva, Israel; 4) Department of Radiology at Soroka Medical Center, Beer Sheva, Israel.

Pelizaeus-Merzbacher-Like disease (PMLD) is a hypomyelinating leukodystrophy, a disorder involving aberrant myelin formation presenting with rotatory nystagmus, progressive spastic paraplegia, severe motor impairment and neurological deterioration within the first months of life. The known forms of the disease are caused by homozygous mutations in GJA12 and HSPD1. Two remotely related Bedouin kindred in southern Israel presented with an autosomal recessive phenotype of PMLD. Homozygosity at the two known loci was ruled out in affected individuals. DNA samples of 5 affected individuals and 7 non-affected obligatory carrier first-degree relatives were analyzed using 250k SNP Affymetrix arrays, and fine mapping was done using microsatellite markers. The phenotype-associated locus was mapped to a 8.94 Mb region on chromosome 4q24 (maximum multipoint LOD score of 4.25). Sequence analysis of 14 candidate genes of the 39 genes in the region unraveled a two-nucleotide (CA) deletion mutation in AIMP1/P43 encoding ARS-Interacting Multifunctional Protein 1. AIMP1 functions as a non-catalytic component of the multi-synthetase complex, catalyzing the ligation of amino acids to their cognate tRNAs. The deletion causes a frameshift mutation resulting in a premature stop codon amputating the 312aa protein after 127 aa, abrogating AIMP1/P43's main catalytic domain .The mutation was not found in 190 control chromosomes.

2196/W

GENETIC ANALYSIS OF FAMILIAL AUTOSOMAL RECESSIVE HYPERCHLORHIDROSIS. *M. Feldshtein[1], S. El-Krinawi[2], B. Yerushalmi[2], D. Landau[2], H. Romi[1], B. Marcus[1], D. Vullo[3], R. Ofir[1], s. Sivan[1], C.T. Supuran[3], O.S Birk[1,4].* 1) The Morris Kahn Laboratory of Human Genetics at the National Institute of Biotechnology in the Negev, Ben Gurion University, Beer Sheva, Israel; 2) Division of Pediatrics,Soroka Medical Center,Beer Sheva, Israel; 3) Universita degli Studi di Firenze, Laboratorio di Chimica Bioinorganica, Rm. 188, Via della Lastruccia 3, I-50019 Sesto Fiorentino (Firenze), Italy; 4) Division of Genetics,Soroka Medical Center,Beer Sheva, Israel.

Cystic fibrosis (CF) is caused by mutations within the cystic fibrosis transmembrane conductance regulator (CFTR). Elevated levels of sweat electrolytes as measured in the "sweat test" are the classical routine diagnostic criterion for this disease. A Bedouin kindred in southern Israel presented with an autosomal recessive phenotype of elevated sweat electrolytes with no clinical evidence of CF. Homozygosity at the CFTR locus was ruled out in affected individuals. We studied 26 individuals (including 7 affected) of 3 related families. DNA samples of 7 affected individuals and 4 non-affected obligatory carrier parents were analyzed using 250k SNP Affymetrix arrays. Homozygosity loci unique to the affected individuals were further analyzed using microsatellite markers. The phenotype-associated locus was mapped to a 5.6 Mb region on chromosome 15q22 with a maximum two-point LOD score [Zmax] = 6.65, recombination fraction [q] = 0.0 at D15S153. Sequence analysis of 5 candidate genes of the 60 genes in the region, unraveled a single missense mutation in CA12, encoding carbonic anhydrase, a ubiquitous enzyme that catalyzes the reversible hydration/dehydration reactions of carbon dioxide. The mutation is within the preserved catalytic domain of the enzyme. Of 300 Bedouin controls, the mutation was found in only one individual in a heterozygous state. The mutant protein showed 71% activity of the wildtype enzyme. Unlike the wildtype enzyme which was not inhibited by chloride, bromide or iodide, the mutant protein was inhibited in the submicromolar range by these anions.

2197/W

Identification of the molecular defect underlying a novel neurological autosomal recessive phenotype. *D. Fine[1], H. Flusser[2], B. Marcus[1], S. Khateeb[1], Z. Shorer[2], S. Sivan[1], O. Birk[1,3].* 1) The Morris Kahn Laboratory of Human Genetics at the National Institute of Biotechnology in the Negev; 2) Division of Pediatric at Soroka Medical Center, Ben-Gurion University of the Negev, Beer Sheva, Israel; 3) Genetics Institute at Soroka Medical Center, Ben-Gurion University of the Negev, Beer Sheva, Israel.

A consanguineous inbred Israeli Bedouin kindred presented with an autosomal recessive phenotype of microcephaly, mental retardation and dysmorphism reminiscent of Cornelia de Lange syndrome, with a long philtrum, hypertelorism, thin lips, synophris, low anterior hairline and narrow forehead. Brain MRI and CT demonstrated agenesis of corpus callosum and/or partial hypoplasia of vermis - not characteristic to Cornelia de Lange syndrome. Homozygosity of affected individuals in loci of 3 genes associated with Cornelia de Lange was ruled out using STS markers. Genome-wide scan of 6 individuals using the GeneChip Human Mapping 250K SNP microarrays was done following fine-mapping using STSs, and candidate genes within this locus are being sequenced in search for the disease-associated gene. A 7Mb region of homozygosity on chromosome 10p harboring 48 genes was identified (maximum MultiPoint LOD score 4.99). Sequencing of 30 of the 48 genes identified a single duplication mutation of 13 nucleotides within the coding region of a novel gene, truncating the encoded 1,039 aa protein to 739 aa. The mutation was not found in 400 Bedouin control chromosomes.

2198/W

Identification of the molecular defect causing non-CF meconium ileus: a likely modifier of cystic fibrosis. *H. Romi[1], I. Cohen[1], S. El-Krinawi[2], D. Landau[2], B. Yerishalmi[2], R. Ofir[1], S. Sivan[1], O.S. Birk[1,3].* 1) The Morris Kahn Laboratory of Human Genetics at the National Institute for Biotechnology in the Negev, Beer-Sheva, Israel; 2) Division of Pediatricsat Soroka Medical Center,Ben-Gurion University, Beer-Sheva, Israel; 3) Genetics Institute at Soroka Medical Center, Ben-Gurion University, Beer-Sheva, Israel.

Meconium ileus (MI) is a form of neonatal intra-luminal intestinal obstruction that occurs in the terminal ileum due to accumulation of thick, viscoid meconium. MI occurs in ~15-20% of CF patients. Eighty to ninety percent of infants presenting with MI will develop CF later in life. A unique autosomal recessive form of non-CF meconium ileus was shown to be prevalent in a large inbred Israeli-Bedouin kindred. Some of the patients required surgical treatment while others managed with conservative treatment. Homozygosity of affected individuals at the locus of CFTR and at the CF modifier 1 locus was rued out. Genome wide homozygosity screening followed by fine mapping demonstrated linkage to a ~ 4 Mb region on chromosome 12p13. Two-point linkage analysis using SUPERLINK software demonstrated maximum LOD score of [Zmax] = 4.16 at recombination fraction [q] = 0.0. A missense mutation was found in one of the 60 genes within the locus, a gene encoding a protein that is expressed specifically in the intestine and that is known to interact with the CFTR protein. In-vitro assays demonstrated that the mutation dramatically decreased the enzymatic activity of the encoded protein. A second homozygous mutation in the same gene, fully abrogating the functional domain of the enzyme, was found in another case of non-CF MI in another inbred Bedouin kindred. Of 240 unrelated Israeli-Bedouin control individuals, only three were heterozygous to the affected allele and none were homozygous for the mutation. We suggest that the gene identified in this study is a likely candidate as a modifier of meconium ileus in CF.

2199/W

Pre and post-axial polydactyly caused by a novel dominant GLI3 mutation in a large kindred. *M. Volodarsky[1], Y. Langer[1], S. Sivan[1], O.S. Birk[2].* 1) The Morris Kahn Laboratory of Human Genetics at the National Institute of Biotechnology in the Negev; 2) Genetics Institute at Soroka Medical Center, Ben-Gurion University of the Negev, Beer Sheva, Israel.

A large Jewish Moroccan kindred presented with 12 cases of isolated polydactyly. Affected individuals had either pre-axial, post-axial or combined polydactyly. Most had also syndactyly. Using polymorphic markers adjacent to candidate genes, linkage to SHH, GJA1, HOXD13, LMBR1, FBLN1 was ruled out. Analysis using markers D7S691 and D7S1526 near GLI3 followed by sequencing of the coding sequence of the gene, demonstrated that the phenotype in this family was due to a novel c.A1802G (p.H601A) mutation in exon 12 of GLI3 abrogating one of the C2H2 type zinc fingers of the encoded protein. The study demonstrates the significant phenotypic variability of GLI3 mutations, with the same mutation leading to pre or post-axial polydactyly.

2200/W

Characterization of the genetic defect underlying an autosomal recessive leukodystrophy. *J. Zolotushko[1], H. Flusser[3], B. Markus[1], M. Heverin[2], I. Björkhem[2], S. Sivan[1], O. Birk[1,3].* 1) The Morris Kahn Laboratory of Human Genetics, National Institute of Biotechnology in the Negev, Ben-Gurion University, Beer-Sheva, Israel; 2) Division of Clinical Chemistry, Karolinska University Hospital, Huddinge, Sweden; 3) Genetics Institute, Soroka Medical Center, Beer-Sheva, Israel.

An Israeli-Bedouin kindred presented with an autosomal recessive phenotype of convulsions near birth, mental retardation and cerebral palsy. Brain MRI demonstrated significant reduction in white matter and agenesis of corpus callosum. Linkage analysis ruled out association with twelve genes known to be associated with inherited defects of white matter or agenesis of corpus callosum. Using 250K SNP microarrays, a region of homozygosity on chromosome 1p33-1p32.3 was identified and further narrowed down to 6.75cM (7.25 Mb) between D1S2824 and D1S200, with a maximum multipoint LOD score of 6. Six candidates of the 62 genes in the linkage interval were sequenced, identifying a novel missense mutation, C307T, in DHCR24, resulting in substitution of arginine to cysteine at amino acid 103 (conserved exposed residue) within the flavin adenine dinucleotide (FAD) binding domain. DHCR24 (24-dehydrocholesterol reductase) encodes an FAD-dependent oxidoreductase expressed in the endoplasmic reticulum membrane, which catalyzes the reduction of the delta-24 double bond of sterol intermediates during cholesterol biosynthesis. Missense mutations in this gene have been associated with desmosterolosis. Plasma sterol quantification in two affected individuals demonstrated a normal cholesterol level, but ~300-fold increased levels of desmosterol proving deficient activity of 24-dehydrocholesterol reductase. The novel DHCR24 mutation we describe leads to excessive desmosterol accumulation much beyond that previously described, and a unique severe novel clinical phenotype.

2201/W

Mutation of THOC6, a member of the THO/TREX complex, causes autosomal recessive mental retardation. *C. Beaulieu[1], L. Huang[2], D. Bulman[3], E. Puffenberger[4], D.R. McLeod[1], A.M. Innes[1], K.M. Boycott[2], J.S. Parboosingh[1].* 1) Department of Medical Genetics, University of Calgary, Calgary, AB, Canada; 2) Department of Genetics, Children's Hospital of Eastern Ontario, Ottawa, ON, Canada;; 3) Ottawa Hospital Health Research Institute, Ottawa, ON, Canada; 4) Clinic for Special Children, Strasburg, PA, USA.

Mental retardation (MR; also known as intellectual disability) is the most frequent handicap affecting 2-3% of children. Autosomal recessive inheritance accounts for at least 25% of unexplained MR and is an emerging field of study with a number of genes now identified. We recently described a novel autosomal recessive neurodevelopmental disorder in four patients from two related Hutterite families. The patients have mild to moderate intellectual disability, head circumference at the 2nd centile, distinctive facial features, and minor malformations of the heart and genitourinary system. The Hutterites are a genetically isolated Anabaptist group living on the North American prairies; their population numbers over 40,000, the majority of whom are descendants of 89 founders. Identity-by-descent mapping identified a 5.1 Mb locus on 16p13.3 containing 173 genes. Genes within the region were prioritized using data mining and expression microarrays. Ninety-seven genes were sequenced. A homozygous variant, p.G46R, in THOC6 was identified in a conserved amino acid; this variant was not present in 150 general population or 500 Hutterite controls. THOC6 is part of the THO/TREX complex involved in the nuclear export of transcripts; knockdown of members of this complex has the greatest effect on the export of inducible heat shock protein mRNA. Expression changes observed on patient microarrays support a detrimental effect of THOC6 function on nuclear export of this group of mRNAs. Additional studies on mRNA export using patient cell lines and zebrafish functional studies are underway. This is the first example of a human disorder associated with the THO/TREX complex and may provide a link between the export of stress inducible transcripts and neurodevelopment.

2202/W

Exome Sequencing in Obligate Carrier Parents of an Autosomal Recessive Nonsyndromic Mental Retardation (NSMR) Sibship Reveals a Novel Mutation in the *TECR* Gene on Chromosome 19p13. *M. Caliskan[1], J.X. Chong[1], L. Uricchio[1], R. Anderson[1], P. Chen[1], C. Sougnez[2], K. Garimella[2], M. DePristo[2], S. Gabriel[2], S. Das[1], D. Waggoner[1,3], D.L. Nicolae[1,4,5], C. Ober[1,6].* 1) Human Genetics, University of Chicago, Chicago, IL; 2) The Broad Institute of MIT and Harvard, Boston, MA; 3) Pediatrics, University of Chicago, Chicago, IL; 4) Medicine, University of Chicago, Chicago, IL; 5) Statistics, University of Chicago, Chicago, IL; 6) Obstetrics and Gynecology, University of Chicago, Chicago, IL.

Mental retardation (MR) is one of the most common disabilities among children, affecting 1-3% of the population. The majority of MR cases occur without other associated abnormalities, a condition that is referred to as NSMR. We previously mapped a novel locus for autosomal recessive NSMR to chromosome 19p13 in a consanguineous family with 5 affected and 9 unaffected children (Nolan et al. 2008; Am J Med Genet 146A:1414). Subsequent genotyping of members of this family with the Affymetrix 500k array defined a 2 Mb critical region, which contained 82 genes. To identify the causal mutation in this family, we included the obligate carrier parents in a larger exome sequencing study. Of the 70 variant sites (33 missense, 37 synonymous) in the critical region, 18 were heterozygous in both parents and considered candidate NSMR mutations. We were able to eliminate 16 of the 18 as causal mutations because they had reported allele frequencies of >0.10 in dbSNP. The remaining 2 candidate mutations segregated with NSMR in the family but only one was predicted by both SIFT and PolyPhen to be a damaging mutation that very likely alters protein function and, therefore, downstream phenotypes. This putatively pathogenic mutation resulted from the substitution of a leucine for a highly conserved proline at amino acid 182 (Pro182Leu) in the TECR protein. TECR is a synaptic glycoprotein that is involved in the synthesis of very long chain fatty acids (VLCFAs). Although the precise mechanism through which the Pro182Leu mutation in TECR leads to mental retardation is not known, genetic diseases involving perturbations to normal synthesis and degradation of VLCFA have significant neurological consequences and mutations in other genes in the VLCFA pathway result in NSMR. The role of TECR in fatty acid synthesis and the phenotypes of affected individuals suggest that the NSMR observed in this family is an inborn error of metabolism in which fatty acids are not properly metabolized, highlighting the importance of normal lipid homeostasis for proper nervous system development.

2203/W

Combination of linkage mapping and microarray-expression analysis identify NF-κB signalling defect as a novel cause for non syndromic autosomal recessive mental retardation. *O. PHILIPPE[1], M. RIO[1], A. CARIOUX[2], JM. PLAZA[3], P. GUIGUE[1], F. MOLINARI[1], N. BODDAERT[1], C. BOLE-FEYSOT[3], P. NITSCHKE[3], A. SMAHI[1], A. MUNNICH[1], L. COLLEAUX[1].* 1) Hôpital Necker-Enfants malades, FRANCE, Paris, France; 2) Plateforme de Génomique de la Fondation Imagine Paris, France; 3) Plateforme de Bioinformatique de l'Université Paris Descartes, Paris, France.

Autosomal-recessive (AR) inheritance accounts for nearly 25 percent of non syndromic mental retardation (MR), but the extreme heterogeneity of such conditions markedly hampers gene identification. In the course of a project aiming to identify ARMR, we ascertained a consanguineous Tunisian family of three MR children with mild microcephaly and usual white-matter abnormalities with discordance between T2 and FLAIR sequences. The combination of autozygosity mapping and RNA expression profiling identified the *TRAPPC9* gene, which encodes a NF-kappaB-inducing kinase (NIK) and IkappaB kinase complex beta (IKK-beta) binding protein, as a likely candidate. Sequencing analysis revealed a nonsense variant (c.1708C>T [p.R570X]) within exon 9 of this gene that is responsible for an undetectable level of TRAPPC9 protein in patient skin fibroblasts. Moreover, TNF-alpha stimulation assays showed a defect in IkBalpha degradation, suggesting impaired NF-kappaB signalling in patient cells. A series of patients have been recruited on the basis of the association of MR, mild microcephaly and T2/FLAIR dissociation. Interestingly recent results of *TRAPPC9* molecular screening in these patients suggest that *TRAPPC9* mutations may define a clinically recognizable phenotype. In conclusion, this study provides the first demonstration of NF-kappaB signalling defect as a cause of autosomal recessive MR. Moreover, our study may shed light on the role of NF-kappa B in the myelination process in the central nervous system.

2204/W

Mutation screening and cellular complementation aid gene identification in mitochondrial complex I deficiency. *T.B.L. Haack[1], F. Madignier[2], K. Danhauser[2], J. Hoser[3], H. Mayr[9], I. Wittig[4], S. Biskup[5], P. Freisinger[10], B. Rolinski[8], E. Lamantea[7], G. Uziel[6], R. Horvath[11], M. Tesarova[12], V. Tiranti[7], W. Sperl[9], M. Zeviani[7], T. Meitinger[1,2], H. Prokisch[1,2].* 1) HealthInstitute of Human Genetics, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany; 2) Institute of Human Genetics, Technische Universität München, Munich, Germany; 3) Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München, Germany Research Center for Environmental Health, Neuherberg, Germany; 4) Molecular Bioenergetics, Medical School, Goethe-Universität Frankfurt, Frankfurt am Main, Germany; 5) CeGaT GmbH, Tübingen, Germany; 6) Unit of Child Neurology, Neurological Institute "Carlo Besta" - IRCCS Foundation, Milan, Italy; 7) Unit of Molecular Neurogenetics, Neurological Institute "Carlo Besta" - IRCCS Foundation, Milan, Italy; 8) Städtisches Klinikum München GmbH, Department Klinische Chemie, Munich, Germany; 9) Department of Paediatrics, Paracelsus Medical University, Salzburg, Austria; 10) Department of Paediatrics, Technische Universität München, Munich, Germany; 11) Medizinisch Genetisches Zentrum München, Munich, Germany; 12) First Faculty of Medicine, Department of Pediatrics, Prague, Czech Republic.

Complex I deficiency is a frequent biochemical condition, accounting for about one third of respiratory chain disorders. Impairment of complex I activity compromises the transfer of electrons derived from carbohydrate and, partly, from fatty acid oxidative catabolism to the downstream protein complexes of the respiratory chain. The broad phenotypic spectrum and seemingly loose association with causative gene defects pose a major hurdle in clinical diagnosis; hence, there is an urgent need for new strategies enabling us to fill the gap between biochemical definition and molecular correlate in complex I disorders. In 150 complex I-deficient index patients, we screened all 45 subunits and 6 assembly factors of complex I by high resolution melting curve analysis and Sanger sequencing and identified causative mutations in 30 cases. We identified for the first time mutations in genes encoding two complex I subunits and one assembly factor. Taking advantage of the defined biochemical defect present in patient-derived fibroblast cell lines, we performed a lentiviral complementation experiment to substantiate the pathogenic role of the mutations. Stable transduction of mutant cell lines with wild-type cDNA demonstrated a significant restoration of complex I activity. As many cases remained unresolved, we initiated a whole exome sequencing approach. With a focus on mitochondrial proteins and the complementation approach, we are able to reduce the number of candidate genes and to descriminate pathogenic from non-pathogenic variants.

2205/W

Identification of a missense mutation for Infantile Hypertrophic Pyloric Stenosis in the transcription factor *FOXF1*. *K.V. Everett, C. Shaw-Smith, R.M. Gardiner, E.M.K. Chung.* Institute of Child Health, University College London, London, United Kingdom.

Purpose: Infantile Hypertrophic Pyloric Stenosis (IHPS) is the most common cause of gastrointestinal obstruction in the first months of life with an incidence of 1-8 per 1000 live births in Caucasians. It affects infants 2-6 weeks after birth with symptoms of projectile vomiting, weight loss and dehydration. Disease pathology is restricted to the pylorus at the base of the stomach associated with smooth muscle hypertrophy. Treatment is by surgery. A genetic predisposition to IHPS is well established. It is commonly inherited as a 'complex' multifactorial trait but monogenic families are well described. We previously mapped the IHPS5 locus to chromosome 16q24 in a family with 8 affected individuals in which IHPS is inherited as a dominant trait with reduced penetrance. The aim of this study was to identify the disease gene, and disease-causing mutation, in this family. **Methods**: *In silico* interrogation of the chromosome 16q24 locus identified the transcription factor *FOXF1* as a candidate gene. *FOXF1* was re-sequenced in an affected individual from this family. Verification and familial segregation of a plausible causal variant was performed using a restriction digest. 570 control chromosomes taken from UK blood donors were genotyped for this same variant to determine population frequency. **Results**: Re-sequencing identified a single missense mutation, R139Q, in the transcription factor gene *FOXF1*. Restriction digest analyses demonstrated that it segregated in the heterozygote state with affected individuals and obligate carriers. This mutation was not detected in 570 control chromosomes. The R139Q mutation alters a conserved residue in the W2 domain of FOXF1, the region of the protein responsible for binding to the minor groove of DNA. **Conclusion**: We hypothesize that the R139Q change which we have identified in FOXF1 will affect DNA-binding and/or trans-activation ability. We predict that this causes IHPS either by altering the efficacy of FOXF1 to trigger the expression of a downstream target gene or by enabling FOXF1 to bind to non-specific targets; both may be part of a pathway involved in controlling smooth muscle development or function in the gut or pylorus. *In vitro* experiments to characterise the effect of the R139Q mutation are being performed. This should shed light on the pathophysiology of IHPS, reveal disease pathways and candidate genes for the condition, and improve the understanding of the role of FOXF1 in the development of the foregut.

2206/W

Initial analysis of whole exome and whole genome sequencing in the NIH Undiagnosed Disease Program. *T.C. Markello[1], D.A. Adams[1], K. Fuentes Fajardo[1], M. Sincan[1], H. Carlson-Donohoe[1], C.J. Tifft[1], T.M. Pierson[1,4], C. Toro[1], J.K. Teer[3], P.F. Cherukuri[3], N.F. Hansen[3], S.S. Ajay[3], H. Ozel Abaan[3], E.H. Margulies[3], P. Cruz[3], J.C. Mullikin[2], W.A. Gahl[1], NISC Comparative Sequencing Program.* 1) Undiagnosed Diseases Program, NIH/NHGRI, Bethesda, MD; 2) NIH Intramural Sequencing Center, NIH, Bethesda, MD; 3) Genome Technology Branch, NHGRI, NIH, Bethesda, MD; 4) Neurogenetics Branch, NINDS, NIH, Bethesda, MD.

The NIH Undiagnosed Diseases Program began analyzing single families with whole exome and whole genome sequencing in October 2009. We have completed sequencing 28 whole exomes and 1 whole genome. The current plan is to complete 60 exomes and 7 genomes by September 2010. The first 8 families chosen for sequencing included 3- four member families with two affected siblings and both parents, 3- three member families with a single affected along with both parents, and one four member family with one affected and one unaffected sibling with both parents. The final family had 4 individuals, with an autosomal dominant parent -child inheritance. One family had both parents and one affected child analyzed by whole exome, and the other affected child by whole genome sequencing. One family involved a consanguineous first cousin mating. 4 families had skeletal phenotypes and 7 of the 8 had significant neuro-developmental phenotypes. Exome capture and sequencing produced a total of 674Mb high quality sequence and an average coverage of 78.4% of the UCSC " known genes" sequence. The single whole genome data was truncated to the rest of the family's exome data. To analyze the sequences, high-density SNP array data previously obtained on these same individuals were used to construct linkage regions for recessive, dominant, and conjoint new mutations; this excluded up to 80% of the genome for recessive cases with two affected children. dbSNP130 data were used as an optional filter. For each variation from the reference sequence every family trio or quartet was examined for loci that followed homozygous, compound heterozygous recessive, or autosomal dominant (germ line mosaic) new mutation inheritance patterns. The final selection criterion used conservation information by the CDPred algorithm to prioritize candidates by the potential deleteriousness of each variation. For the single case of consanguinity, there were 113 variants that met criteria, 27 that were not in dbSNP130, and only 2 that had significant CDPred scores. One variation was identified and verified to be a homozygous mutation in AFG3L2, the first recessive diagnosis involving this gene. For the other families we have between 68 and 201 viable candidates that pass all exclusion constraints. These candidates are being evaluated using additional bioinformatics and laboratory assays. Analysis of these data and the remaining whole exome and whole genome evaluations are ongoing.

2207/W

A repeat mediated homozygous deletion in *ADAMTSL4* leads to isolated ectopia lentis. *A. Rump[1], J. Artelt[1], T.F. Neuhann[2], S. Tinschert[1], T.M. Neuhann[1].* 1) University of Technology Dresden, Institute of Clinical Genetics, Dresden, Germany; 2) Praxis Prof. Neuhann & Kolleagues, Munich, Germany.

To date, only two different homozygous mutations in *ADAMTSL4* have been reported in patients with isolated ectopia lentis, each mutation in one consanguineous family. We report eight individuals from seven non-consanguineous and non-related families with isolated ectopia lentis. All of them have an identical homozygous *ADAMTSL4* mutation not reported so far: a deletion of 20 bp within exon 6 (NM_019032.4:c.759_778del20). In a screen of 360 ethnically matched unaffected individuals, we found two further heterozygous mutation carriers. This deletion is flanked by a perfectly matching 8 bp direct repeat as well as two perfect DNA polymerase α frameshift hotspots, suggesting a repeat mediated recurrent mutation event. However, the deletion always comes along with the same SNP-haplotype in all individuals (affected as well as carriers), suggesting a single founder. These findings might best be explained by a "mixture" of both scenarios: a repeat mediated mutation due to the local DNA sequence environment occurred in a small number of individuals, i.e. there is a small group of different founders for the very same mutation. Our results further support the association of *ADAMTSL4* null-mutations to isolated ectopia lentis. Screening of *ADAMTSL4* should be considered in all patients with isolated ectopia lentis, with or without family history. In patients from non-consanguineous families, we propose a two-step diagnostic approach starting with an examination of exon 6 before sequencing the entire coding region of *ADAMTSL4*.

2208/W

Molecular studies of the PANK2 gene in patients with PKAN. *F. Annesi[1], M. Doco-Fenzy[2], G. Lesca[3], P. Tarantino[1], E.V. De Marco[1], D. Civitelli[1], F.E. Rocca[1], G. Provenzano[1], V. Greco[1], V. Scornaienchi[1], A. Gambardella[1,4], A. Quattrone[1,4], G. Annesi[1].* 1) Inst Neurological Sci, National Research Council, Mangone, Cosenza, Italy; 2) Service de Génétique, Hopital-Maison Blanche, CHU-Reims, 45 rue Cognacq Jay, 51092 REIMS cedex, FRANCE; 3) Service de Génétique moléculaire et clinique, CHRU de Lyon - Hôpital Edouard Herriot, 5 Place d'Arsonval, 69437 LYON CEDEX 03, FRANCE; 4) Institute of Neurology, University Magna Graecia, Catanzaro, ITALY.

Pantothenate kinase-associated neurodegeneration (PKAN) is an autosomal recessive disorder characterized by progressive dystonia, rigidity, choreoathetosis, spasticity, retinitis pigmentosa, optic atrophy, parkinsonism and iron accumulation in the brain. Clinical data suggest two forms of PKAN: a classic form characterized by early onset and rapid progression and an atypical form with later onset and a more slowly progressive course. Many patients with classical and atypical PKAN have mutations in the gene encoding pantothenate kinase 2 (PANK2) and a specific magnetic resonance imaging (MRI) pattern called eye-of-the-tiger. In this study we performed a mutational analysis of the PANK2 gene in 10 PKAN patients from Italy and France. Brain MRI examinations were not available for all the patients. The entire coding region (seven exons) was investigated for point mutations by sequencing analysis; furthermore, we used PCR real time to identify any possible exonic rearrangements. In 9 patients no PANK2 mutations were identified; only one patient showed an already described point mutation, but in the heterozygous state. Our results excluded PANK2 point mutations and exonic rearrangements in our patients, both in atypical and classic forms. This confirms the genetic heterogeneity in PKAN and therefore the importance of investigating the role of other responsible genes.

2209/W

Autosomal-dominant striatal degeneration is caused by a mutation in the phosphodiesterase 8B gene. *S. Appenzeller[1], A. Schirmacher[2], H. Halfter[2], S. Baeumer[2], M. Pendziwiat[2], V. Timmerman[3], P. De Jonghe[3], K. Fekete[4], F. Stoegbauer[5], P. Luedemann[6], M. Hund[7], E.S. Quabius[8], E.B. Ringelstein[2], G. Kuhlenbaeumer[1].* 1) Department Molecular Neurobiology; Institute of Experimental Medicine; University of Kiel; Kiel; 24105; Germany; 2) Department of Neurology; University of Münster; Münster; 48149; Germany; 3) Department of Molecular Genetics; VIB and University of Antwerpen; Antwerpen; 2610; Belgium; 4) Department of Neurology; University of Debrecen; Debrecen; 4012; Hungary; 5) Department of Neurology; Klinikum Osnabrück; Osnabrück; 49076; Germany; 6) St.Franziskus Hospital Ahlen; Ahlen; 59227; Germany; 7) Zürcher Höhenklinik Wald; Faltigberg; 8639; Switzerland; 8) Department of Immunology; University of Kiel; Kiel; 24105; Germany.

Statement of Purpose: The purpose of this study was the identification of the Autosomal Dominant Striatal Degeneration (ADSD) causing mutation and the analysis of its functional consequences. ADSD is characterized by bradykinesia, dysarthria and muscle rigidity resembling idiopathic Parkinson's disease. Methods: 17 members of a German ADSD family underwent neurologic examination and received MRI examinations. DNA was isolated from white blood cells. All individuals, seven of whom are affected, were included in the initial genome wide scan and the fine mapping. Coding exons and intronic flanking regions of all 21 protein coding genes in the candidate region were analyzed for sequence variations by direct DNA-sequencing and RFLP-analysis. Phosphodiesterase 8B (PDE8B) activity of mutated and wildtype PDE8B was determined using the cAMP-Glo™ Assay. Protein expression was determined qualitatively by western blot analysis. PDE8B expression in different human tissues was measured by quantitative real time PCR and qualitative RT-PCR. Subcellular localization was determined using confocal microscopy. Summary of results: We mapped ADSD to a 3.25 Mb candidate region on chromosome 5q13.3-14.1. A maximum LOD score of 4.1 (Θ=0) was obtained at marker D5S1962. We found one sequence variation resulting in a frameshift (c.94G>C+c.95delT) in the PDE8B gene. The mutation alters the amino acid sequence of the PDE8B-protein C-terminal of amino acid 31 and introduces a premature stop after 63 amino acids. The sequence variation segregates with ADSD and was neither found in 500 controls nor in the database dbSNP. No other potentially ADSD causing mutation was detected. The mutation results in a loss of cAMP-degrading activity. PDE8B expression is higher in putamen than in pallidum, total brain, and peripheral tissues. We did not observe any difference in the localization of wild type versus mutant protein. Conclusion: Our results strongly argue that ADSD is caused by the PDE8B mutation. However, these data would be strengthened further if a second family with ADSD had been available for the study. PDE8B degrades cAMP, a second messenger implied in dopamine signaling. Dopamine is one of the main neurotransmitters involved in movement control and is deficient in Parkinson's disease. We believe that the functional analysis of PDE8B will help to further elucidate the pathomechanism of ADSD as well as contribute to a better understanding of movement disorders.

2210/W

Differential expression of miRNA-411 in FSHD myoblasts. *N. Harafuji[1], S. Rongye[1], P. Schneiderat[2], M. Walter[2], YW. Chen[1,3].* 1) Center for Genetic Medicine Research, Children's National Medical Center, Washington DC; 2) Friedrich-Baur-Institute, Department of Neurology, Ludwig-Maximilians-University of Munich, Germany; 3) Department of Integrative Systems Biology and Department of Pediatrics, George Washington University, Washington DC.

Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant muscular dystrophy, which is linked to the contraction of the D4Z4 array at chromosome 4q35. Previous studies suggest that this shortening of the D4Z4 array causes an abnormal expression of genes, and defects in cell cycle regulation and differentiation of myotubes. In this study, we hypothesized that misregulation of microRNAs (miRNAs) in FSHD is involved in the disease progression. To identify miRNAs misregulated in FSHD myoblasts, we performed miRNA expression profiling using TaqMan Human MicroRNA Array v1.0 (Applied Biosystems). The profiles of eight primary human myoblasts (four FSHD and four controls) at two time points, 0 hour and 48 hours post differentiation (PD) were analyzed, respectively. We identified 26 and 11 miRNAs significantly changed in expression in FSHD myoblasts at 0 and 48 hours PD, respectively. The expression change of miR-411 at the 0 time point was further validated using TaqMan MicroRNA Assays (Applied Biosystems). To localize the miR-411 in myoblasts, we performed in situ hybridization with Locked Nucleic Acid (LNA)-Digoxigenin (DIG) labeled probes (Exiqon). The miR-411 was localized in the cytoplasm with stronger expression in the FSHD myoblasts. We further examined miR-411 expression in a mouse model of FSHD which overexpressing Pitx1 in skeletal muscles (Pandy and Chen, ASHG abstract) and localized the miR-411 expression in the atrophic fibers. Our results showed that miR-411 was upregulated in FSHD primary myoblasts and may be involved in the muscle atrophy process in FSHD.

2211/W

Candidate Mutation Discovery In Autosomal Dominant Diseases. *M. Bainbridge[1,2], D. Baldridge[3], I. Newsham[1], Y. Wu[1], M. Wang[1], D. Muzny[1], J.L. Jefferies[4], B.H. Lee[3], R.A. Gibbs[1].* 1) Human Genome Sequencing Center, Baylor College Med, Houston, TX; 2) Structural and Computational Biology and Molecular Biophysics, Baylor College Med, Houston, TX; 3) Molecular and Human Genetics, Baylor College Med, Houston, TX; 4) Pediatric Cardiology, Baylor College of Med, Houston, TX.

Rare coding variants in the human genome have been implicated in the development of a multitude of Mendelian diseases. Recent studies of large scale whole genome and targeted sequencing have shown that rare variants are significantly more common than originally thought. Our ability to distinguish disease causing mutations from benign mutations in targeted sequencing studies is thus hampered by the prevalence of rare non-synonymous SNPs, especially for dominantly inherited diseases. To alleviate this problem, we have developed a collection of human variants from multiple requencing projects conducted at the HGSC. Currently this collection contains over 120,000 singleton, coding non-synonymous variants which have not been described in either the Thousand Genomes project or in dbSNP. Using this resource, we were able to eliminate 50-75% of the candidate mutations in two separate whole exome sequencing projects to yield a short candidate list of causative mutations for autosomal diseases Osteogenesis Imperfect type V (OIV), and Left Ventricular Non-Compaction (LVNC). Our work outlines strategies for discovery of candidate mutations for autosomal dominant diseases and highlights the importance targeted sequencing across large, phenotypically-normal, populations.

2212/W

Linkage of a large Chinese family with nevoid basal cell carcinoma syndrome to a novel locus at chromosome 22q13. *T. Zhang[1], W. Chen[1], Q. Xing[2], W. Guo[1], Y. Lu[1].* 1) Dept. of Oral and Maxillofacial Surgery, Ninth People's Hospital,Shanghai Jiao Tong University School of Medicine, Shanghai, China; 2) Institutes of Biomedical Sciences Fudan University, Shanghai, China.

BACKGROUND Nevoid basal cell carcinoma syndrome (NBCCS,OMIM109400)is an autosomal dominant disorder characterized by a wide range of developmental abnormalities and a predisposition to neoplasms including multiple basal cell carcinomas (BCCs), keratocystic odontogenic tumor (KCOT), palmer and plantar pits and skeletal deformities. Recent evidences have indicated that patched gene, PTCH, is a NBCCS susceptibility gene. Our previous study has excluded PTCH as the causative gene in a large Chinese family with NBCCS, but the gene responsible is still unknown. OBJECTIVE To localize the pathogenic gene of the NBCCS family. METHODS Clinical data and blood samples were obtained from the extended pedigree with five generations including 10 affected members. A total genome scan was performed with a 500K SNP Affymetric DNA microarray for 21 individuals. After initial generation of a genome-wide, two-point LOD score, linkage was confirmed and the critical interval was established by genotyping of short tandem repeat (STR) microsatellite markers (D22S1177, D22S272, D22S423, D22S276,D22S1157, D22S1171, D22S282, D22S1140, D22S1168, D22S928, D22S1160). RESULTS A genome-wide linkage scan localized the disease interval to the long arm of chromosome 22, with a maximum two-point parametric LOD score of 4.72. Haplotype analyses refined the critical interval to 22q13.1-13.31, spanning a 6-MB region. CONCLUSION We have thus identified a new pathogenic gene locus for NBCCS on chromosome 22q13.1-13.31. The identification of the disease-causing gene will provide further insights into the pathogenesis of NBCCS.

2213/F

The SPATAX (Spastic PAraplegia and cerebellar ATAXia) network. *A. Brice[1,2], A. Mantonnier[1], M. Koenig[3], C. Depienne[1,2], S. Forlani[1], C. Tallaksen[4], G. Stevanin[1,2,5], A. Durr[1,2], all members of the SPATAX network.* 1) CR-icm, INSERM / UPMC UMR_S975, NEB, Paris, France; 2) APHP, Pitie-Salpetriere Hospital, Dpt of Genetics and Cytogenetics, Paris, France; 3) IGBMC, Illkirch, France; 4) Dep. of Neurology, Oslo University Hospital, Ulleval, Oslo, Norway; 5) EPHE, Paris, France.

Cerebellar ataxias and spastic paraplegias are two closely related groups of rare neurodegenerative diseases resulting from dysfunctions of the spinal cord andor the cerebellum or its afferent or efferent structures. These clinical signs are often associated in complex forms of these diseases. They are thus diagnosed in the same clinical centers and are studied according to similar protocols. This is why in 2001, Drs Alexandra Durr (Paris) and Chantal Tallaksen (Oslo), created the SPATAX network (Spastic PAraplegia and cerebellar ATAXia) to centralize biological material and coordinate research within the first international network on these rare diseases. The SPATAX network counts 36 teams of clinicians and scientists distributed in 17 countries, mainly from Europe, North Africa, Middle-East and South-America. Thanks to a very active collaboration, SPATAX members have collected a large number families and sporadic cases presenting these pathologies (more than 4500 sampled patients centralized in Paris), together with a very precise description of the symptoms according to a common clinical diagnosis chart validated within the network. This allowed important progresses in the knowledge of these very complex pathologies by the establishment of phenotype-genotype correlations in large series of patients. Since 2003, SPATAX reported 4 new loci for autosomal recessive spastic paraplegias (SPG28, SPG30, SPG32, SPG46), one new locus of dominant spastic paraplegias (SPG37), two loci in dominant ataxias (SCA25, SCA31) and 4 new loci among recessive ataxias (SAX2, ataxia with epilepsy, MSS, JBTS3). An effective collaborative work within the network allowed to identify simultaneously three new genes of spastic paraplegias, SPG11, SPG15 and SPG48. Similarly, in recessive ataxias, after the identification of the AOA2 gene, responsible as AOA1, of cases associating ataxia and oculomotor apraxia, we identified the ADCK3 gene. So, the continuous efforts of the network makes possible the establishment of the genetic diagnosis for more and more patients together with the design of rational diagnosis strategies.

2214/F

***De-novo* translocation involving *PTK2* and *THOC2* genes in a patient with cerebellar hypoplasia and mental retardation.** *E. Di Gregorio[1,2], M. Rolando[3], L. Verdun Di Cantogno[4], E. Grosso[2], S. Cavalieri[1], G. Stevanin[5,6], O. Zuffardi[7,8], F. Retta[1], C. Marelli[5], N. Ventura[9], A. Durr[5], A. Brussino[1,2], A. Brusco[1,2].* 1) Gen Biol & Biochem, Univ Torino, Torino, Italy; 2) S.C.D.U. Medical Genetics, Az. Osp. San Giovanni Battista, Torino, Italy; 3) Servizio di Neuropsichiatria Infantile, ASL TO3, Italy; 4) S.C.D.U. Anatomia Patologica, Az. Osp. San Giovanni Battista, Torino, Italy; 5) INSERM, UMR_S975 (formerly U679), Paris, France; 6) UPMC Univ Paris 06, UMR_S975, Centre de Recherche Institut du Cerveau et de la Moelle, CNRS 7225, Pitié- Salpêtrière Hospital, Paris, France; 7) Genetica Medica, Università di Pavia, Pavia, 27100 PV, Italy; 8) Istituto di Ricovero e Cura a Carattere Scientifico C. Mondino, Pavia, 27100 PV, Italy; 9) Caenorhabditis elegans laboratory, Department of Experimental Medicine and Biochemical Sciences, University of Rome “Tor Vergata”.

Nonprogressive congenital ataxias are rare and heterogeneous neurological syndromes. We report the case of a girl with cerebellar hypoplasia, and a non progressive form of congenital ataxia and mental retardation (MR). Karyotype showed a *de novo* translocation involving chromosomes Xq25 and 8q24. We defined the boundaries of the translocation using FISH analysis, and determined the breakpoints with long range PCR. Array-CGH (244K) excluded further deletion/duplication. On chromosome 8, the translocation interrupts intron 2 in the 5’ UTR of the Protein Tyrosine Kinase 2 gene (*PTK2*), which encodes for a tyrosine kinase involved in cellular migration and in central nervous system development and myelination. A half dose *PTK2* mRNA expression was shown by real time RT-PCR in patient’s fibroblasts. On the X chromosome, the translocation juxtaposes, in the same orientation, the promoter and exons 1-2 of *PTK2* to the THO Complex 2 gene (*THOC2*). THOC2 protein is involved in spliced m-RNA export from the nucleus to the cytoplasm. Using RT-PCR and a combination of a forward primer in *PTK2* exon 1, and a reverse primer in exons 2 or 6 of *THOC2*, we demonstrated that a fusion transcript is produced from the X-derivative: it contains exon 1 of *PTK2*, and continues in THOC2, skipping its exon 1. Because THOC2 exon 1 contains the first ATG (Met) codon, the fusion transcript is not predicted to give a protein. A wild-type *THOC2* mRNA is however produced, because the translocation leaves intact the wild-type promoter. Using real-time RT-PCR, we showed that in patient’s lymphoblasts *THOC2* mRNA was apparently up regulated (2.5 times), but this increase is mostly due to the fusion product (2.0) than to the wild type transcript (0.5). *THOC2* is highly expressed in Purkinje cells (BrainAtlas), compared to a widespread expression of *PTK2*. Using DHPLC screening, no mutation was found in *PTK2* (9 males, 9 females) and *THOC2* (9 males) genes in unrelated patients with a neuroradiologic/clinical profile similar to our patient. C.elegans *ptk-2* mutant does not have phenotype. Preliminary data for thoc-2 mutant show that animals that reach adulthood are sterile, with vulva defects, and barely move. The lin-12/Notch pathway independently specifies vulva development and neuronal functions regulating locomotion. In conclusion, we hypothesize that in our patient the fusion *PTK2-THOC2* transcript, driven by *PTK2* promoter, is causing a tissue specific downregulation of the wild type *THOC2* gene.

2215/F

Clinical features of Spinocerebellar ataxia type 31 (SCA31) in Japanese population. *Y. Ichikawa[1], K. Araki[2], F. Katada[2], T. Fukutake[2], S. Tsuji[1], J. Goto[1].* 1) Dept Neurology, Univ Tokyo, Tokyo, Japan; 2) Dept Neurology, Kameda Medical Center, Chiba, Japan.

Background: Autosomal dominant cerebellar ataxias (ADCAs) are heterogeneous neurodegenerative diseases characterized by progressive cerebellar ataxia occasionally accompanied with other findings. Spinocerebellar ataxia type 31 (SCA31) were mapped on 16q22.1 and caused by the insertion mutation. The C/T substitution in the 5'UTR of *PLEKHG4* was specifically linked to SCA31 patients. **Objectives:** To reveal frequency of SCA31 among Japanese ADCAs and clinical features of SCA31. **Patients and Methods:** The 366 Japanese ADCA families were analyzed on the referral basis. The gene loci of SCA1, 2, 6, 7, 8, 12, 17, Machado-Joseph disease (MJD) / SCA3, and dentarorubral-pallidoluysian atrophy (DRPLA) were examined by the fragment analyses. The insertion mutation of SCA31 was analyzed using the published primers (Sato *et al*., *AJHG*, 2009). Detection of the C/T substitution in *PLEKHG4* was accomplished by DHPLC analysis and direct sequencing method. **Results:** Among 366 Japanese ADCA families, MJD was the most common ADCA (105families: 28.7%), followed by SCA6 (73families: 19.9%) and DRPLA (56families: 15.3%). SCA31 ranked as the fourth frequent ADCA (50families: 13.7%). We examined 53 patients from 49 SCA31 families whose clinical information was available. The average age at onset of SCA31 was 57.7±6.8 years old (40-73 years). Significant correlation between the size of insertion and the age of onset was not revealed among our patients. All patients showed ataxic gait or dysarthria as their initial symptoms (ataxic gait: 83%, dysarthria: 17%). Although it has been reported that the characteristic clinical feature of SCA was slowly progressive pure cerebellar ataxia, pyramidal signs including hyperactive deep tendon reflexes or Babinski's positive signs were shown in 42% of the SCA31 patients. The C/T substitution in *PLEKHG4* was presented in 52 patients from 48 SCA31 families. Two patients from two independent families did not have C/T substitution but C/C genotype in *PLEKHG4*. They are suggested as recombinant cases. One of the two C/C type patients, 60 year-old female, showed depression, frontal signs, hyperactive tendon reflexes and rigidity besides cerebellar ataxia. **Conclusion:** SCA31 accounted for 14% of Japanese ADCA families. Pyramidal signs were shown in around 42% of the SCA31 patients. Among 50 SCA31 families, two families (4%) did not carry C/T substitution in *PLEKHG4*.

2216/F

Siblings of pathologically proven multiple system atrophy: an application of whole genome analysis toward finding strong genetic factors for sporadic diseases. *H. Ishiura[1], B. Ahsan[1], J. Mitsui[1], Y. Takahashi[1], Y. Fukuda[1], Y. Ichikawa[1], Y. Nakahara[1], K. Hara[2], H. Takahashi[2], A. Kakita[2], O. Onodera[2], M. Nishizawa[2], J. Goto[1], S. Tsuji[1].* 1) Department of Neurology, The University of Tokyo, Tokyo, Japan; 2) University of Niigata, Niigata, Japan.

Background Multiple system atrophy (MSA) is a sporadic neurodegenerative disorder characterized by various combinations of autonomic dysfunction, cerebellar symptoms, parkinsonism and pyramidal signs. MSA has been classified into two subtypes, MSA-C (characterized by predominant cerebellar ataxia) and MSA-P (characterized by predominant parkinsonism). Although MSA is a sporadic disorder, previous studies suggest that MSA-P is more prevalent in Europe or North America than in Japan, raising the possibility of involvement of some genetic factors. We have recently identified rare familial aggregations in MSA. Identification of the causative gene for MSA in these multiplex families would help to elucidate the molecular mechanisms of familial MSA, and, furthermore, bring insights into the disease mechanisms of sporadic MSA. **Method** A family with 2 siblings with pathologically confirmed MSA was examined. Their parents were first cousins, raising the possibility of autosomal recessive mode of inheritance. Linkage analysis was performed using SNP-HiTLink after genotyping with Affymetrix' SNP6.0 arrays. Whole genome resequencing of the proband was performed using an Illumina GAIIx system. **Result** With assumption of autosomal recessive mode of inheritance, linkage analysis showed the candidate region of ~70Mb on 4 chromosomes. A total of 187.5 Gb of paired end reads was obtained. 92.5% was mapped properly, and 89.9% was mapped uniquely. Average coverage was 58X. A total of 3.49M SNPs was called. In comparison with the SNP array calls, 99.8% called by the sequence analysis was concordant. Among the 3.49M SNPs, 3.15M SNPs (90.2%) were registered in dbSNP and exonic SNPs were 19,432, which were comparable with those of previous reports. Of the 3.49M SNPs, 67.3K SNPs were found in the candidate region, 248 were located in exons or splice sites, 134 were nonsynonymous or at splice sites, and only 4 were novel. **Conclusion** We conducted whole genome resequencing of a patient with familial MSA. The 4 novel nonsynonymous variants are considered as candidates for pathogenic mutation for MSA, which are being investigated.

2217/F

New mutation in the *PRKCG* gene in three Portuguese patients with autosomal dominant spinocerebellar ataxia. *C. Martins[1,2], J. Pinto-Basto[1,2,3], J. Sequeiros[1,2,3], I. Alonso[1,2].* 1) UnIGENe, IBMC, Univ. do Porto, Porto, Portugal; 2) CGPP, IBMC, Univ. do Porto, Porto, Portugal; 3) ICBAS, Univ. do Porto, Porto, Portugal.

Spinocerebellar Ataxia type 14 (SCA14) is an autosomal dominant neurodegenerative disorder characterized by progressive gait and limb ataxia, dysarthria and nystagmus, although other neurological symptoms may also be present. SCA14 is caused by mutations in the *PRKCG* gene which encodes PKCγ, a member of a family of serine/threonine kinases, which role is to regulate several cellular processes such as signal transduction, cell proliferation and differentiation and synaptic transmission. PKCγ has an N-terminal regulatory region composed by a C1 domain (with 2 Cys-rich sub-domains, C1A and C1B) responsible for diacylglycerol binding and a C2 domain, which mediates Ca^{2+} and phospholipids binding, and a C-terminal catalytic region composed by C3/C4 domains. We have studied the coding and the intronic flanking sequences of the 18 exons that compose *PRKCG* gene in a series of 54 index cases of Portuguese families with autosomal dominant spinocerebellar ataxia (ADCA), with or without other associated symptoms and negative for the presence of an expansion at the SCAs 1, 2, 3 (MJD), 6, 7, 17 and DRPLA *loci*. PCR and sequencing analysis were performed. Three patients presented new missense mutations at the regulatory region, one in exon 3 (at PKCγ C1A sub-domain) and two in exon 4 (at C1B). One of the mutations identified in exon 4 was confirmed in an additional patient from this family. Though the analysis of co-segregation of the mutation and the disease in the families is not concluded, several bioinformatic softwares indicated a pathogenic effect for the mutations detected. Clinically, all the cases presented progressive pure cerebellar ataxia and late age-at-onset of the disease (above the age of 53). These results confirm exon 4 as a mutational hot-spot where, until now, have been described 15 of the 26 *PRKCG* mutations found in SCA14 patients. Additionally, patients with ADCA, already tested for the most common SCA types, should be screened for mutations at *PRKCG* exon 3 (corresponding to PKCγ C1A sub-domain) and exons 4 and 5 (C1B sub-domain).

2218/F

Screening of 38 Nonsyndromic Autosomal Recessive Deafness Genes Identifies Mutations in 63% of Families in Turkey. *M. Tekin[1], A. Sirmaci[1], D. Duman[2], F.B. Cengiz[2], H. Ozdag[3].* 1) Dept Human Genetics, Univ Miami, Miami, FL; 2) Div Pediatric Genetics, Ankara Univ, Ankara, Turkey; 3) Biotechnology Institute, Ankara Univ, Ankara, Turkey.

More than 60% of prelingual deafness is genetic in origin, and of these up to 93% are monogenic autosomal recessive traits. Causal mutations have been identified in one of 38 different genes in a subset of patients with nonsyndromic autosomal recessive deafness (NSARD). In this study we screened 50 unrelated Turkish families with at least three affected children with NSARD born to consanguineous parents. Probands from all families were negative for mutations in the GJB2 gene, two large deletions in the GJB6 gene and the m.1555A>G substitution in the MTRNR1 gene. Each family was subsequently screened via autozygosity mapping with Affymetrix 10K SNP arrays. If the phenotype cosegregated with a haplotype flanking one of the 38 genes, mutation analysis of the gene was performed. We identified 23 different autozygous mutations in 11 genes, other than GJB2, in 27 of 50 families, which along with GJB2, explains deafness in 63% of families with NSARD. Relative frequencies of genes following GJB2 (18.9% from previous data) were MYO15A (9.7%), TMC1 (6.4%), TMIE (6.4%), OTOF (4.8%), MYO7A (4.8%), CDH23 (3.2%), SLC26A4 (1.6%), PCDH15 (1.6%), LRTOMT (1.6%), SERPINB6 (1.6%), and TMPRSS3 (1.6%). 18 of 23 mutations were reported for the first time in this study. All identified mutations cosegregated with deafness in families and were negative in at least 100 Turkish hearing controls. Novel deafness genes are yet to be discovered in the remaining 23 families (37%). This study was supported by Tubitak 105S464 and 108S045 grants and funds from UM to M.T.

2219/F

Re-sequencing analysis of candidate region for a neurodegenerative disorder by massively parallel sequencing. *T. Kaname[1,2], A. Tsujino[3], K. Yanagi[1], K. Hayashi[4], M. Tsukahara[2,5], K. Fujimori[2,6], I. Kikuzato[2,7], M. Teruya[2,8], Y. Imada[2,5], M. Nezuo[2,5], S. Yano[2,5], Y. Sato[2,7], Y. Miwa[2,5], T. Niikawa[9], K. Yoshiura[4], K. Naritomi[1].* 1) Dept Med Genet, Univ Ryukyus, Nishihara, Japan; 2) Okinawa Cutting-Edge Genome Project; 3) Dept Clinical Neuroscience and Neurology, Nagasaki Univ Graduate Sch Biomed Sci, Nagasaki, Japan; 4) Dept Hum Genet, Nagasaki Univ Graduate Sch Biomed Sci, Nagasaki, Japan; 5) TTC, Okinawa, Japan; 6) AIST, Tsukuba, Japan; 7) OSTC, Okinawa, Japan; 8) OITC, Okinawa, Japan; 9) Res Inst Personal Health Sci, Health Sciences University of Hokkaido, Hokkaido, Japan.

The genes underlying mendelian disorders have been identified through positional cloning, a process of meiotic mapping, physical mapping, and candidate-gene sequencing. Before the process of candidate-gene sequencing during positional cloning, it is important to narrow the candidate region for the disease, which requires survey of the genome in large number of families or patients. Due to the performance of the conventional sequencer, it is difficult to identify causative mutations for uncharacterized mendelian disorders, such as a disease consists of small number of familial cases. Next-Generation Sequencing technologies have brought new approaches of genetic analyses for personal genome or human diseases. The technologies can accurate giga-base order of sequence within a single run, allowing for complete resequencing of whole candidate region of a genetic disease in patients, whose responsible gene is not identified. Amyotrophic lateral sclerosis is a neurodegenerative disorder characterized by the death of motor neurons in the brain, brainstem, and spinal cord, resulting in fatal paralysis. It is well known that the ALS has genetic heterogeneity. Approximately 10% of ALS cases are familial. Many types of ALS, which have not been identified causative mutations, are still remaining. We analyzed candidate region in a familial ASL patient by massively parallel sequencing using next-generation sequencer. The candidate fragments were enriched by array-capture method. Then, a fragment library was constructed and sequenced to a depth >200-fold using the SOLiD 3 plus system. SNP calling program extracted 190 SNPs in the candidate region. Comparison of the calling SNPs and the dbSNP database allowed us to find novel four variations in the patient. In addition to the novel SNPs, 18 of non-synonymous were detected.

2220/F

HPRT deficiency causes broad transcriptional aberrations in cellular and signaling pathways. *T. Kang, T. Friedmann.* Department of Pediatrics, UCSD School of Medicine, La Jolla, CA.

HPRT deficiency in humans leads to the severe neurological and metabolic disorder of Lesch Nyhan Disease (LND). We have virtually no understanding of the mechanisms by which the underlying purine defects in LND produce the neurological phenotype. We have carried out extensive microarray-based analysis of global transcriptional patterns in human fibroblasts made HPRT deficient by expression of an shRNA targeted to HPRT. Gene ontology (GO) and gene set enrichment analysis (GSEA) have identified aberrant gene expression in a variety of cellular developmental and signaling pathways, including those related to the Alzheimer's-presenilin pathways. We have also used ChIP-Seq methods to examine the effects of HPRT deficiency on epigenetic gene regulation, and have identified a number of genes whose activation or inactivation by promoter interaction with methylated histones H3K4me3 and H3K27me3 is affected by HPRT deficiency. We conclude from these studies that HPRT plays a crucial role in regulation of gene expression in many cellular processes, apparently to some extent by epigenetic mechanisms. These new insights clarify some of the complex systems defects and mechanisms responsible for neuropathology in human HPRT deficiency.

2221/F

A novel homozygous intronic DARS2 mutation in a family with congenital leukoencephalopathy. *S. Miyatake[1], N. Miyake[1], S. Yamashita[2], K. Kurosawa[3], N. Matsumoto[1].* 1) Department of Human Genetics, Yokohama City University Graduate School of Medicine, Yokohama, Japan; 2) Department of Neurology, Kanagawa Children's Medical Center, Yokohama, Japan; 3) Division of Medical Genetics, Kanagawa Children's Medical Center, Yokohama, Japan.

Leukoencephalopathy is an inclusive condition presenting the impairment of the white matter of the brain, and has several different forms and causes. We analyzed a one Japanese-Chinese consanguineous family with severe congenital encephalopathy. Three of four children are affected in this family and two of the affected deceased at 8 years and 2 years old, respectively. Their common clinical phenotypes include psychomotor retardation, cerebellar ataxia and peripheral neuropathy. There were no abnormal biochemical findings. The level of lactate and pyruvate were within normal range in both blood and cerebrospinal fluid. Based on their consanguinity suggesting that this disease inherits in autosomal recessive fashion, we performed homozygosity mapping using three affected children, one unaffected child and unaffected parents by GeneChip Human Mapping 10K XbaI (Affymetrix). Then, we identified only an informative homozygous region on chromosome 1 spanning 8 Mb. Among this region, DARS2 (aspartyl-tRNA synthetase 2) gene is included, which is the causative gene for leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation (LBSL, MIM #611105). Although our cases did not show the elevation of lactate and pyruvate levels, their lesions and clinical features were very similar to LBLS. Thus we performed the direct sequence for DARS2 gene as a primary candidate gene, and identified a novel homozygous intronic mutation. This change was not observed in normal 184 control samples. We extracted mRNA from fibroblast derived from one patient and the father (mutation carrier) and confirmed the exon skipping by RT-PCR and sequencing. Furthermore, we showed complete loss and the decrease to half level of DARS2 protein in affected and carrier (parent) compared with the normal control fibroblast by immunoblotting, respectively. We concluded that the novel homozygous intronic DARS2 mutation is a real culprit for LBLS in a Japanese-Chinese consanguineous family.

2222/F

Parkin mutations and Juvenile Parkinson disease in Portuguese patients. *S. Morais[1], J. Pinto-Basto[1,2], J. Sequeiros[1,2], I. Alonso[1].* 1) CGPP and UnIGENe - IBMC, Univ. Porto, Porto, Portugal; 2) ICBAS, Univ.Porto, Porto, Portugal.

Parkinson disease (PD) is one of the most common movement disorders, the aetiology of which remains mostly unknown. The majority of PD cases are sporadic, although the discovery of genes linked to rare familial forms has provided valuable insights into disease mechanisms. Autosomal recessive juvenile Parkinson disease (AR-JP) is genetically heterogeneous and, apart from age at onset, clinically indistinguishable from idiopathic PD. AR-JP presents with rigidity, bradykinesia and resting tremor, usually before age 50 years. Abnormal behavior or psychiatric symptoms may also occur. Among the recessive forms, mutations in *PARK2*, mapping to chromosome 6q25.2-27, are found in approximately 50%; of the cases. PARK2 is composed by 12 exons and encodes parkin, an E3 ubiquitin ligase. We received samples from 155 Portuguese patients showing PD features with variable age at onset, and performed *PARK2* mutation analysis for diagnostic testing. Mutation screening was performed by PCR amplification of all coding regions, followed by bidirectional direct sequencing. In patients in whom no mutation was found, we also perform MLPA (multiplex ligation-dependent probe amplification) to detect possible large deletions/duplications. We confirmed the clinical diagnosis in 22.6% (35/155) of the patients. Age at onset of these cases ranged from 3 to 42 years-old. Sixteen different mutations were found, including missense mutations, small deletions or insertions, and large deletions. Homozygous mutations were found in 65.7%. Large deletions were present in 48.6% of the patients; the most frequent mutation - c.155delA - was present in 41.4% of the cases. Age at onset is an important clinical feature when considering the molecular genetic diagnosis of juvenile PD, but its variation is still considerable. Our results show that the c.155delA mutation is highly recurrent in the Portuguese population. Large deletions are responsible for juvenile PD in about half of these patients, making MLPA an essential approach in this molecular diagnosis, in addition to direct sequencing.

2223/F

Neurofibromatosis caused by neurofibromin mutations in a large cohort of Portuguese patients. *R. Bastos-Ferreira[1, 2], J. Pinto-Basto[1, 2], M. Barbosa[3], M. Reis-Lima[4], I. Alonso[1], J. Sequeiros[1, 2].* 1) CGPP and UnIGENe, IBMC, Porto, Portugal; 2) ICBAS, University of Porto, Porto, Portugal; 3) Centro de Genética Médica Jacinto Magalhães, INSA, Porto, Portugal; 4) HPP Boavista, Porto, Portugal.

Neurofibromatosis type 1 (NF1) is a common autosomal dominant disease with complete penetrance, but highly variable clinical expression. This condition is characterized by café-au-lait spots, axillary and inguinal freckling, neurofibromata, Lisch nodules, optic gliomas, distinct bone lesions and, in a subset of patients, learning disability/development delay. NF1 is caused by mutations in the *NF1* gene, one of the largest in the human genome. Mutations are scattered throughout the gene and include point mutations and small and large rearrangements, including whole gene deletions. In 50% of the patients mutations are *de novo*. *NF1* is located on chromosome 17q11.2, spanning over 250Kb, and is composed by 57 exons. Its product, neurofibromin, is a tumor suppressor protein, with 8,457 aminoacids. Our aim was to collect a large sample of NF1 patients, with detailed clinical data, and perform mutation analysis of the *NF1* gene, in order to establish genotype-phenotype correlations. We have ascertained 72 Portuguese patients with a clinical diagnosis of NF1 and performed neurofibromin mutation analysis, for diagnostic testing. Mutation screening was performed by PCR amplification of all coding and flanking regions, followed by bidirectional direct sequencing. In the Human genome there are several *NF1* pseudogenes which can lead to an incorrect diagnosis. In order to overcome this, we have introduced additional techniques such as RT-PCR and *NF1* isolation. Until now, we have performed mutational analysis in 27 clinically diagnosed NF1 patients and have found four nonsense mutations and one large gene rearrangement confirming the clinical diagnosis in five patients. All mutations result in truncated proteins, much smaller than the normal neurofibromin. In the remaining patients no mutations were found so far. Our sample of patients shows that neurofibromin truncated mutations may be frequent in Portuguese patients and that large gene rearrangements can also be present in a significant percentage of patients. NF1 has a large phenotypic heterogeneity and mutation screening in this gene can be invaluable to confirm and establish an early diagnosis, and to allow proper genetic counselling, including the offer of prenatal diagnosis.

2224/F

Autossomal Dominant Spastic Paraplegia: mutational characterization of a large sample of Portuguese patients. *A.F Brandão[1,2], A. Lopes[1], J.L. Loureiro[2,3], J. Pinto-Basto[1,2,4], P. Coutinho[2,3], J. Sequeiros[1,2,4], I. Alonso[1,2].* 1) CGPP, IBMC, Porto, Portugal; 2) UnIGENe, IBMC, Porto; 3) Serviço de Neurologia, Hospital de São Sebastião, Feira; 4) ICBAS, Universidade do Porto, Porto.

Hereditary spastic paraplegias (HSPs) are a group of rare neurodegenerative disorders with clinical and genetic heterogeneity. Clinically, can be divided into: pure, characterized mainly by slowly progressive weakness and spasticity in the lower limbs, and complex, with additional features. Genetically, three modes of inheritance have been described: autosomal dominant (AD), autosomal recessive (AR) and X-linked (XL). Among AD-HSP, mutations in *SPG4* and *SPG3A* are described to account for about 50% of cases. SPG4 encodes spastin, an ATPase belonging to the AAA protein family and SPG3A encodes atlastin-1, a Golgi transmembrane GTPase. Recently, mutations in the receptor expression enhancing protein 1 (*REEP1*) have been found to cause the third most frequent form of AD-HSP: SPG31. We have clinically ascertained 145 Portuguese patients corresponding to 74 families showing HSP with dominant inheritance and 71 patients without known family history of HSP and performed mutation analysis in the 3 genes, taking into account clinical information. Mutation screening was performed by PCR amplification of all coding and flanking regions, followed by bidirectional direct sequencing. In patients in whom no mutation was found, we have also performed MLPA to detect large deletions or duplications. By molecular analysis we identified 12 mutations in *SPG4*, 2 in *SPG3A* and 1 in *REEP1* (SPG31). In *SPG4*, we found 8 missense mutations, 5 of which are novel, a 3 bp duplication, 1 frameshift mutation and 1 intronic deletion. By MLPA, a large genomic non-contiguous deletion in *SPG4* was identified in a family with a complex phenotype and early disease onset. This large rearrangement was confirmed in two additional patients from this family. In *SPG3A*, both mutations identified are missense and affect atlastin-1 highly conserved residues, in patients with early disease onset. We have also found the first SPG31 Portuguese family, showing a *REEP1* nonsense mutation, in a patient with a pure phenotype. This novel mutation leads to a truncated protein. In conclusion, this work enlarges the number of patients and mutations identified in AD-HSPs and allow the estimation of SPG4, SPG3A and SPG31 mutation frequency. Until now, in Portugal, SPG4 represents 15% of the cases, SPG3A 5% and SPG31 1%, frequencies significantly different from the described in other populations.

2225/F

31 novel mutations in SPG11/spatacsin identified using both direct sequencing and MLPA. *G. Stevanin[1,2,3], E. Denis[2], E. Mundwiller[1], E. Ferdiko[2], C. Cazeneuve[2], E. Leguern[1,2], A. Durr[1,2], A. Brice[1,2], C. Depienne[1,2].* 1) CR-icm, INSERM / UPMC UMR_S975, NEB, Paris, France; 2) APHP, Département de Génétique et Cytogenetique, Groupe Hospitalier Pitie-Salpetriere, 75013 Paris, France; 3) EPHE, Paris, France.

Background: mutations in SPG11/spatacsin are the major cause of autosomal recessive spastic paraplegia with thin corpus callosum. Recently genomic rearrangements were also involved. Methods: We used 60 unrelated patients with spastic paraplegia and thin corpus callosum +/- mental retardation or cognitive delay, all referred to the Department of Genetics of the Pitie-Salpetriere Hospital for SPG11 diagnosis. The SPG11 gene was investigated using direct sequencing of all exons and MLPA (multiplex ligation-dependent probe amplification). Results: 40 different truncating SPG11 mutations, 31 of which were novel, were identified in 26 index patients (43.3%). 35 mutations were point mutations or small indel and 5 were genomic rearrangements that introduced premature termination codons in the protein sequence and were compatible with a degradation of the corresponding mRNA by the nonsense-mediated mRNA decay. In addition, 2 heterozygous missense variants which altered highly conserved amino-acid residues of the protein were found associated with a truncating mutation. Analysis of the affected relatives and parents, when possible, showed that the mutations segregated with the disease and that heterozygous compound mutations were inherited each from a healthy parent. Finally, we identified new missense polymorphisms that did not segregated with the disease. Conclusions: These findings expand the SPG11 mutation spectrum and highlight the importance of screening the whole coding region with both direct sequencing and a quantitative method. Rearrangements accounted for 12,5% of the mutated cases and can now be easily detected using MLPA. Rare missense polymorphisms are frequent in SPG11, complicating interpretation of diagnosis.

2226/F

Mottled hypopigmentation on the trunk in a Japanese case of PCWH with p.Q377X nonsense mutation in SOX10 gene. *K. Fukai[1], T. Oshimo[1], Y. Abe[2], Y. Hozumi[2], A. Tanaka[3], K. Yamanishi[4], M. Ishii[1], T. Suzuki[2].* 1) Dermatology, Osaka City Univ, Osaka, Japan; 2) Dermatology, Yamagata Univ. Yamagata, Japan; 3) Pediatrics, Osaka City Univ, Osaka, Japan; 4) Dermatology, Hyogo College of Medicine.

We reported a case of PCWH with SOX10 nonsense mutation on exon 5. The patient had a mottled hypopigmented macules on the truck, which was not described before. We also reviewed the cutaneous changes of PCWH and WS4 associated with SOX10 mutations. A Japanese boy was born after a 39-weeks pregnancy. He was the second child of healthy nonconsanguineous parents, with a healthy elder brother. There was no family history of dyschromatosis or neurological diseases. He had blue irises, nystagmus, and growth deficiency from birth. When he was a 3-month-old baby, his hair was light brown color, which was significantly lighter than most of the Japanese infants. Mottled hypopigmentation of the skin was apparent on his abdomen, inguinal and the thigh. At the age of 2 years, Hirschsprung disease was noted and the 15cm of the colon was surgically removed. Meanwhile, the otolaryngological examination revealed an anomaly of internal ear, the malfunction of the three semicircular canals, and severe deafness. At the age of three, he can manage to pull himself up by holding on to something but cannot walk, indicating severe delay of motor development. Heterochromia of the ireses has been pointed out by Ophthalmologists. We identified a single nucleotide substitution (1129C®T) in the last exon5 of SOX10 gene. This substitution changes codon 377 from CGA (Arginine) to the stop codon TGA (p.Q377X). So far, 22 cases with PCWH or WS4 associated with SOX10 mutations have been described. The so-called white forelock was noted in 5 cases with severe phenotype of PCWH. Therefore, the presence of white forelock may be indicative for PCWH with severe neurological changes. In this case, the hypopigmented macules were present not only on the abdomen, but also on the back, and the margin of the leukoderma is obscure. This is clearly different from the white macules of piebaldism, which is associated with KIT mutation. The presence of white macules on the back may suggest the significance of the SOX10/MITF system for the post-natal survival of melanocytes within epidermis. Dermatologists, especially pediatric dermatologists, should be aware that mottled hypopigmentation on the trunk is possibly a suggestive cutaneous sign for WS4 or PCWH.

2227/F

Jaffe-Campanacci syndrome and neurofibromatosis type 1, revisited: case report and review of the literature from the geneticist's perspective. *S.L. Ruppert, D.R. Stewart.* National Human Genome Research Institute, National Institutes of Health, Bethesda, MD.

Jaffe-Campanacci syndrome (JCS) is a disorder characterized by multiple non-ossifying fibromas (NOFs) of the long bones in association with café-au-lait macules (CALMs). NOFs are sharply demarcated osteolytic lesions located eccentrically in the metaphysial area. With completion of skeletal growth they stop enlarging and tend to heal spontaneously. If the repair is incomplete, however, pathologic fracture may occur. The CALMs seen in JCS patients typically have smooth ("Coast of California") borders. Axillary freckling has been reported in some JCS patients, but other signs of neurofibromatosis type 1 (NF1), including neurofibromas and Lisch nodules are reportedly absent. Mental retardation is a variable feature of the disorder. JCS was first described in 1958 and later named in 1982. The incidence and genetic etiology of JCS are not known. All reported cases to date are sporadic and without recurrence. No formal diagnostic criteria have been proposed. The literature includes several case reports of patients with both NF1 and multifocal NOFs, including a small number of families with more than one affected individual. Many of these patients have Lisch nodules and/or pathologically proven neurofibromas as well as CALMs and axillary freckling. There is only one report in the literature of a child with NF1 and multifocal NOFs who had genetic testing; this patient was found to have an intragenic NF1 gene deletion. We present a case report of a 26-year-old Caucasian male who received a clinical diagnosis of NF1 at age 6 years due to multiple CALMs and faint axillary freckling. There is no family history of NF1. At age 13 years he sustained bilateral femoral fractures that were retrospectively attributed to NOFs. No Lisch nodules or neurofibromas were noted at that time and it was therefore suggested that the findings were more supportive of a diagnosis of JCS. On examination at age 25 years the patient was found to have approximately 10 skin lesions consistent with cutaneous neurofibromas and Lisch nodules in both eyes. Genetic testing showed a nonsense mutation in the NF1 gene. The overlap between JCS and NF1 is striking. It would be valuable to patients, healthcare providers and researchers alike to understand whether the disorders are, in fact, allelic. This would improve genetic counseling and management of affected patients and would also raise awareness of multifocal NOFs as an established feature of NF1.

2228/F

Copy Number Variation and Loss of Heterozygosity In Saudi Patients With ASD. *N. Al Tassan[1], J. Shinwari[1], L. Al Sharif[1], D. Khalil[1], A. Almuslamani[2], H. Khalak[1], M. Ghannam[1], B. Meyer[1], M. Nester[2], M. Aldosari[2].* 1) Department of Genetics, KFSH & RC, Riyadh, Saudi Arabia. MBC-03-06 P.O Box 3354, Riyadh 11211 Saudi Arabia; 2) Department of Neurosciences , King Faisal Specialist Hospital. P.O Box 3354, Riyadh 11211 Saudi Arabia.

Autistic Spectrum Disorders (ASD) represents a genetically heterogeneous complex developmental disorder. Although there is no defined single genetic cause, in <10% of reported cases ASD has been found to be associated with a recognized cause, such as fragile X syndrome [FXS] (Xq27.3), tuberous sclerosis [TSC] (9q or 16p) and Angelman (AS)/Prader-Willi (PW) syndrome (15q11-q13 chromosome abnormality). Several approaches have been used to find candidate genes linked to/ or associated with ASD. These include genome wide scans, linkage studies of multiplex families, cytogenetic studies and copy number variation [CNV]. These different approaches have yielded a number of associated and susceptible genes and high risk loci in several chromosomes which include; 1p, 2q32, 5q, 6q21, 7q22,11p12-p13,13q, 16p13, 17q,19p and X=ACq13-q21. Single base pair substitutions in NLGN3, NLGN4, SHANK3 and PTEN genes were identified in rare cases of ASD with different degrees of severity. Here we report on the results of CNV and LOH in families with 2 or 3 affected individuals with ASD from the Saudi Arabia, where there is a high rate of consanguinity and endogamy. Clinical assessment of these patients classified them on the extreme end of the Autism spectrum. Analysis of 250K and/or 6.0 microarray data revealed a number of candidate loci in these families in which some may represent novel loci. Our data confirms the genetic heterogeneity and complexity of the disorder.

2229/F

A novel analysis of genomic rearrangements in multifactorial Hirschsprung disease. *Q. Jiang, Y-Y. Ho, A. Chakravarti.* Institute of Genetic Medicine, Baltimore, MD.

Hirschsprung disease (HSCR, OMIM 142623) is a developmental neurocristopathy characterized by the absence of intramural ganglion cells along variable lengths of the gastrointestinal tract. HSCR is a typical multifactorial disorder with an incidence of 1/5,000 live births, male predominance (sex-ratio of 4:1), sibling recurrence rate of 4%, variable expression and non-mendelian inheritance. To date, 12 genes (RET, GDNF, NRTN, SOX10, EDNRB, EDN3, ECE1, ZFHX1B, PHOX2B, TCF4, KIAA1279, SEMA3D (described at this meeting)) with segregating mutations and variants have been identified in syndromic, familial and sporadic cases. However, many other additional genes, identified through studies of mouse models of HSCR, are likely to exist. We have identified a total of 67 such proven and candidate HSCR genes. Approximately 12% of HSCR cases are likely to harbor a visible chromosomal change: the incidence of smaller chromosomal mutations and polymorphisms (CNVs) are unknown. In this study, we custom designed an array for comprehensive CGH analysis of dosage alterations in all of these 67 HSCR genes. We used the Agilent technology to assess genomic dosage using 45K probes across ~6 mb of the 67 genes and examined 11 isolated and 7 syndromic HSCR patients (16 males, 2 females). Of these, 8 cases were short-segment HSCR, 3 long-segment HSCR, 4 cases were total colonic aganglionosis and 3 cases were unclassified. To test the consistency of the technology and possible batch effect, we performed technical replication experiments in different batches. Under stringent criteria for calling CNVs, we identified 63 changes in the 18 patients (3.5 CNVs/patient). The most common loss, 8 out of 18 patients (44.4%), was a 2 kb deletion of the 5'UTR and translation start site of ZFHX1B (ZEB2), a gene mutant in Mowat-Wilson syndrome. The most common gain, 15 out of 18 patients (83.3%), was a 2 kb duplication containing SEMA3A and including a conserved sequence covering exon3 that encodes part of the sema domain. These changes do not exist in extant databases. Further validation of these results and a screening of a larger number of patients is currently underway.

2230/F

Large *SPG11* gene rearrangements cause Hereditary Spastic Paraplegia type 11 in Portuguese patients. *M.C. Pereira[1,2], J. Loureiro[2,3], J. Pinto-Basto[1,2,4], P. Coutinho[2,3], J. Sequeiros[1,2,4], I. Alonso[1,2].* 1) CGPP, IBMC, Porto, Portugal; 2) UnIGENe, IBMC, Porto, Portugal; 3) Serv. Neurologia, Hosp. São Sebastião, Feira, Portugal; 4) ICBAS, Universidade do Porto, Portugal.

Hereditary spastic paraplegias (HSPs) compose a group of heterogeneous genetic disorders both clinical and genetically. Among the autosomal recessive forms of these neurodegenerative diseases, SPG11 is the most common one, and is mainly characterized by early onset rapidly progressive spasticity of the lower limbs, the presence of a thin corpus callosum and cognitive impairment. In order to better understand SPG11 distribution among the Portuguese population we genetically characterized a group of 43 patients with spastic paraplegia. Mutation screening was performed by direct sequencing of the PCR amplified coding regions and flanking intron boundaries of the *SPG11* gene, to detect point mutations, followed by MLPA analysis for detection of large gene rearrangements (deletions or duplications). We were able to identify 8 different mutations (5 frameshift and 3 large deletions), confirming SPG11 in 9 patients. Amongst these, we found through direct sequencing a novel 4 base pair small deletion and though MLPA analysis three new large gene rearrangements, comprising two different single exon deletions and an interesting case of a heterozygous complex entire gene rearrangement. All result in a truncated spatacsin leading to disease through a loss of function mechanism. Our results, together with other previously reported SPG11 Portuguese families, show that point mutations, like c.529_533delATATT and c.733_734delAT, seem to be very prevalent in our population. Nevertheless, gene dosage analysis proved to be an important resource in SPG11 molecular diagnosis revealing that a considerable amount of SPG11 cases can arise from large gene rearrangements.

2231/F

Copy number variation as a marker of genomic instability and disease severity in neurofibromatosis type 1 (NF1). *J.D. Schiffman[1,2], B. Demarest[1], L. Cannon-Albright[3], D. Viskochil[2], D.A. Stevenson[2].* 1) Department of Oncological Sciences, University of Utah, Salt Lake City, UT; 2) Department of Pediatrics, University of Utah, Salt Lake City, UT; 3) Department of Internal Medicine, University of Utah, Salt Lake City, UT.

Background: Neurofibromatosis Type 1 (NF1) affects 1 in 3000 children and is caused by mutations in the *NF1* gene. NF1 individuals have a wide spectrum of clinical manifestations including bone dysplasias, cafe-au-lait macules, neurocognitive defects, and cancer. The severity of phenotype differs within and between NF1 families. Unlinked genomic modifiers of phentoype have been suggested, but not yet identified. Copy number variations (CNVs) recently have been described to affect risk in other complex diseases. Objective: To perform genome-wide CNV analysis in NF1 individuals as markers of increased genomic instability and modifiers of disease severity. Methods: DNA was extracted from NF1 cases (n=45) and run on Affymetrix SNP 6.0 microarray chips. SNP 6.0 CEL files were obtained from normal controls (n=101) from a previous, unrelated GWAS investigation (Shi et al. 2009). CNVs were identified using Nexus Copy Number software (BioDiscovery, Inc.). Total number CNVs/genome, total number CNVs within cancer genes/genome, and total structural variation (size)/genome were compared between NF1 cases and controls using Wilcoxon Rank Sum test. We also divided NF1 cases into mild (n=15) vs. severe (n=21) phenotypes in cases with enough clinical data and analyzed CNV differences between both categories. Results: NF1 cases had 118 ± 44 mean total CNVs vs. 54 ± 12 CNVs in controls ($p<2.2x10e-16$), 20 ± 13 mean CNVs within cancer genes vs. 8 ± 3 in controls ($p<2.5x10e-15$), and 32.5 ± 24 MB mean total structural variation/genome vs. 7.3 MB ± 5 in controls ($p<2.2x10e-16$). The slope of acquired cancer gene CNVs in NF1 cases was steeper than controls, corresponding to 26% increase in CNVs within cancer genes for every additional CNV ($p=4.23x10e-5$). We found 16 distinct CNV loci that differed between NF1 cases with mild vs. severe phenotypes with $p<0.05$, and 3 distinct loci with $p<0.01$. Conclusions: CNVs differ in total number, size, and location between NF1 individuals and healthy controls. In addition, this NF1 cohort had an increased number of cancer gene CNVs for every increase in over-all number of CNVs/genome. Furthermore, genome-wide CNVs differed between NF1 individuals with mild vs. severe phenotypes. These differences in genomic structural variation may reflect underlying genomic instability and explain the increased cancer risk in the NF1 population. We are currently in the process of validating the results in a larger cohort with additional controls.

2232/F

Molecular Diagnosis of Amyotrophic Lateral Sclerosis (ALS): Evaluation of Four Autosomal Dominant Genes. *M. Chen, S.D. Batish, C. Stanley, E. Couchon, C. Braastad, J. Jones, M. Ito.* Athena Diagnostics, Inc. Worcester, MA.

Amyotrophic lateral sclerosis (ALS) is an adult-onset, progressive neurodegenerative disorder characterized by the death of motor neurons in the brain, brainstem, and spinal cord, with eventual fatal paralysis. ALS prevalence in the United States is 4 to 8 per 100,000 individuals. There are more than twelve types of ALS with significant clinical overlap, and while the majority of cases are sporadic, approximately 5-10% of cases are familial. Heritable forms of the disorder show multiple modes of inheritance, with autosomal dominant being most prevalent. In May 2009, Athena Diagnostics, Inc., began offering DNA testing for ALS; the test menu includes four genes responsible for autosomal dominant forms of ALS, including *Superoxide Dismutase 1* (*SOD1*), *Fused in Sarcoma/Translated in Liposarcoma* (*FUS*), *Angiogenin* (*ANG*), and *TAR DNA-Binding Protein* (*TARDBP*). Herein, we report DNA test results for approximately 500 unrelated families in which the diagnosis of ALS was suspected based on clinical findings. In the *SOD1* gene, we identified 10 different previously published mutations in 24 patients; furthermore, two different missense mutations of unknown clinical significance were found in three additional cases. Molecular testing of *FUS* was positive in 11 individuals, with identification of three different previously published mutations in six individuals and five different frame-shift mutations predicted to be disease-associated. Interestingly, all five novel frame-shift mutations were located in one particular exon (exon 14). In addition, *FUS* sequencing identified five novel missense mutations of unknown clinical significance. Two different *ANG* mutations were identified in three patients, and one *ANG* variant of unknown clinical significance was found in two unrelated individuals. Finally, *TARDBP* sequencing identified four different previously published mutations in five individuals. In conclusion, *SOD1* mutations were found in 55% of test-positive individuals, *FUS* mutations accounted for 25% of positive test results, and *ANG* and *TARDBP* mutations were responsible for approximately 7% and 12% of genetically-linked ALS cases, respectively. When compared to *SOD1* testing alone, the addition of three new genes to Athena's ALS testing menu has almost doubled test sensitivity. The clinical sensitivity is approximately 8%, which is similar to other published reports.

2233/F

A new locus (SPG46) maps to chromosome 9 in a Tunisian family with a complicated autosomal recessive hereditary spastic paraplegia with mental impairment and thin corpus callosum. *A. Boukhris[1,2,3], I. Feki[2], N. Elleuch[2], M.I. Miladi[2], A. Boland-Augé[4], J. Truchetto[1], E. Mundwiller[1], D. Zelenika[4], A. Brice[1,3], C. Mhiri[2], G. Stevanin[1,3].* 1) CR-icm, INSERM / UPMC UMR_S975, NEB, Paris, France; 2) Department of Neurology, Habib Bourguiba University Hospital, Sfax, Tunisia; 3) AP-HP, Pitié-Salpêtrière Hospital, Department of Genetics and Cytogenetics, Paris, France; 4) Centre National de Génotypage, Evry, France.

Hereditary spastic paraplegia (HSP) with thin corpus callosum (TCC) and mental impairment is a frequent subtype of complicated HSP, often inherited as an autosomal recessive (AR) trait. It is clear from molecular genetic analyses that there are several underlying causes of this syndrome, with at least 6 genetic loci identified to date. However, SPG11 and SPG15 are the 2 major genes for this entity. Our objective was to map the responsible gene in a large consanguineous AR-HSP-TCC family of Tunisian origin excluded for linkage to the SPG7, SPG11, SPG15, SPG18, SPG21 and SPG32 loci. A genome-wide scan was undertaken using 6000 SNP markers covering all chromosomes. The genome-wide search identified a single candidate region on chromosome 9, exceeding the LOD score threshold of +3. Fine mapping using additional markers narrowed the candidate region to a 45.1-Mb interval (15.4 cM). Mutations in 3 candidate genes were excluded. Phenotypic presentation in 5 patients was suggestive of a complex HSP that associated an early onset spastic paraplegia with mild handicap, mental deterioration, congenital cataract, cerebellar signs and TCC. The mapping of a novel AR-HSP-TCC locus further demonstrates the extensive genetic heterogeneity of this condition. We propose that testing for this locus should be performed, after exclusion of mutations in SPG11 and SPG15 genes, in AR-HSP-TCC families, especially when cerebellar ataxia and cataract are present.

2234/F

Genetic heterogeneity in congenital mirror movements. *S. Gulsuner[1], H. Uysal[2], R. Bilgen[2], K. Bilguvar[3], M. Gunel[3], T. Ozcelik[1].* 1) Department of Molecular Biology and Genetics, Bilkent University Faculty of Science, Ankara, Turkey; 2) Department of Neurology, Akdeniz University Faculty of Medicine, Antalya, Turkey; 3) Department of Neurosurgery, Yale University School of Medicine, New Haven, CT, United States.

Mirror movements (MM) in humans are rare and they generally accompany conditions such as Klippel-Feil or Kallmann syndromes, Friedreich ataxia, phenylketonuria, Parkinsonism, schizophrenia, central nervous system invasions and epilepsies. They are characterized by involuntary contralateral movements of upper extremities during voluntary movements. Here, we describe a three-generation consanguineous family with multiple members displaying variable degrees of MM with or without juvenile myoclonic epilepsy. Pedigree analysis suggests autosomal dominant inheritance. High-resolution microarray genotyping of 16 individuals from the family and analysis of the data according to autosomal recessive model revealed stretches of 9 homozygous regions ranging from 1.04 Mb to 1.7 Mb on chromosomes 1, 3, 4, 7, 8, 16 and 17. Linkage analysis with a dominant model and full or 90 percent penetrance parameters independently resulted in positive LOD scores for three chromosomal regions: chromosome 3 (1.28), chromosome 15 (1.71) and chromosome 22 (3.62). Minimal critical regions for each locus were determined by haplotype analysis. Three chromosome 22 genes, SYN3, TIMP3 and LARGE, were sequenced in two affected individuals but no mutation detected. Recently, mutations in DCC were shown to cause congenital mirror movements in two chromosome 18 linked families (Science 328:592, 2010). However, chromosome 18 is not implicated under any inheritance model in our family. These findings suggest that there is genetic heterogeneity in MM. At present we are performing next-generation exome sequencing to identify the genetic basis of this condition in our family.

2235/F

Identification of mutations in the *NAGLU* gene in a mild form of Mucopolysaccharidosis IIIB through targeted high-throughput sequencing.

K.K. Selmer[1], G.D. Gilfillan[1], P. Strømme[2], K. Brandal[1], H.S. Hjorthaug[1], D. Misceo[1,3], S. Nakken[4], T. Hughes[1,3], S.K. Braekken[5], R. Lyle[1], D.E. Undlien[1,3]. 1) Department of Medical Genetics, Oslo University Hospital, Oslo; 2) Department of Pediatrics, Oslo University Hospital, Oslo; 3) Institute of Medical Genetics, University of Oslo, Oslo; 4) Department of Tumor Biology, Institute of Cancer Research, University of Oslo, Oslo; 5) Department of Neurology, Oslo University Hospital, Oslo, Norway.

Ultra high-throughput sequencing techniques (HTS) have proven useful in the identification of genetic causes of Mendelian disease by targeted resequencing and exome sequencing. When available linkage information exists, linked regions can be captured directly and the familial occurrence reduces the possibility of genetic heterogeneity and the uncertainty of mode of inheritance. We identified a Norwegian family consisting of healthy parents and eight children, of whom four were similarly affected, with static developmental delay from the age of two. From their mid-twenties peripheral and night vision was reduced and their cognitive abilities started to slowly regress. At present the patients are between 47 and 61 years old. They are severely demented with loss of expressive verbal language and ambulation is very restricted. As no etiological diagnosis had been set, linkage analysis was performed. Autosomal recessive inheritance was assumed and power analyses showed an expected maximum LOD score of 2.3. Linkage analysis was performed using Affymetrix 10K SNP arrays and subsequent calculations done in Merlin showed two linkage peaks reaching a LOD score of 2.3 on chromosomes 9 and 17. The linked regions were 31 Mb and contained more than 180 genes. Eighteen candidate genes were sequenced, but no mutations were detected. Using custom NimbleGen Sequence Capture Arrays we captured the linked coding regions and performed HTS on chromosome 9 with Roche 454 and on chromosome 17 with Illumina Genome Analyzer II. We identified 124 new single nucleotide variants (SNVs) located in coding regions. When considering potential compound heterozygous SNVs, two mutations identified in NAGLU had previously been observed in patients affected by Mucopolysaccharidosis IIIB (MPS IIIB, also called Sanfilippo syndrome B). MPS IIIB is an autosomal recessive disorder caused by an enzyme deficiency of α-N-acetyl-glucosaminidase due to mutations in NAGLU. This diagnosis was confirmed in the Norwegian family by the finding of elevated heparan sulfate in the urine and low levels of α-N-acetyl-glucosaminidase activity in cultured fibroblasts. The phenotype in this Norwegian family is much milder than what is usually described in MPS IIIB, as such patients rarely reach the age of 30. Our findings show how targeted HTS might accelerate the identification of disease causing mutations in Mendelian disease, and also add examples to the rarely described attenuated form of MPS IIIB.

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Evidence of association of SNPs in *MSH6* with café-au-lait macule burden in neurofibromatosis type 1.

A. Pemov[1], H. Sung[2], J.L. Sloan[3], S. Ruppert[1], J. Mullikin[4], P. Cruz[4], A.F. Wilson[2], D.R. Stewart[1]. 1) Genetic Disease Research Branch, NHGRI, NIH, Bethesda, MD; 2) Inherited Disease Research Branch, NHGRI, NIH, Baltimore, MD; 3) Genetics and Molecular Biology Branch, NHGRI, NIH, Bethesda, MD; 4) Genome Technology Branch, NHGRI, NIH, Rockville, MD.

Background. The cause of the variation in phenotypic severity in neurofibromatosis type 1 (NF1) is unknown and may be due to genetic modifiers. We hypothesized that variation in *germline* gene expression of certain genes correlates with variation in the severity of quantifiable phenotypic features of NF1. **Methods.** We performed whole-genome transcriptional profiling (Illumina HumanRef-8 arrays) in lymphoblastoid cell lines from 79 individuals affected with NF1 and 23 controls. A single observer quantified severity in multiple NF1 sub-phenotypes, including café-au-lait macules (CALM) number. We examined the correlation of the 6 NF1 sub-phenotypes with the level of each of the 22,177 transcripts. To control for multiple testing, we calculated a False Discovery Rate (FDR), in addition to a nominal *P*-value of the significance of the regression. We filtered for FDR (< 0.3), expression range (~2X) and expression level (mean log2 > 6.0). We validated 22 QTTs by quantitative PCR on low-density microfluidic arrays (ABI). By qPCR, 9 QTTs remained statistically significant (nominal *P*-value < 0.05). Exons, 5'-UTR, 3'-UTR and limited intronic regions of three genes (*MSH6, MED21, DPH2*), whose expression significantly correlated with CALM burden were subject to Sanger sequencing in 99 patients (79 from original set and 20 new). Two additional genes (*MSH2, MLH1*) which products are known to form functional complexes with MSH6 were also subject to sequencing analysis. The genotypes of the sequenced SNP variants were regressed against CALM burden. **Results.** We found evidence of association for two non-coding *MSH6* SNPs (rs3136316, MAF ~0.20 and rs1800934, MAF ~0.20) with CALM burden (nominal *P* <0.05). We also identified four rare (MAF < 0.05) SNPs in the 3'-end of *MSH6*, which when collapsed in a single group, correlated significantly with CALM burden (*P* = 0.047). **Conclusions.** We identified six SNPs in *MSH6* that are associated with the number of café-au-lait macules in NF1. Functional tests and genotyping of rs3136316 and rs1800934 in a second independent cohort of phenotyped patients is underway. *MSH6* is involved in mismatch repair. Our findings are of interest since CALMs arise secondary to bi-allelic inactivation of *NF1*, the same pathogenic mechanism as NF1-associated tumors. If validated, *MSH6* would be the first identified modifier gene of NF1.

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Continued UBE-1 Mutation Screening in X-linked Lethal Infantile SMA (XL-SMA) and Investigations of Functional Defects in Ubiquitination.

M.E. Ahearn[1], L. Baumbach[1], J. Ramser[2], K.O. Yariz[1], T. Prior[3], S. Srinivasan[1], Z. Nawaz[1], H. Kierstead[4], A. Meindl[2]. 1) Univ Miami Medical School, Miami, FL; 2) Gynaekologische Tumorgenetik Ismaningerstr, Munich, Germany; 3) Ohio State University, Columbus, OH; 4) University of California at Irvine, Irvine, CA.

X-linked infantile SMA (MIM 301830) is a lethal form of SMA with early onset/congenital contractures and/or fractures. We have previously described three novel UBE1 (Ubiquitin-Activating Enzyme E1) Exon 15 variants, found in five unrelated X-linked SMA families: two missense mutations (c.1617 G>T, p.Met539Ile; c.1639 A>G, p.Ser547Gly) present each in one family, and one synonymous C>T substitution (c.1731 C>T, p.Asn577Asn) identified in three additional unrelated families. Both missense mutations occur in conserved amino acids within a conserved protein domain, while the 1731 C>T SNP alters methlyation in a hyper-methylated region of the gene. Over the last two years, we have identified at least fifteen new potential cases of XL-SMA, many of which represent sporadic cases. We continue to screen for UBE1 mutations in this cohort, as well as in a set of males under age 2 who have SMA clinically, but fail to have detectable SMN-1 gene deletions. We will present our latest information regarding genotype-phenotype correlations. Importantly, UBE1 is responsible for the activation of ubiquitin, the first step of the ubiquitin-proteasome pathway (UPP). "Activated" ubiquitin is transferred from UBE1 to a family member of the ubiquitin carrier protein enzymes (E2s) by a thioester linkage (E2-S-Ub). We have begun a series of in vitro and in vivo experiments to evaluate possible functional effects of UBE1 mutations on the UPP. To examine in vitro possible functional effects on ubiquitin activation, we are generating HA-Tagged UBE1 cDNAs (wild-type and mutant, containing each of the three novel variants detected in XL-SMA patients) for transformation and expression in E. coli. Purified UBE1 proteins are quantified for use in ubiquitin-activation and E2 charging assays. Two types of in vivo experiments are also being performed: one using a mammalian cell line harboring a temperature-sensitive (ts) UBE1 mutation which will be transfected with plasmids expressing wild-type or mutant UBE1 protein(s), and secondly, human motor neuron cultures which will be transfected with known UBE1 mutations to assay effects of mutations on ubiquitination and neuronal development. In addition, we are evaluating overall ubiquitination activity in several XL-SMA patient cell lines (as compared to controls), as another measure of in vivo effects. These important experiments should shed important new insights into pathogenesis of human UBE1 mutations.

2238/F

Variant in SPG4 linked to early onset hereditary spastic paraplegia. *C. DiVincenzo, C.D. Braastad, C.D. Elzinga, I.D. Karbassi, J.G. Jones, S.D. Batish.* Athena Diagnostics/ThermoFisher Scientific, Worcester, MA.

Variants found in the SPG4 (spastin) gene are associated with autosomal dominant hereditary spastic paraplegia (HSP). Typically symptoms of HSP present in young adulthood, though a varied age of onset has been seen ranging from infants to the elderly. No mechanism has been identified as the cause of this widely varying age of onset[Durr 1996],[Yip 2003]. The ability to link a specific variant with a predictive age of onset could prove useful in diagnostic testing to better assess a clinical diagnosis as well as family member risk and potential treatment. If more variants are able to be linked with a particular range of onset, it may help to determine a mechanism for such a varied age of onset. Through the course of diagnostic testing for hereditary spastic paraplegia (HSP) at Athena Diagnostics, we have identified 14 individuals carrying the variant R499H. Of these patients, two are related (mother and daughter) and the remaining twelve are seemingly unrelated. This variant is inherited maternally in one family tested at Athena, but appears to have arisen de novo in other patients (paternity not confirmed). Individuals with de novo occurrences of this variant have an average age of onset of 6 years old (SD = 3.8 years), consistent with early-onset HSP. Interestingly, in the family where R499H is inherited, both mother and daughter experienced onset of symptoms within the first two years of life. There are also three independent patients whose cases were previously published by others, 1 familial case, 2 sporadic cases, but no definitive familial studies were described[Park 2005],[Depienne 2006],[Crippa 2006]. The R499H variant is found in Exon 13 of SPG4. It lies within the AAA cassette domain of the protein. This region is highly conserved and is thought to be involved with intracellular processes, specifically microtubule interactions[Errico 2002]. The change of arginine to histidine at position 499 was predicted as damaging to the SPG4 protein by both SIFT and PolyPhen.

2239/F

A Genetic Mechanism for Facioscapulohumeral muscular dystrophy. *R.J.L.F. Lemmers[1], P.J. van der Vliet[1], R. Klooster[1], S. Sacconi[2], P. Camano[3], J.G. Dauwerse[1], L. Snider[4], K. Straasheijm[1], G.J. van Ommen[1], G.W. Padberg[5], D. Miller[6], S.J. Tapscott[4], R. Tawil[7], R.R. Frants[1], S.M. van der Maarel[1].* 1) Department of Human Genetics, Leiden University Medical Center, Leiden, Netherlands; 2) Centre de reference pour les maladies Neuromusculaires and CNRS UMR6543, Nice University, Nice, France; 3) BioDonostia Health Research Institute, Hospital Donostia, San Sebastián, Spain; 4) Division of Human Biology, Fred Hutchinson Cancer Research Center, Seattle, WA; 5) Department of Neurology, Radboud University Medical Center Nijmegen, Nijmegen, Netherlands; 6) Department of Pediatrics, University of Washington, Seattle, WA; 7) Department of Neurology, University of Rochester Medical Center, NY.

Facioscapulohumeral muscular dystrophy (FSHD) affects 1:20,000 people and is clinically characterized by progressive and often asymmetric weakness and wasting of facial, shoulder girdle and upper arm muscles. FSHD is most often caused by contraction of the D4Z4 macrosatellite repeat array, but only when this array resides in a specific genetic background of chromosome 4q. Repeat contractions on other, non-permissive 4q backgrounds and contractions of its homologue on chromosome 10q are non-pathogenic. D4Z4 repeat contractions cause a local chromatin relaxation, resulting in the transcriptional deregulation of genes within or close to D4Z4. The candidate gene DUX4 has been identified within each D4Z4 unit and encodes for a double homeobox protein. Different from DUX4 transcripts originating from internal units, the DUX4 transcript from the last D4Z4 unit is spliced to a unique last exon in the region immediately flanking D4Z4. This exon contains a poly(A) signal which presumably stabilizes the distal transcript. Distal DUX4 transcripts can be observed in FSHD myotubes. We discovered sequence polymorphisms in the FSHD locus that separate FSHD-permissive chromosome 4 backgrounds from non-permissive genetic backgrounds of chromosomes 4 and 10. These polymorphisms are restricted to specific sites in the FSHD locus. We show evidence that these polymorphisms functionally affect the regulation of the FSHD locus thereby for the first time not only providing an explanation for the striking haplotype-specificity of FSHD, but also uncovering a genetic mechanism that unifies all patients with FSHD.

2240/F

Preliminary whole genome sequencing analysis of monozygotic twins with a concordant unique neurological phenotype. *K.V. Fuentes Fajardo[1], S.S. Ajay[3], T.C. Markello[1], D.A. Adams[1], C. Toro[1], M. Sincan[1], H. Carlson-Donohoe[1], P.F. Cherukuri[3], N.F. Hansen[3], H. Ozel Abaan[3], J.C. Mullikin[2], W.A. Gahl[1], E.H. Margulies[3], NISC Comparative Sequencing Program.* 1) Undiagnosed Diseases Program, NHGRI/NIH, Bethesda, MD; 2) NIH Intramural Sequencing Center, NIH, Bethesda MD; 3) Genome Technology Branch, NHGRI, NIH Bethesda MD.

Advances in Next Generation Sequencing technology have made whole genome sequencing a practical reality for clinical research applications. Other investigators have reported on whole genome sequencing of monozygotic twins discordant for multiple sclerosis. No discordant variant was identified as the cause of MS in the affected twin. We have an opportunity to sequence concordant twins. Two of the NIH Undiagnosed Diseases Program's initial choices for whole genome sequencing were a 24 year-old monozygotic twin pair concordant for the unique phenotype that includes early childhood neuro-cognitive deficits, rare seizures, and slowly progressive movement disorder characterized by myoclonus with ataxia. A detailed neurologic evaluation, including metabolic screening, candidate gene sequencing and mitochondrial DNA studies was not diagnostic. We will present the interim analysis of genomic variations we have found in one or both twins and contrast and compare them to the reference human genome sequence; an a priori unknown variant, that could be anywhere in the human genome, must be in both twins. The search will be one of the most rigorous tests of whether whole genome sequencing can be successfully used to identify a disease-causing variant, or whether the intrinsic background false positive rate will overwhelm our current analytic tools.

2241/F

Identification of genomic deletions spanning the *PCHD19* gene in two unrelated girls with Intellectual Disability and Seizures. *J.B. Vincent[1], A. Noor[1], A. Janson[2], M. Ayub[3], C.F. Morel[2].* 1) Neurogenetics Sect, R30, Ctr Add/Mental Hlth,Clarke Div, Toronto, ON, Canada; 2) The Fred A. Litwin Family Centre in Genetic Medicine, University Health Network, Toronto, Ontario, Canada; 3) St. Luke Hospital, Durham, UK.

Recently, missense and truncating mutations in the gene *PCDH19* have been reported to cause Female-Restricted Epilepsy with Mental Retardation (EFMR). EFMR (MIM#300088) is an X-linked disorder characterized by early onset seizures and Intellectual Disability (ID). Interestingly, unlike typical X-linked mode of inheritance, the phenotype is restricted to females, and males are unaffected carriers. Previously, using sequence analysis, several mutations in PCHD19 have being shown to cause EFMR. Here, we report two unrelated female patients with deletions spanning PCHD19 genes. These deletions were identified by genome-wide Copy Number Variation (CNV) analysis using high density microarrays and were validated by qPCR. In first case, we have identified a denovo 603 Kb heterozygous deletion in a female patient with fits (since one year of age), ID and additional clinical features including hyperactivity and aggressive behavior. The deletion spans entire *PCDH19* gene and four other Refseq annotated genes (*TNMD*, *SRPX2*, *TSPAN6* and *SYTL4*). In another unrelated female patient, we have identified a 3 Mb interstitial deletion at Xq21.33-q22.1 which spans entire *PCHD19* gene and two other annotated genes (*LOC442459* & *TNMD*). This patient had first episode of seizures at the age of 8 months, second seizure occurred at 13 months, with subsequent bouts of seizures occurring approximately every 25 days. Other clinical features include developmental delay, ID and aggressive behavior. The *PCDH19* is highly expressed in brain and protein encoded by this gene belongs to the cadherin superfamily. Cadherins play a crucial role in the neuronal cell adhesion and several members of this family have been previously implicated in ID and autism (*CDH15*, *PCDH9* and *PCDH10*). In conclusion, we have demonstrated that CNVs at the *PCDH19* locus can also cause EFMR. Moreover, our findings provide further support for the involvement of *PCHD19* in EFMR.

2242/F

The role of the JAK-Stat3 pathway in inherited photoreceptor (PR) degenerations (IPDs). *C.K. Jiang[1], M.J. Szego[1], A.N. Bramall[1], L.R. Pacione[1], W.W. Hauswirth[2], R.R. McInnes[1,3].* 1) Program in Stem Cell and Developmental Biology, Hospital for Sick Children Research Institute, Toronto, M5G1L7; 2) Department of Ophthalmology, University of Florida, Gainesville, Florida 32610-0284, USA; 3) The Lady Davis Institute, Jewish General Hospital, McGill University, Montreal H3T1E2.

The critical biochemical changes that occur in the retina, in response to an IPD mutation, are largely unknown. To identify the molecules that confer or resist the risk of death, we examined the retinal mRNA population in the *Rds*$^{+/-}$ mouse IPD model by microarray and qPCR analysis, at an early stage of degeneration (7 weeks old) when 85-90% of the mutant PRs were still alive. We identified 9 up-regulated mRNAs encoding proteins that constitute a putative JAK-Stat3 cytokine signaling pathway, of which the nexus is the transcription factor *Stat3* (2.3-fold increased, n=3, p<0.05). By qPCR, *Stat3* mRNA was also found increased in two other IPD models: *TgRHO(P347S)* (2.4-fold, n=3, p<0.05) and *Rd1*$^{-/-}$ (3.0-fold, n=3, p<0.05). By immunoblotting, we found comparable increases of both the STAT3 protein and its active form, pSTAT3: 2.8-fold (n=3, p<0.01) and 6.3-fold (n=3, p<0.01) in 7 weeks old *Rds*$^{+/-}$ retinas; 4.0-fold (n=3, p<0.01) and 3.6-fold (n=3, p<0.01) in 3 weeks old *TgRHO(P347S)* retinas; and 1.9-fold (n=3, p<0.05) and 2.6-fold (n=3, p<0.01) in 12 days old *Rd1*$^{-/-}$ retinas, respectively. By immunostaining of *Rds*$^{+/-}$ retinas, we determined that the increased STAT3 and pSTAT3 proteins were localized predominantly to Müller glia cells, less to mutant PRs. We confirmed the increase in *Stat3* expression in mutant PRs using laser capture microdissection vs. age matched wild type retinas: by qPCR, *Stat3* mRNA was found increased 2.4 fold (n=3, p<0.05) in 7 weeks old *Rds*$^{+/-}$ PRs and 5.4 fold (n=3, p<0.05) in 12 days old *Rd1*$^{-/-}$ retinas. Since Stat3 signaling can mediate apoptotic responses in other contexts, we asked whether the increased *Stat3* expression in mutant PRs was pathogenetic. We used subretinal injections of an AAV2-CBA-Cre virus, whose expression is PR-specific, to delete *Stat3* from *Stat3*$^{flox/flox}$*; Rd1*$^{-/-}$ mutant PRs. The effect of PR *Stat3* deletion was variable; but overall, PR survival increased: 14/18 AAV2-CBA-Cre injected retinas had significantly increased PR survival vs. controls, with an average increase of 16.7%±8.7% (n=14; p<0.05), ranging from 6.9% to 41.1%. We conclude that these results suggest that the increased expression of *Stat3* in mutant PRs is pathogenetic in the *Rd1*$^{-/-}$ retina. Whether this proapoptotic role of *Stat3* is a general response to PR mutation will be evaluated through specific deletion of the PR-*Stat3* gene in different IPD models (eg. *TgRHO(P347S)* and *Rd1*$^{-/-}$ model), using PR-specific *Opsin-Cre* mice crossed to *Stat3*$^{flox/flox}$ mice.

2243/F

Aberrant microRNA expression in HPRT-deficient human neuroblastoma cells: A regulatory role for a housekeeping gene? *G. Guibinga, G. Hrustanovic, T. Friedmann.* Department of Pediatrics, UCSD School of Medicine, La Jolla, CA.

The mechanisms responsible for neuropathology in the HPRT-deficiency disorder Lesch Nyhan Disease (LND) are poorly understood. We have previously presented evidence that the underlying purine biosynthetic defect in HPRT-deficient human teratocarcinoma cells results in aberrant expression of transcription factors vital for neurogenesis in vitro, including down-regulation of LMX1a an En1, among others. The composite effect of these changes are reflected by disrupted neurogenesis. We have now examined the expression of microRNAs in wild type and HPRT-deficient human SH-Sy5y neuroblastoma cells at basal state and during retinoic acid-induced in vitro differentiation and have detected aberrant expression of a number of microRNAs. One such dysregulated microRNA is miR-181a which has LMX1a and En1 as potential regulatory targets. In HPRT-deficient SH-Sy5y cells, miR-181a is significantly up-regulated and LMX1a and En1 are both expressed at markedly reduced levels. Over-expression of miR-181a leads to significant repression of En1 and Lmx1a gene expression. Furthermore, treatment of cells with an inhibitor of miR-181a leads to significantly increased expression of both Lmx1a and En1. These and other data suggest that aberrant microRNA expression in HPRT deficiency may play a causal role in the development of the neurological defects in LND.

2244/F

Myostatin expression in different mice models for neuromuscular disorders. *D. Zilbersztajn-Gotlieb, D. Ayub-Guerrieri, P.C.G. Onofre-Oliveira, P. Martins-Machado, A.F. Santos, A.B. Martins-Bach, M. Vainzof.* Universidade de Sao Paulo, Sao Paulo, Sao Paulo, Brazil.

Myostatin is a TGFβ superfamily member and is a negative regulator of skeletal muscle growth. Inhibition of myostatin has been considered as a potential therapeutic target against muscle degeneration in muscular dystrophies. The expression of myostatin in the dystrophic process is not clearly known, and the aim of the present study is to analyze the relative expression of myostatin in four strains of mice model for muscular dystrophies, as compared to normal controls. The relative expression of myostatin was analyzed using real time PCR, in the gasctrocnemius and dyaphragm muscles of adults (3 months old) mice from the following strains of dystrophic mice: *Dmd*^{mdx} (dystrophin deficient, mild phenotype, model for Duchenne muscular dystrophy), *Lama2*^{dy2J}*/J* (laminin-α2 deficient, severe phenotype, model for Congenital Muscular Dystrophy 1A), *SJL*, dysferlin deficient model for Limb Girdle Muscular Dystrophy 2B, *Large*^{myd} (defect in glycosylation of α-DG, severe phenotype in older animals, model for Congenital Muscular Dystrophy 1D), and controls *C57Bl*. In normal *C57Bl* controls, the expression of myostatin in the gastrocnemius was significantly different that the observed in diaphragm muscles. Comparing to normal controls and considering the two different muscles (gastrocnemius and diaphragm), higher or lower levels of expression was observed in the 4 mice models. The present study showed variation among relative expression in different muscular dystrophies and types of muscles, in mice models with different mutations in genes expressing muscle proteins. Our results suggest that each primary defect in the studied muscular dystrophies has its own pathway of muscle degeneration/regeneration. These data have significant importance and must be considered for future investigations using the modulation of the expression of myostatin aiming therapies. Financial support: FAPESP-CEPID, INCT-CNPq, FINEP, ABDIM.

2245/F

Genes and Disease: Molecular Genetic Approach to Identify Gene Mutations Causing Distal Hereditary Motor Neuropathy. *O.M. Albulym[1,2], A. Antonellis[3], M. Kennerson[1,2,4], S. Reddel[1,2,4], G. Nicholson[1,2,4].* 1) Northcott Neuroscience Lab., ANZAC Research Institute, Concord, NSW, Australia; 2) Sydney Medical School, University of Sydney, NSW 2008 Australia; 3) University of Michigan Medical School, Department of Human Genetics, Department of Neurology, Ann Arbor, MI; 4) Molecular Medicine Laboratory, Concord Hospital, NSW 2139, Australia.

Hereditary motor neuropathies (HMN), also known as distal spinal muscular atrophy (SMA) or spinal Charcot-Marie Tooth (spinal CMT), mainly affect motor neurons in the peripheral nervous system. They can be subdivided into proximal HMN and distal hereditary motor neuropathy (dHMN). Distal hereditary motor neuropathies are a group of clinically and genetically heterogeneous disorders characterized by weakness and wasting of distal muscles of the upper and lower limbs [1,2]. Mutations in several genes have been identified for dHMN. They include Berardinelli-Seip congenital lipodystrophy (BSCL2), small heat shock 27kDa protein 1 (HSPB1), small heat shock 22kDa protein 8 (HSPB8), glycyl-tRNA synthetase (GARS), senataxin (SETX), and dynactin 1 (DCTN1) [1]. Recently, a mutation causing HMN has been identified in the small heat shock 27kDa protein 3 gene (HSPB3) [3]. We aim to (i) investigate the frequency and distribution of known mutations and (ii) identify new pathogenic mutations in six genes in an Australian cohort of dHMN patients consisting of 119 familial and isolated cases. These genes are BSCL2, HSPB1, HSPB8, GARS, SETX and DCTN1. Exonic and exonic-intronic boundaries of BSCL2, HSPB1, SETX, DCTN1 and HSPB8 genes were amplified with 2.5X HRM Master Mix and scanned by a 96-well Light-Scanner (Idaho Technology). All exons of GARS gene were analyzed by direct sequencing. The genes SETX and DCTN1 are being scanned. We have detected one BSCL2 mutation (N88S) in three patients (2.5%), and two different mutations in GARS (H418R and S581L) in two patients (1.7%). No mutations were detected in DCTN1, HSP22, HSP27, and SETX. Our findings indicate that by screening these six genes we have accounted for mutations in 4.2% of our dHMN families. We concluded that the yield of mutations in DCTN1, HSP22, HSP27, and SETX is low in these patients. This study also provides further evidence for genetic heterogeneity of dHMN. [1] Irobi, J., P. De Jonghe, et al. (2004). “Molecular genetics of distal hereditary motor neuropathies.” Hum Mol Genet 13 Spec No 2: R195-202. [2] Irobi, J., I. Dierick, et al. (2006). “Unraveling the genetics of distal hereditary motor neuronopathies.” Neuromolecular Med 8(1-2): 131-146. [3] Kolb, S. J., P. J. Snyder, et al. (2010). “Mutant small heat shock protein B3 causes motor neuropathy: utility of a candidate gene approach.” Neurology 74(6): 502-506.

2246/F

A novel *ARX* gene mutation in a female individual with early onset epileptic encephalopathy. *W. Andreoli[1], G. Di Rosa[2], S. Rossato[1], S. Sartori[1], R. Polli[1], A. Murgia[1].* 1) Pediatrics, University of Padua, Padua, PD, Italy; 2) Child Neuropsychiatry Unit, University Hospital of Messina, Messina , Italy.

The *ARX* (Aristaless Related Homeobox) gene belongs to a large family of homeodomain transcription factors thought to play an essential role in neural development. This gene is located on the short arm of the X chromosome (Xp22.13) and consists of five exons that encode a protein of 562 amino acids. Mutations in the *ARX* gene are associated with a wide range of disorders from severe developmental abnormalities of the brain structure to syndromic and non-syndromic forms of X-linked mental retardation (XLMR) that can be associated with normal or abnormal brain morphology. Mental retardation and epilepsy often occur together. In the course of a mutational scanning, performed in a cohort of subjects with epileptic encephalopathy of the first year of life, we have detected a novel frame-shift mutation in exon 5 of the *ARX* gene, predicted to yield a protein with truncation of the 5 COOH-terminal amino acids (p.Thr487GlufsX5). The mutation has been detected in a 2 year-old female individual with a very severe neurodevelopmental delay and a clinical history of infantile spasms and focal seizures with onset at about three months of age. The brain magnetic resonance imaging (MRI) is normal and no dysmorphic features or genitalia alterations are reported. An X-inactivation analysis of the androgen receptor (AR) locus was performed which showed a ratio of 70:30 consistent with a skewed inactivation pattern. Analysis of the mutation segregation on the parents' DNA is currently underway. The novel truncating mutation we have detected is the most C-terminal alteration described in the *ARX* gene, nevertheless it truncates an extremely conserved region possibly relevant for protein-protein interactions and is therefore highly likely pathogenic. The majority of female carriers of *ARX* mutations so far reported in literature have normal brain MRI scan or partial to complete agenesis of the corpus callosum, with normal cognition or mild mental retardation. The patient we report is the first description an affected female, showing a severe phenotype with neurodevelopmental impairment and early epileptic encephalopathy, but absence of malformations or dysmorphic features.

2247/F

Molecular characterization of a cohort of Stargardt and Stargardt-like affected individuals and families. *M.B. Gorin[1,2], A. Martinez[1], Y.-Q. Gao[1], M.C. Ortube[1], D.B. Farber[1].* 1) Dept Ophthalmology, Jules Stein Eye Inst - UCLA, Los Angeles, CA; 2) Dept Human Genetics, David Geffen Sch. of Med - UCLA, Los Angeles, CA.

We performed mutation analyses of the ABCA4, PRPH2, VMD2 and ELOVL4 genes, in a cohort with the diagnosis of Stargardt or Stargardt-like Disease. We sequenced the ABCA4 gene for 99 affected individuals and 24 family members from 92 families. A subset of individuals (41) was initially screened by SSCP of the ABCA4 exons prior to complete DNA sequencing of the exons and exon-intron junctions. We compared the detection of probable and definite disease-causing mutations identified by sequencing with the predicted detection that would be observed with the ABCR400 chip and the ability of the SSCP to detect sequencing-confirmed variants. For the 99 affected individuals, sequencing identified 123 variants that were represented in 1087 occurrences (average: 11 per person). Two or more mutations (including both cis and trans configurations) were found for 44 individuals. Thirty-two individuals had only a single ABCA4 mutation. Sixty-three different ABCA4 mutations were identified of which 43 are currently included in the ABCR400 chip. If we assume 100% sensitivity for the ABCR400 chip, we would have identified 30 affected persons with either 2 or more ABCA4 mutations and 40 affected people with a single ABCA4 mutation. The ABCR400 chip would have identified 92% of the affected people who were positive with at least one of the disease-causing variants detected by sequencing and 68% of those affected persons identified by sequencing with 2 or more mutations. In contrast, SSCP was a poor screening method for ABCA4, detecting only 31% of all of the variant occurrences and 48% of the mutations that were identified by direct sequencing. Seven individuals (5 families) had a mutation in PRPH2. One of these individuals was found to have two mutations (trans) in the ABCA4 gene and one mutation in the PRPH2 gene. No mutations were found in the sequencing of VMD2 and ELOVL4 for those individuals with either one or no ABCA4 mutations (definite and/or probable). Even with complete exon sequencing, a number of clinical Stargardt cases are not identified with mutations in known Stargardt-related genes. This is partly due to genetic heterogeneity, as evidenced by the identification of several individuals with PRHP2 mutations and the excess number of individuals (17) with no identifiable ABCA4 or PRPH2 mutations. In addition to the need to find more comprehensive methods for identifying ABCA4 mutations, the genetic heterogeneity for this group of disorders has yet to be defined.

2248/F

Frequency of SMA in Western India. *A. Gupta, J. Sheth, F. Sheth.* Department of Molecular Geneti, FRIGE's Institute of Human Genetics, Ahmedabad, Gujarat, India.

Spinal muscular atrophy (SMA) is an autosomal recessive disorder with a frequency of 1:6,000 to 1:10,000 live births. It is characterized by degeneration of lower motor neurons and occasionally bulbar motor neurons leading to progressive limb and trunk paralysis as well as muscular atrophy. In 98%; cases it is due to allelic deletion of exon 7 and exon 8 of the SMN1 and SMN2 gene. Due to overlapping phenotype of SMA with other motor neuron disease; it remains undiagnosed and require high index of clinical suspicion followed by confirmative molecular diagnosis. Our study is aimed at to identify the frequency of SMN gene deletion in children and adults with motor neuron disease. Molecular analysis was performed on 33 patients in the age range of 2 months to 50 years having clinical phenotypes of lower motor neuron disease and bulbar dysfunction in adults; poor sucking ability, reduced swallowing and respiratory failure in children. Genomic DNA isolated from blood was used to detect the SMA1 and SMN2 gene deletion in exon 7 and exon 8 using restriction enzyme Dra1 and Dde1 respectively. Twenty two of 33 (67%) suspected as SMA type I (Werdnig Hoffmann) were in the age range of 0-6month, 4 (12%) suspected as type II were in the age of 7-18months, 1 (3%) suspected as type III (Kugelberg-Welander) were in the age of >18months, and 4 (12%) as type IV in the age range of 32-50 years. The analysis of both exon 7 and 8; homozygous deletion were observed in 82%; of SMA I [n=18], 50%; SMA II [n=2] and confirmed diagnosis was observed in one case clinically suspected with SMA III. One of the four suspected SMA IV patients showed homozygous deletion of exon 7 in SMN2 gene. In 9 cases no deletion were found in any of the exons. Our study suggest that; with the advent of molecular techniques, SMN gene deletion studies are the first line of investigations in those with impaired lower motor neuron function and are highly prevalent in children.

2249/F

A novel ASPA gene mutation in Canavan Disease. *H. Onay, A. Durmaz, A. Vahabi, H. Akin, F. Ozkinay.* Department of Medical Genetics, Ege University , Izmir, Turkey.

Human aspartoacylase (ASPA) gene which catalyzes the deacetylation of N-acetyl-L-aspartate is mutated in Canavan disease (CD), is a severe autosomal resesif leukodystrophy. CD is characterized by macrocephaly, ataxia, severe motor and mental retardation, dysmyelination and progressive spongial atrophy of the brain. To date more than 50 mutations have been described in patients having Canavan Disease. We present a novel mutation within the ASPA gene in a family with as CD. A couple applied for genetic counselling with a history of lost child having a clinical diagnosis of CD. No molecular analysis was performed on the child. The proband was a 1-year-old girl and died due to CD. She was hospitalized for motor mental retardation. Brain magnetic resonance imaging (MRI) revealed increased T2 signal intensity and MR spectroscopy showed elevated brain N-acetyl-aspartate confirming the diagnosis of CD. Sequencing analysis for ASPA gene was perfomed on the parents. Heterozygous Y88X mutation was detected in both parents. This mutation was not described before in CD and results in premature stop codon. Here we present a case of CD having a novel mutation in ASPA gene.

2250/F

Novel mutations of ZFHX1B responsible for the typical cases of Mowat-Wilson syndrome. *Y. Yamada[1], K. Yamada[1], S. Mizuno[2], N. Furuya[3], M. Matsuo[4], M. Urano[4], Y. Hiraki[5], K. Kurosawa[3], K. Saito[4], N. Wakamatsu[1].* 1) Dept Gen, Inst Dev Res, Aichi Human Service Ctr, Kasugai, Aichi, Japan; 2) Ctrl. Hosp., Aichi Human Service Ctr., Kasugai, Aichi, Japan; 3) Kanagawa Children's Med., Ctr., Yokohama, Kanagawa, Japan; 4) Inst. Med. Genet., Tokyo Women's Med Univ., Shinjuku, Tokyo, Japan; 5) 5Hiroshima City Children's Med. Ctr., Hiroshima, Japan.

The mutations of *ZFHX1B* cause an autosomal dominant disorder of Mowat-Wilson syndrome associated with profound mental retardation, delayed motor development and specific facial features such as hypertelorism, often with microcephaly, epilepsy, congenital heart disease, and Hirschsprung disease. We performed molecular analysis of *ZFHX1B* to confirm diagnosis of Mowat-Wilson syndrome. To screen for mutation, DNA fragments including each exon of *ZFHX1B* from patients and normal controls were amplified by PCR and subjected to direct sequencing analysis. In this study, performed after our last reports (Yamada et al. ASHG, 2005 and Ohstuka et al. J. Child Neurol., 2008), 11 novel mutations were found in the typical patients with Mowat-Wilson syndrome, 2 nonsense mutation (2400C>G, S800X; 2761C>T, R921X) and 9 frame-shift mutations including three mutations inserted one base (1338insC, 446fs455X; 1427insA, 476fs481X; 2351insT, 784fs794X), five mutations deleted one base (460delG, 154fs211X; 1169delT, 390fs395X; 1417delA, 473fs486X; 2282delC, 761fs486X; 2718delT, 906fs929X), and a mutation deleted four bases (1270delAGCC, 427fs438X). We have identified 43 mutations in *ZFHX1B* up to the present. In 41 typical cases with profound mental retardation and specific facial features, 18 nonsense mutations and 23 frame-shift mutations were detected. These mutations generated truncated protein without the zinc finger domain located in the C-terminal region.

2251/F

Identification of a novel locus for juvenile primary lateral sclerosis by homozygosity mapping. *A. Al-Saif[1], S. Bohlega[2].* 1) Department of Genetics, King Faisal Specialist Hospital & Research Centre, Riyadh, KSA; 2) Department of Neurosciences, King Faisal Specialist Hospital & Research Centre, Riyadh, KSA.

Primary lateral sclerosis is a rare progressive motor neuron degenerative disorder affecting the upper motor neurons. It leads to weakness and spasticity of limbs, trunk and bulbar muscles. The age of onset is between 35 and 66 years but a subtype of this disorder (juvenile primary lateral sclerosis, JPLS) begins in early childhood and inherited as an autosomal recessive disorder. Mutations in ALS2 gene were previously identified in some JPLS families. In a consanguineous family with four JPLS patients (age of onset between 2 and 3 years), we performed homozygosity mapping using SNP genotyping arrays and identified a novel locus on chromosome 8 (p11.21-p12). The four affected individuals shared homozygosity of this region but none of their seven normal siblings. Identification of the genetic defect in this region will help understanding the pathophysiology of primary lateral sclerosis and motor neuron degeneration.

2252/F

Mutant HTT mRNA toxicity in the pathogenesis of Huntington Disease. *M. Banez-Coronel[1,2], S. Porta[4], B. Kagerbauer[1,2], E. Mateu[1,2], I. Ferrer[3,4], X. Estivill[1,2], E. Martí[1,2].* 1) Centre for Genomic Regulation, Barcelona, Catalonia, Spain; 2) Centro de Investigación Biomédica en Red en Epidemiología y Salud Pública (CIBERESP), Barcelona, Catalonia, Spain; 3) Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Barcelona, Catalonia, Spain; 4) Institut Neuropatologia, Servei Anatomia Patològica, IDIBELL-Hospital Universitari de Bellvitge, Universitat de Barcelona, Barcelona, Catalonia, Spain.

Huntington's disease (HD) is a dominantly-inherited neurodegenerative disorder that results from expanded CAG repeats in the exon 1 of the huntingtin gene. It is widely accepted that the abnormally extended polyglutamine tract in the Huntingtin protein (HTT) produces toxic activities that lead to progressive striatal and cortical neurodegeneration; however many aspects of HD pathology still remain unexplained. A range of neurodevelopmental and neurodegenerative diseases associated with trinucleotide repeat expansion appears to be caused by RNA-mediated mechanisms including RNA toxicity or aberrant splicing (1-3). Here we have investigated the possible deleterious effect of the mutated HTT exon1 mRNA and the mechanisms related. Vectors expressing translated and untranslated (wild type and CAG expanded) forms of the exon 1 of the human HTT gene were transfected into differentiated neuroblastoma cells and cell viability was evaluated at different time-points after transfection. The results revealed that mutant HTT is able to exert a cytotoxic response at the RNA level. The expression of the expanded HTT mRNA led to the generation of small CAG-repeated RNAs (sCAG) in human cells. In consonance with previous in vitro studies (4), Dicer depletion prevented the production of sCAG in cells trasfected with mutant HTT. This was accompanied by a dramatic reduction in the subsequent cytotoxicity in vivo, suggesting an important role of sCAG in HTT-mediated pathogenesis. In consonance, an increased expression of sCAGs was also found in human brain samples from HD patients when compared to control individuals. Finally, the transfection of exogenous sCAGs (siCAG7) in differentiated neuroblastoma cells also resulted in strong toxicity. On the other hand, the administration of sCAG on different human cells had a mild effect on non-neuronal cell metabolism. This result support a link between the generation of sCAG and the cell loss specificity observed in HD. Our results suggest an additional mechanism that may take place in HD pathogenesis, where the abnormal expression of CAG-repeated small RNAs may initiate a cell-type specific, transcriptome-dependent detrimental response. 1. Baralle, J. M. et al. Am. J. Hum. Genet. 83, 77-88 (2008). 2. Miller, J. W. et al. EMBO J. 19, 4439-4448 (2000). 3. Li, L.B. et al. Nature 453, 1107-1111 (2008). 4. Krol, J. et al. . Mol. Cell 25, 575-586 (2007).

2253/F

Pathogenic consequences of *ATP7A* mutations causing X-linked distal hereditary motor neuropathy. *S. Chu[1, 2], G.A. Nicholson[1, 2, 3], R. Chaudhry[1], R. Hyland[6], A. Shanu[2, 4], J.F.B. Mercer[5], R.M. Llanos[5], P.K. Witting[2, 4], S. Myers[6, 7], M.L. Kennerson[1, 2, 3].* 1) Northcott Neuroscience Laboratory, ANZAC Research Insitute, University of Sydney, Concord, Australia; 2) Department of Medicine, University of Sydney, Camperdown, Australia; 3) Molecular Medicine Laboratory, Concord Hospital, Concord, Australia; 4) Redox Biology Group, Department of Pathology, University of Sydney, Camperdown, Australia; 5) Centre for Cellular and Molecular Biology, School of Life and Environmental Sciences, Deakin University, Burwood, Australia; 6) Neuro Cell Biology Laboratory, Molecular Medicine Research Group, School of Biomedical and Health Sciences, University of Western Sydney, Campbelltown, Australia; 7) School of Medicine, University of Western Sydney, Campbelltown, Australia.

Distal hereditary motor neuropathies (distal HMN) are a clinically and genetically heterogeneous group of disorders affecting the peripheral motor nerves. We have identified two missense mutations (P1386S and T994I) in the copper transport gene *ATP7A* that cause X-linked distal hereditary motor neuropathy on chromosome Xq13-q21 (distal HMNX). Male patients with these mutations present with variable age onset distal muscle wasting of both upper and lower limbs but show no clinical or biochemical abnormalities of Menkes Disease (MD) or occipital horn syndrome (OHS), disorders typically associated with *ATP7A* gene mutations. The *ATP7A* gene encodes a P-type ATPase that plays an important role in copper homeostasis and the delivery of copper to cuproenzymes. The ATP7A protein resides in the *trans*-golgi network (TGN) in basal copper levels and trafficks to cytoplasmic vesicles and the plasma membrane when copper levels are elevated. Our studies show a trafficking defect in patient fibroblasts where the mutant ATP7A protein fails to traffic from the TGN in elevated copper conditions. While mechanisms underlying the MD and OHS phenotypes have been explored, the underlying cause of axonal degeneration of the motor neurons in distal HMNX is poorly understood. We hypothesise that mutant ATP7A disrupts the availability of ATP7A laden vesicles and that excess cytoplasmic copper over time activates pathways of axonal degeneration. We aim to develop a neuronal cell model by transfecting T994I and P1386S expression constructs into SH-SY5Y cells. Copper loading experiments will be performed to confirm the trafficking defect in a neuronal cell model. The mechanism of oxidative stress as a potential cause of axonal degeneration will be explored by examining the activity of the copper enzyme, cytoplasmic Cu/Zn superoxide dismutase (SOD1) and testing gene markers of oxidative stress under normal and elevated copper conditions in both the neuronal cells and patient fibroblasts expressing the mutant ATP7A protein.

2254/F

***Hsp70i* and the CMT peripheral neuropathic phenotype.** *J. Saliba[1], M. Khajavi[1], W. Wojciech[1], J.R. Lupski[1, 2, 3].* 1) Molec & Human Gen, Baylor College Med, Houston, TX; 2) Pediatrics, Baylor College of Med, Houston, TX; 3) Texas Children's Hospital, Houston, TX.

Charcot Marie Tooth disease (CMT) is the most common hereditary peripheral neuropathy with a prevalence of 1 in 2,500. CMT is most often the result of a 1.4 Mb duplication of the 17p11.2-p12 region of chromosome 17 that includes the dosage sensitive peripheral myelin protein 22 gene (*PMP22*). In addition, a number of point mutations in over two dozen genes, including *PMP22*, have been discovered to be associated with CMT. Numerous point mutations within myelin genes can cause severe peripheral neuropathies through protein accumulation within the ER, which leads to cellular stress and ultimately apoptosis of the Schwann cells. Therapeutic methods attempting to regulate *PMP22* expression have shown promise, however these methods may not be effective in the alleviation of cellular stress. Previously, the oral administration of curcumin, a small molecule derived from the curry spice tumeric, has been shown to significantly decrease the percentage of apoptotic Schwann cells and partially alleviates the severe neuropathic phenotype of *Trembler-J* (*Tr-J*) mice in a dose dependent manner. Comparative expression profiling of the sciatic nerves of curcumin treated and untreated *Tr-J* mice revealed a significant increase in the expression of the heat shock protein, Hsp70i. Additionally, reduction of Hsp70i dosage in *Tr-J* mice results in a more severe peripheral neuropathy and upon curcumin treatment a rise in mortality. Based on these findings, we investigated the possible therapeutic role of Hsp70i further by producing F1 generation and an F2 generation *Tr-j* mice that over express *Hsp70i* on a mixed background. Within the F1 generation one copy of the *Hsp70i* transgene leads to a visibly healthier phenotype and improved neuromotor function. In addition, to further assess the role of *Hsp70i* expression in the alleviation of cellular stress and the peripheral neuropathic phenotype, other small molecular compounds known to induce *Hsp70i* expression are being screened for their potential benefits.

2255/F

Founder Effect in French Canadian Action Myoclonus-Renal Failure Syndrome (AMRF). *E. Andermann[1], D. Amrom[2], J. Mulley[3], M.A. Bayly[4], M. Jomphe[5], M. Talani[2], M. Pierre[6], S. Berkovic[7], F. Andermann[8], L. Dibbens[9].* 1) Neurogenetics Unit, Montreal Neurological Hospital and Institute; Departments of Neurology & Neurosurgery and Human Genetics, McGill University, Montreal, Canada; 2) Neurogenetics Unit, Montreal Neurological Hospital and Institute; Department of Neurology & Neurosurgery, McGill University, Montreal, Canada; 3) Department of Genetic Medicine, Women's and Children's Hospital, North Adelaide, Australia; 4) Epilepsy Research Program, SA Pathology, Women's and Children's Hospital, North Adelaide, Australia; 5) BALSAC Project, Université de Québec, Chicoutimi, Canada; 6) Neurogenetics Unit, Montreal Neurological Hospital and Institute, McGill University, Montreal, Canada; 7) Epilepsy Research Center, Department of Medicine (Neurology), University of Melbourne; Austin Health, Heidelberg West, Melbourne, Australia; 8) Epilepsy Service, Montreal Neurological Hospital and Institute; Depts of Neurology & Neurosurgery and Pediatrics, McGill University, Montreal, Canada; 9) Epilepsy Research Program, SA Pathology, Women's and Children's Hospital, North Adelaide, Australia; School of Pediatric and Reproductive Health, University of Adelaide, Australia.

BACKGROUND: AMRF, an autosomal recessive form of progressive myoclonus epilepsy with renal failure, was initially described in three French Canadian families in 1986. Subsequently, additional families were reported in other continents. Disease onset is usually in the late teens or early twenties, and begins with tremor and proteinuria, progressing to action myoclonus, tonic-clonic seizures and renal failure. The gene for this disease was recently found to be SCARB2/LIMP2, a lysosomal protein. OBJECTIVES: To characterize the SCARB2 mutations in French-Canadian patients with AMRF, and their family members and to confirm founder effect in these families. METHODS: The original French Canadian families were contacted and family histories updated. Additional families were also ascertained. Blood was obtained on 85 family members, and exon 7 of the SCARB2/LIMP2 gene was sequenced for the c.862C>T (Q288X) mutation found in one of the original French Canadian families. Haplotyping and genealogical studies to find common ancestors employing the BALSAC database were also performed. RESULTS: All five original AMRF patients have died. Four new patients in two families were homozygous for the Q288X mutation, but the mother of one of the original patients was found to carry another SCARB2 mutation, suggesting allelic heterogeneity. In one of the new families, both parents are second degree cousins to one of the original probands and to one another. Twenty-eight unaffected individuals in four families who were not obligate carriers were found to be heterozygous for the Q288X mutation. This included one carrier couple, who were later found to have an affected son. Over 20 other married-in spouses of these mutation carriers had negative sequencing of the entire SCARB2 gene. Haplotype analysis resulted in a shared haplotype of over 7.2 Mb, strongly suggesting identity by descent. Genealogies of 4 families have been traced to the beginning of the17th century, and thirty-two ancestors common to all 4 families have been identified. CONCLUSIONS: The finding of the same SCARB2 mutation and common haplotype in at least four French Canadian families who were previously not known to be related strongly suggests a founder effect. Carrier screening for the founder mutation in this population can be carried out without sequencing the entire SCARB2 gene. This has important implications for prenatal diagnosis, genetic counseling and prevention of this severely debilitating disease.

2256/F

Dentatorubral pallidoluysian atrophy: clinical and genetic analysis of a Sicilian pedigree. *G. Annesi[1], M. D'Amelio[2], P. Tarantino[1], V. Lo Re[2], P. Ragonese[2], G. Salemi[2], A. Quattrone[3], G. Savettieri[2], P. Aridon[2].* 1) Istituto di Scienze Neurologiche, Mangone (CS), CS, Italy; 2) Dept. di Biomedicina Sperimentale e Neuroscienze Cliniche, Università di Palermo, Palermo,Italy; 3) Institute of Neurology, University Magna Graecia, Catanzaro, Italy.

Objectives: To describe clinical, radiological and genetic features of a three generation Sicilian family affected by Dentatorubral-Pallidoluysian atrophy (DRPLA). Material and Methods: We obtained clinical and genealogical information from twelve people of a three generation pedigree. Five out of seven affected living people were examined and blood samples were obtained. All family members signed an informed consent form. Neurological evaluations, neurophysiological and neuropsychological examinations, neuroimaging studies were performed. Genomic DNA was extracted from peripheral blood by standard methods. Triplet expansion in DRPLA was detected by PCR amplification with fluorescent primers. PCR products were separated onto a capillary sequencer (3130XL genetic analyzer-Applied Biosystems) and the length of specific DRPLA fragments was confirmed by direct sequence analysis. Results: The proband of the family, a 48-year-old man, at the age of 37, began to experience episodes of loss of consciousness with generalized seizures. Afterwards he developed an unsteady gait, involuntary movements of the head, face and limbs, in addition to cognitive and psychiatric symptoms. Neurological examination was characterize by choreiform movement, myoclonus and cognitive deterioration. His gate was ataxic and tandem walking was impossible. All laboratory tests were normal. Cranial MRI showed mild diffuse atrophy. An electroencephalogram revealed abnormalities in the right frontal and temporal lobes. Somatosensory, motor and brainstem auditory evoked potentials were altered. Two sisters of the proband reported similar symptoms with different degree of gait, speech disturbance and choreoathetosis. The first son of the proband, a nephew and two young first cousins, at the age of 15 started to complain of myoclonic epilepsy, and variable degree of cognitive disturbance. Molecular analysis of all affected people revealed a heterozygous CAG repeats expansion in ATN-1 gene, confirming the DRPLA diagnosis. All the heterozygous expanded alleles were fully sequenced. Conclusions and Discussion: We provided a detailed description of the main clinical, genetic and radiological features of the second Italian family with DRPLA. Our results further support ATN-1 gene as causative of DRPLA. As a whole, the phenotype mainly overlapped known DRPLA cases but our data show that the disease may have a great variability of age, type of onset and course of the disease.

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Depletion of CTCF, even in the absence of expanded GAA triplet-repeats, is sufficient to reproduce the epigenetic silencing of the *FXN* gene seen in Friedreich ataxia. *S.I. Bidichandani, A.M. Castro, Y.K. Chutake.* Dept Biochemistry/Molec Biol, Oklahoma Univ Hlth Sci Ctr, Oklahoma City, OK.

Friedreich ataxia (FRDA) patients are homozygous for an expanded GAA triplet-repeat sequence in intron 1 of the *FXN* gene. The expanded GAA triplet-repeat results in deficiency of *FXN* gene transcription, which is reversed via administration of histone deacetylase inhibitors indicating that transcriptional silencing is due to an epigenetic abnormality. FRDA patients show depletion of the chromatin insulator protein CTCF (CCCTC-binding factor) in the 5'UTR of the *FXN* gene in FRDA, and coincident heterochromatin formation involving the +1 nucleosome via recruitment of heterochromatin protein 1. It has been suggested that epigenetic silencing of the *FXN* gene is likely due to spreading of heterochromatin from the expanded GAA triplet-repeat. Here we show that knockdown of CTCF in a human cell line (HEK293; ChIP assays confirmed depletion of CTCF at the *FXN* locus) that does not contain expanded GAA triplet-repeats at the *FXN* locus resulted in recruitment of heterochromatin protein 1 to nucleosome +1 and *FXN* transcriptional deficiency, as is seen in FRDA. We previously showed that FRDA patients have overexpression of *FAST-1*, an antisense transcript that overlaps with nucleosome +1. Indeed, CTCF knockdown in HEK293 cells also reproduced the *FAST-1* overexpression seen in FRDA. Together, these data indicate that depletion of CTCF is sufficient to explain the epigenetic silencing of the *FXN* gene seen in FRDA, and is a critical mediator of the molecular pathogenesis of FRDA.

2258/F

Molecular analysis of EIF2B genes in patient with late onset CACH/VWM syndrome. *B-Z. Yang[1], S.E. Sparks[2], N. McNeill[1], C.K. Brasington[2], J-H. Ding[1].* 1) Inst Metabolic Disease, Baylor Res Inst, Dallas, TX; 2) Levine Children's Hospital at Carolinas Medical Center, Charlotte, NC.

Childhood ataxia with central nervous system hypomyelination or leukoencephalopathy with vanishing white matter syndrome (CACH/VWM) is an autosomal recessive disorder characterized by episodes of rapid deterioration during and/or after infection, leading to a progressive vanishing of the white matter replaced by cerebrospinal fluid. Clinical phenotypes include: 1. fatal infantile/congenital form; 2. early childhood form associated with extra-neurological symptoms and 3. juvenile and adult forms (ovarioleukodystrophy), all of which are mostly caused by eukaryotic initiation factor 2B (eIF2B) defect leading to the term of eIF2B-related leukoencephalopathies. In this study, we report a patient with adult form of CACH/VWM. Patient is a 25-year old male who initially presented with neurological deterioration following a traumatic head injury at 18 years of age. He subsequently had 4 more instances of head trauma leading to neurological deterioration and loss of ambulation at 22 years of age. A seizure disorder, controlled with antiepileptics began after the second head injury. A brain MRI demonstrated diffuse leukodystrophy. On exam he is wheelchair dependent, has slow, dysarthric speech, truncal and intentional ataxia, and signs of a peripheral neuropathy. Once available, the brain MRI was further reviewed and showed signs consistent with CACH/VWM. To investigate the molecular defect, genomic DNA was extracted from patient's blood, and all exons and flanking intronic regions of the five EIF2B genes were amplified. The PCR products were purified and sequenced. Sequence analysis revealed that the patient is a compound heterozygous for a c.728C>T (p.P243L) mutation and a novel mutation c.715G>C in exon 8, which results in an Aspartic acid to Histidine substitution at codon 239 (p.D239H) in the EIF2B4 gene. The novel mutation c.715G>C was verified by a PCR/restriction test, but was not detected in the normal control subjects. In addition, this patient also carries a variation c.1759A>G (p.I587V) in the EIF2B5 gene, for which both parents are heterozygous. Sequence analysis confirmed that this patient's father carries c.1759A>G and mutation c.728C>T; mother with c.1759A>G and novel mutation c.715G>C. Further studies will improve our understanding of genetic causes in late onset CACH/VWM patients.

2259/F

Protein-RNA interactive network of X-linked Mental Retardation (XLMR) genes. *N. Zhong[1, 2], XG. Tao[2].* 1) Dept Human Gen, New York State Inst Basic Res, Staten Island, NY; 2) Peking University Center of Medical Genetics.

To better understand the biological mechanism associates with X-linked Mental Retardation (XLMR), a protein-RNA network was constructed with a yeast tri-hybrid (Y3H) system, followed by in vitro gel mobility assays. 2115 pairs of protein-RNA interaction were performed by constructing RNA sequence of 3' UTR fragment derived from 47 XLMR genes and 45 genes encoding neuron synapses or neuronal development with Y3H. 275 positive interactions were identified. Our results showed that proteins SERPINI1, OFD and ZNF41 have the most connections with 3'UTR of RNAs, suggesting that these proteins may locate in the centre of the network and play important roles in regulation. Potentially they might be a candidate for some XLMR diseases whose underlying gene is yet unidentified. HOPA 3'UTR and PQBP1 3'UTR were interacted by common synaptic or neuron developmental proteins. A cluster, composed of 3'UTR of OTC, PAK3, KIAA1202 and proteins of GPC3, MID1, FTSJ1, HADH2, showed the associations between synaptic or neuron developmental genes and XLMR. Analysis with systems biology approach, eight clusters of protein-RNA interaction, including one cluster of 14 nodes with 19 edges, one of 7 nodes with 9 edges, one of 6 nodes with 13 edges, three of 4 nodes with 4 edges, one of 5 nodes with 6 edges, and one of 6 nodes with 6 edges, were identified. In addition, three clusters of RNA-RNA association, two of which are 4 nodes with 6 edges and one of 4 nodes with 4 edges, and two clusters of protein-protein association, one is 4 nodes with 6 edges and the other, 5 nodes with 10 edges, were identified. In summary, our study may have open a new avenue to investigate the XLMR pathogenic mechanism.

2260/F

Convergent transcription of an ataxin-7 antisense RNA represses sense promoter activity through Dicer-1 dependent processing of double-stranded RNA. *P. Ladd[1], B.L. Sopher[2], V.V. Pineda[2], G.N. Filippova[3], A.R. La Spada[1,4,5,6].* 1) Pediatrics, UCSD, La Jolla, CA; 2) Laboratory Medicine, Univ of Washington, Seattle, WA; 3) Fred Hutchinson Cancer Research Center, Seattle, WA; 4) Cellular & Molecular Medicine, UCSD, La Jolla, CA; 5) Institute for Genomic Medicine, UCSD, La Jolla, CA; 6) Rady Children's Hospital, San Diego, CA.

Spinocerebellar ataxia type 7 (SCA7) is a polyQ disease characterized by retinal and cerebellar degeneration. SCA7 is caused by expansion of a CAG repeat in the ataxin-7 gene. Both the ataxin-7 CAG repeat tract and start site of translation are located in exon 3. We identified an alternative promoter (P2A) located ~1 kb 5' to exon 3. Promoter activity and boundary were confirmed by luciferase reporter assays. We also identified an antisense (AS) non-coding RNA, SCANT1 (for SpinoCerebellar Ataxia Noncoding Transcript 1) that is convergently transcribed across exon 4, exon 3, and P2A. RNase A/T1 dsRNA duplex assays on fibroblast RNAs revealed dsRNAs in the region of bidirectional transcription. Northern blot analysis of human tissues detected small RNAs (20-60 nt), suggesting that small processed RNA fragments are being produced in this region. To understand the regulation of promoter P2A, we tested if expression of SCANT1 in trans could reduce the luciferase activity of the promoter fragment, and noted no effect. To test if SCANT1 transcription regulates P2A in cis, we performed gene trap assays with ataxin-7 P2A - exon 3(CAG10) - exon 4 genomic fragment constructs with a 3' IRES-luciferase (luc) in sense orientation, and a Renilla luciferase (Rluc) in AS orientation. The AS promoter was replaced with a tet-regulatable element (TRE) to yield the TRE-only ataxin-7 construct, and a second version created by cloning a polyA transcription termination signal ("polyA trap") in AS orientation into exon 3. We confirmed the integrity of the polyA trap by comparing TRE-only and TRE-polyA-trap ataxin-7 vectors, and observed marked reduction of Rluc/luc for the TRE-polyA-trap. After doxycycline treatment, we also measured ataxin-7 expression by qPCR, and observed significant de-repression of ataxin-7 sense expression in TRE-polyA-trap transfected cells. To determine if RNA processing machinery is involved in SCANT1 regulation of P2A, we performed Dicer-1 knockdown on cerebellar astroctyes with an integrated ataxin-7 genomic fragment, and detected a significant increase in ataxin-7 sense expression. No evidence for Drosha or argonaute protein involvement in SCANT1 regulation of ataxin-7 expression was seen. Our findings indicate that AS-mediated repression of the ataxin-7 sense promoter operates by convergent transcription, yielding dsRNAs that are processed by Dicer-1, providing evidence for Dicer-1-dependent co-transcriptional silencing in higher organisms.

2261/F

Transgenic expression of the ALS4 senataxin R2136H mutation yields a neurological phenotype in mice. *C.L. Bennett[1], Y. Chen[2], B.L. Sopher[6], G.A. Garden[6], A.R. La Spada[1,3,4,5].* 1) Pediatrics, Univ of California, San Diego, La Jolla, CA; 2) Department of Lab Medicine, University of Washington, WA; 3) Cellular & Molecular Medicine, Univ of California, San Diego, La Jolla, CA; 4) Institute for Genomic Medicine, San Diego, La Jolla, CA; 5) Rady Children's Hospital, San Diego, CA; 6) Department of Neurology, University of Washington, Seattle, WA.

Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disorder characterized by upper and lower motor neuron degeneration, leading to fatal paralysis. The majority of ALS cases are sporadic (SALS); however, various familial forms of ALS (FALS) represent up to 10% of all cases. Despite years of study, the mechanistic basis of motor neuron loss in ALS remains ill-defined. Very recently, a pair of discoveries has demonstrated that ALS can be caused by mutation in either of two DNA/RNA binding proteins (TDP-43 and FUS/TLS). This has led to a paradigm shift positing that altered RNA transcript production and processing are responsible for ALS motor neuron disease pathology. Thus far, however, accurate recapitulation of ALS-like neuromuscular phenotypes in mouse models expressing mutations in these two genes has not been achieved. ALS4 is a juvenile-onset, autosomal dominant form of FALS, characterized by limb weakness, muscle wasting, pyramidal signs, and relentless disease progression. We discovered that gain-of-function mutations in the senataxin (SETX) gene are responsible for ALS4. Interestingly, recessive loss-of-function mutations in SETX also produce a neurological phenotype - in this case, an inherited ataxia, known as ataxia with oculomotor apraxia type 2 (AOA2). The senataxin protein contains a conserved superfamily 1, DNA/RNA helicase domain in the C-terminus, indicating a key role in RNA processing. Furthermore, senataxin appears to regulate RNA polymerase II transcription termination of short transcripts, and may play a role in transcription-coupled DNA repair. To determine how SETX mutations produce a toxic gain-of-function, we derived transgenic mice using the murine prion protein (MoPrP) vector, as MoPrP drives expression throughout neurons and all other cell types in the CNS, including the different cell types that comprise the neuromuscular system. We generated both wild-type transgenic mice (PrP-SETX-wt) and transgenic mice expressing a known ALS4 mutation (PrP-SETX-R2136H). PrP-SETX-R2136H transgenic mice display a slowly progressive neurological phenotype, based upon significantly impaired rotarod performance in comparison to PrP-SETX-wt mice, beginning at six months of age. PrP-SETX-R2136H transgenic mice also exhibit reduced stride length and hind limb drag on ledge test, suggesting that they suffer neuromuscular disease. Our findings thus indicate that ALS4 can be accurately recapitulated in transgenic mice.

2262/F

A novel splicing alteration in *UBA1* results in significant reduction of *UBA1* expression in X-linked infantile SMA. *J. Ramser[1], C. Lenski[1], A. Meindl[1], M. von Rhein[2], B. Wirth[3], M.E. Ahearn[4], L. Baumbach[4].* 1) OB/GYN, Klinikum r.d. Isar, Technische Universitaet Muenchen, Munich, Germany; 2) Department of Neuropediatrics, University Children's Hospital Mainz, Mainz, Germany; 3) Institute of Human Genetics, Center for Molecular Medicine Cologne, University of Cologne, Cologne, Germany; 4) Dr. John T. Macdonald Department of Human Genetics, University of Miami Miller School of Medicine, Miami, USA.

X-linked infantile spinal-muscular atrophy (XL-SMA; MIM #301830) is an X-linked disorder presenting with the clinical features hypotonia, areflexia and multiple congenital contractures (arthrogryposis) associated with loss of anterior horn cells and infantile death. Recently we have identified *UBA1* (also named *UBE1*) which plays a key role in the Ubiquitin-Proteasome System as the underlying disease gene for XL-SMA (Ramser *et al.*, AJHG, 2008). In addition to two missense mutations in *UBA1* exon 15 in two XL-SMA families, we have identified a synonymous C to T substitution (c.1731 C>T, p.Asn577Asn) in the same exon in another three unrelated families. Here we report our further investigations which demonstrate that this synonymous substitution leads to a significant reduction of *UBA1* expression. Applying quantitative PCR in white blood cells of an affected male we demonstrate a reduction to one fifth normal steady-state levels of *UBA1* mRNA as compared to six healthy male controls. Additionally, analysis of a total of eight female carriers from three independent XL-SMA families displayed a reduction of *UBA1* mRNA to one half, compared to eight female healthy controls. Furthermore, *in silico* analyses revealed the c.1731 C>T substitution targets a specific exonic splice enhancer (ESE) motif that can bind SC35, a protein involved in splicing, and could lead in close proximity to the generation of a second putative ESE which functions as a SRp55 binding site. This indicates a possible modification of the splicing process as the underlying cause for the detected reduced expression. Indeed, current quantitative PCR analyses of a weakly expressed *UBA1* transcript lacking exon 15 (delta-exon 15 variant) revealed a 16-fold increase of expression of this variant in white blood cells of an affected individual as compared to four male healthy controls. Female carriers displayed a three fold expression increase as compared to female healthy controls. Since the absolute expression level of the delta-exon 15 variant is even in disease state still rather low as compared to the wild type transcript (estimated two percent) we do not expect to detect the increase of the variant to be pathogenic. We propose that the synonymous c.1731 C>T mutation causes the generation of aberrantly spliced RNA products (at the expense of the wild type transcript) which are likely a target of nonsense-mediated RNA decay (NMD). This possibility is being further investigated.

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IQCB1 (NPHP5) mutations in Leber congenital amaurosis with very late nephronophthisis. *I. Perrault[1], R. Salomon[2], V. Morinière[2], K. Tory[3], N. Delphin[1], E. Silva[4], H. Dollfus[5], C. Hamel[6], C. Antignac[2], A. Munnich[1], JM. Rozet[1], S. Saunier[2], J. Kaplan[1].* 1) Dept Gen - Hopital Necker, INSERM U781, Paris, Cedex 15, France; 2) Dept Gen - Hopital Necker, INSERM U574, Paris, Cedex 15, France; 3) 1st Department of Pediatrics, Semmelweis University, Budapest, HONGRIE; 4) Center for Hereditary Eye Diseases Department of Ophthalmology, University Hospital of Coimbra Av Bissaya Barreto 3000-075 Coimbra Portugal; 5) Clinique Ophtalmologique, Hôpitaux Universitaires de Strasbourg, Strasbourg, France; 6) Inserm U583 Physiopathologie et thérapie des déficits sensoriels et moteurs. Hôpital St Eloi - Montpellier , France.

Purpose: Leber congenital amaurosis (LCA) is the earliest and most severe retinal degeneration with onset in the first months of life. Hitherto, mutations in 13 genes were reported to account for 2/3 of LCA. Mutations in one of them, CEP290 (NPHP6), result in a variety of phenotypes ranging from isolated retinal degeneration to pleiotropic disorders including nephronophthisis (Senior-Løken syndrome; SNLS) and cerebellar vermis aplasia (Joubert syndromes; JBTS). Recently, genetic and physical interactions were reported between CEP290 and IQCB1 (NPHP5) which mutations have been reported to account for SNLS. This finding prompted us to look for IQCB1 mutations in LCA patients. Patients and Methods: We considered 240 LCA patients with no cerebellar or renal alterations when ascertained. Direct sequencing of LCA genes found no mutation. Probands' DNAs were screened for mutations by direct sequencing using primers designed from the intronic sequences flanking the 13 IQCB1 coding exons. Clinical data were updated for renal function. Results: 22 IQCB1 disease alleles were identified in 11 families (13 patients; 8 different mutations, 5/8 novel). Consistent but highly variable hyperopia (+3 to +10) was noted. Patients of 7 families were affected with LCA type II (rod-cone dystrophy). Three unrelated cases presented with type I (cone-rod dystrophy), 2/3 of whom were mentally retarded with normal brain MRI. Fundus showed multiple white-dots and/or snail track aspect in the mid-peripheral retina (n=5). Update of renal history revealed nephronophtisis symptoms with early to very late onset in 6/11 families (9 patients; 4 y<end-STAGE disease renal>55 y) while 5/11 families are under investigations (n=7 patients). Discussion: Owing to its onset at birth, LCA is the presenting symptom in several multisystemic disorders which diagnosis is usually made when extraocular symptoms are overt i.e. in the first two decades of life. However, here we report that in SNLS the age at onset of renal failure may vary widely from patient to patient even within families, ranging from early childhood to very late adulthood(>55y). The observation of non exceptional late-onset renal failure requires a close follow-up of patients with IQCB1 mutations whatever their age and more generally of patients whose genotype is unknown. Conversely, the identification of mutations in all LCA genes but CEP290, warrants the absence of risk to develop syndromic LCA making extra-ocular investigations useless.

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Molecular Analysis of PKD2 Splicing Induced by a Novel Missense Mutation in Autosomal Dominant Polycystic Kidney Disease. *Y. Tan[1], J. Blumenfeld[2, 3], A. Michaeel[1], S. Donahue[2, 3], M. Balina-Marin[3], T. Parker[3], D. Levine[3], H. Rennert[1].* 1) Dept Pathology/Laboratory Med, Weill Cornell Medical Col, New York, NY; 2) Departments of Medicine, Weill Cornell Medical College, , New York, NY; 3) The Rogosin Institute, New York, NY.

Autosomal dominant polycystic kidney disease (ADPKD) is a common, clinically heterogeneous heterogeneous multi-systemic disorder characterized the development of renal cysts,chronic kidney disease, and various extra-renal manifestations. The disease is caused by mutation in either one of two principal genes, PKD1 and PKD2. Here we report the identification of a private missense change in the PKD2 gene (p.Arg440Ser), using SURVEYOR Nuclease-WAVE-sequencing mutation analysis, in a patient presenting with kidney cysts and a family history of ADPKD. This mutation in the first position of exon 6 resulted in premature termination and destruction of a conserved acceptor splice site. Quantitative reverse-transcription (qRT)-PCR analysis demonstrated that the aberrantly spliced PKD2 transcript was present at substantial higher amounts in the patient's peripheral blood leukocytes, and, in varying degrees, in other patients with ADPKD as well as in normal control individuals. Our study presents is the first case of a missense change that also affects RNA splicing in ADPKD. We hypothesize that differential expression of aberrantly spliced variants contributes to the clinical variability observed in ADPKD.

2265/F

Genes in the Ureter Budding Pathway: Exploratory Association Study in a Dutch cohort of Vesico-Ureteral Reflux Patients. *A.M. van Eerde[1], K. Duran[1], E. van Riel[1], C.G.F. de Kovel[1], B.P.C. Koeleman[1], N.V.A.M. Knoers[2], K.Y. Renkema[2], H.J.R. van der Horst[3], A. Bökenkamp[4], J.M. van Hagen[5], L.H. vd Berg[6], K. Wolffenbuttel[7], W.F. Feitz[8], T.P.V.M. de Jong[9], J.C. Giltay[1], C. Wijmenga[1,10].* 1) Department of Medical Genetics, University Medical Center Utrecht, Netherlands; 2) Department of Human Genetics, Radboud University Nijmegen Medical Center, Netherlands; 3) Pediatric Urology, VU University Medical Center, Amsterdam, Netherlands; 4) Pediatric Nephrology, VU University Medical Center, Amsterdam, Netherlands; 5) Department of Human Genetics, VU University Medical Center, Amsterdam, Netherlands; 6) Department of Neurology, Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, Netherlands; 7) Pediatric Urology, Sophia Children's Hospital, Erasmus Medical Center, Rotterdam, Netherlands; 8) Pediatric Urology Centre, Department of Urology, Radboud University Nijmegen Medical Center, Netherlands; 9) Pediatric Renal Center, University Medical Center Utrecht and Academic Medical Center Amsterdam, Netherlands; 10) Department of Genetics, University Medical Center Groningen and University of Groningen, Netherlands.

Vesico-ureteral reflux (VUR) is the retrograde passage of urine from the bladder to the upper urinary tract. 8.5% of end stage renal disease in children is due to VUR. VUR is a complex genetic developmental disorder. Ectopic embryonal ureter budding has long been implicated in the pathogenesis of VUR. We performed an extensive candidate-pathway association study for primary VUR by using a two-stage, case-control design, investigating genes in the ureter budding pathway.

We recruited a Dutch VUR patient cohort (n=409). Controls (n=1446) were included from existing cohorts (e.g. blood bank). The cases were not only analyzed as one group, but we also extracted two extreme phenotype subgroups. Sub-group one consisted of clear cut primary VUR cases (~50% of the cohort); sub-group two contained patients with a duplex collecting system and VUR(~25% of the cohort).

Based on a review of the literature, we selected 44 genes known to be involved in the embryonal ureter budding pathway. A set of 567 tagging single nucleotide polymorphisms (SNPs) for these genes was genotyped in half of the case cohort and controls (stage one). The 14 SNPs that reached the lowest p value (p value cut-off: 0.005) in stage one, were genotyped in the other half of the case cohort and controls (stage two).

The results suggested associations with VUR in six genes. For four genes (gene, odds ratio (95% confidence interval)): GENE1, 0.77 (0.65 - 0.91); GENE2, 1.27 (1.07 - 1.51); GENE3, 1.28 (1.03 - 1.59) and GENE4, (0.77 (0.62 - 0.96), the effect, or trend towards the effect, was present in the joint results of both phenotype sub-groups. For GENE5, 0.80 (0.69 - 0.94), mainly sub-group one with primary VUR cases contributed to the overall effect. The GENE6 gene (1.52 (1.11 - 2.06) was only associated in sub-group two (with duplex collecting systems and VUR). With p-values not reaching the multiple-testing threshold of significance (10-5), these results warrant replication in larger cohorts. This exploratory study seems to confirm the hypothesis that common genetic variants in the ureter budding pathway do contribute to the genetic susceptibility for primary VUR.

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Clinical and Molecular Characterization of a Kidney Disease Resulting From Dominant Renin Gene Mutation and Response to Treatment with Fludrocortisone. *S. Kmoch[1,2], M. Živná[1,2], H. Hļková[1], K. Hodaňová[1,2], P. Vyletal[1,2], J. Sikora[1], J. Sovová[1], M. Elleder[1,2], J. Živný[3], T. Hart[4], J. Adams[5], K. Kapp[6], R. Haws[7], S. Moe[8], L. Cornell[9], S. Hart[5], A. Bleyer[10].* 1) Institute for Inherited Metabolic Disorders, First Faculty of Medicine, Charles University in Prague,; 2) Center for Applied Genomics, First Faculty of Medicine, Charles University in Prague,; 3) Institute of Pathophysiology, First Faculty of Medicine, Charles University in Prague, Prague,; 4) Human Craniofacial Genetics Section, NIDCR, NIH, Bethesda MD; 5) Office of the Clinical Director, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD; 6) ZMBH (Center for Molecular Biology Heidelberg), University of Heidelberg; 7) Specialty Pediatrics, Marshfield, WI; 8) Department of Medicine, Indiana University School of Medicine, Indianapolis, IN; 9) Division of Anatomic Pathology, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN; 10) Section on Nephrology, Wake Forest University School of Medicine, Winston-Salem, NC.

Mutations within the hydrophobic part (h-region) of the renin (REN) signal sequence have been recently identified in three unrelated families with the autosomal-dominant inheritance of early-onset anemia, polyuria, hyperuricemia and progressive chronic kidney disease (Živná et al.; Am.J.Hum.Gen. 2009, 85:204-13). We report fourth family with this syndrome and a novel heterozygous mutation c.58T>C resulting in the amino acid substitution of cysteine for arginine in the preprorenin signal sequence (p.Cys20Arg) occurring in all affected members. The mutation occurs in the conserved polar C-terminal part (c-region) of the preprorenin signal sequence which decreases its overall hydrophobicity and alters its cleavage site score profile and cleavage site probability. In-vitro studies showed that the mutation indeed abolishes endoplasmic reticulum cotranslational translocation and post translational processing of the mutant protein. Transient expression of p.Cys20Arg preprorenin in HEK293 cells leads to massive accumulation of non-glycosylated, non-granular and thus secretion incompetent preprorenin in cytoplasm of transfected cells. We propose that this mechanism likely reduces viability of renin producing cells which leads to attenuation of renin biosynthesis and dysregulation of intra-renal RAS (renin-angiotensin system). This is reflected by low plasma renin activity, low serum aldosterone, irregular expression of RAS components and ultrastructural damage of the kidney. Treatment with fludrocortisone in an affected 10 year old child resulted in an increase in blood pressure and estimated glomerular filtration rate. Nephrologists should consider REN mutations in families with autosomal dominant inheritance of chronic kidney disease, especially if they suffer from anemia, polyuria and hyperuricemia in childhood.

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Unravelling the unexpected genetic heterogeneity of nephronophthisis in the Hutterite population. *C.M. Loucks[1], A.M. Innes[1], A.W. Wade[2], K.M. Boycott[3], P. Frosk[1], C.L. Beaulieu[1], E.G. Puffenberger[4], J.S. Parboosingh[1].* 1) Dept of Medical Genetics, University of Calgary, Calgary, AB, Canada; 2) Division of Pediatric Nephrology, University of Calgary, Calgary, AB, Canada; 3) Dept of Genetics, Children's Hospital of Eastern Ontario, Ottawa, ON, Canada; 4) Clinic for Special Children, Strasburg, PA, USA.

Nephronophthisis (NPHP) is a genetically heterogenous group of autosomal recessive disorders characterized by renal cysts, polyuria, polydipsia, secondary enuresis and anemia. It is the most frequent genetic cause of end-stage renal disease in children and is seen in 1 in 50 000 live births in Canada. NPHP is classified as a ciliopathy due to the identification of causative mutations in 10 genes localizing to nonmotile cilia (*NPHP1-9, 11*), with the majority of cases being attributed to a recurrent homozygous deletion of *NPHP1*. Within the endogamous Hutterite population the frequency of NPHP is even higher and may approach 1 in 2750. As the majority of Hutterites are descended from 89 ancestors this was expected to be the result of a single founder mutation. The 'common' *NPHP1* deletion was found in four Hutterite NPHP patients, but five other patients were initially found to lack the homozygous *NPHP1* deletion, suggesting the presence of a second founder mutation in the population. Three of these patients are closely related, and the causative mutation was expected to lie within a homozygous chromosomal region inherited from a common ancestor. Initially, no homozygous regions were found surrounding the known NPHP genes using microsatellite markers, suggesting the possibility of a novel locus, but a 10K SNP array revealed an apparent homozygous region surrounding *NPHP1*. Sequencing *NPHP1* in these patients showed a single base pair deletion, resulting in a premature stop codon in the final exon expected to disrupt normal cell-cell junctional targeting of the encoded protein. Interestingly, these three patients who appeared homozygous on the SNP array were found to be compound heterozygotes for the large 'common' deletion and the single base pair deletion, while the two remaining patients did not carry either *NPHP1* mutation, indicating that at least one more NPHP mutation is present in the population. A 10K SNP array on one of these patients revealed multiple stretches of homozygosity including one surrounding *NPHP3*. Sequencing of *NPHP3* is underway, and if a mutation is not found consideration will be given to genes in other homozygous regions implicated in renal dysfunction and/or expressed in the ciliary proteome. Identifying the full spectrum of mutations causing NPHP in the Hutterites will allow for more comprehensive clinical genetics service for this population and will further our understanding of the complexities of ciliopathies in this population.

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Identification of a chromosomal locus associated with recessive Primary Ciliary Dyskinesia in a Bedouin family. *M. Mazor[1,2], S. Alkrinawi[3], V. Caspi[2], E. Manor[4], J.C Beck[5], V. Sheffield.[5], M. Aviram[3], R. Parvari[1,2].* 1) Developmental human Genetics, Ben Gurion University, Beer-Sheva, Negev, Israel; 2) The National Institute for Biotechnology in the Negev, Israel; 3) Pediatric Division Soroka medical Center, Beer-Sheva, Israel; 4) Institute of Genetics, Soroka Medical Center, Beer Sheva 84105 and Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva 84101, Israel; 5) Dept. of Pediatrics - Division of Medical Genetics; Howard Hughes Medical Institute, University of Iowa, Iowa City, IA 52242, USA.

Primary ciliary dyskinesia is a rare autosomal recessive genetic disorder, caused by inherited defects of ciliary structure and function. The clinical features reflect the distribution of dysmotile cilia and include neonatal and chronic respiratory distress due to lack of coordinated ciliary movement. In approximately half of PCD patients, there is apparent randomization of left-right axis development, or situs inversus totalis proposed to result from defective function of embryonic nodal cilia. We have characterized a consanguineous Bedouin family from the Negev, who has two siblings diagnosed with situs inversus and respiratory symptoms and numerous healthy siblings. Linkage to the 13 genes known to be associated with the disease was negated. Further genome wide linkage analysis using the Affymetrix GeneChip mapping 250K array and microsatellite markers was performed. Homozygosity mapping identified a chromosomal region larger than 16cM. Genotyping the region by analyzing polymorphic markers to all family members has defined a locus of 30Mb on chromosome 18q with a Lod score of 3.0 for multipoint analysis. Prioritizing genes for search of the mutation and initial sequencing, was preformed according to the databases of proteome collections, and derived from evolutionarily distant organisms which combines independently assembled ciliary, basal body and centrosome. Identification of additional genes involved in cilia function will provide new insights into the molecular mechanisms of the cilia and help to develop much needed novel techniques to diagnose subjects with PCD.

2269/F

Deleterious mutations in *RRM2B* causing severe mitochondrial DNA depletion in infancy. *S. Seneca[1,2], J. Smet[3], R. Van Coster[3], W. Lissens[1,2], S. Van Dooren[1], M. Bonduelle[1,2], I. Liebaers[1,2], L. De Meirleir[4,2].* 1) Center for Mediacal Genetics, UZ Brussel, Brussels, Belgium; 2) Vrije Universiteit Brussel (VUB), Brussels, Belgium; 3) Division of Paediatric Neurology and Metabolism, University Hospital Ghent, Ghent, Belgium; 4) Division of Paediatric Neurology, UZ Brussel, Brussels, Belgium.

Mitochondrial DNA (mtDNA) copy number reduction, known as the mitochondrial DNA depletion syndrome (MDS), is a common cause of severe mitochondrial disorders of infancy and early childhood. MDS results from defects in nuclear encoded factors involved in mtDNA maintenance and within the past years mutations in the *POLG1*, *DGUOK*, *MPV17*, *PEO1*, *SUCLG1*, *TK2* and *SUCLA2* genes have proven to be implicated in the pathogenesis of this disorder. The clinical phenotypes associated with the different gene alterations vary considerately but present either as a hepatocerebral or a myopathic syndrome. We have identified a homozygous p.E85 deletion in exon 3 of the *RRM2B* gene in a neonate. The patient, born to first-cousin Caucasian parents, presented with lactic acidosis, severe hypotonia, deafness, blindness and hyperammonemia. Muscle biopsy showed RRF, a combined respiratory chain defects and massive subcomplexes of ATP synthase both with traditional spectrophotometry and BN-PAGE. Western blotting using antibodies against selective OXPHOS subunits indicated the preservation of nuclear encoded complex II. She died at 2 months of age. Mutations of the *RRM2B* gene, encoding the cytosolic R2 subunit of a p53 controlled ribonucleotide reductase (p53R2), have been reported to cause severe depletion of muscle mtDNA by Bourdon et al. 2007. Indeed, < 5% residual amount of mtDNA was measured in muscle tissue of our patient. Aberrations in these 8 genes count only for a minority of all MDS cases. It is expected that other genes involved will be identified.

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A genetic risk factor behind chronic obstructive pulmonary disesase (COPD). *J. Klar[1], P. Blomstrand[2], J. Badhai[1], B. Schemkel[2], C. Brunmark[3], H. Falk Håkansson[3], C. Sollie Brange[3], B. Bergendahl[2], N. Dahl[1].* 1) Department of Genetics and Pathology, Rudbeck Laboratory, Uppsala University and University Hospital, Uppsala, Sweden; 2) Department of Clinical Physiology, Ryhov County Hospital, Jönköping, Sweden; 3) AstraZeneca R&D, Lund, Sweden.

Chronic obstructive pulmonary disease (COPD) is a complex disorder and one of the leading causes of death in developed countries. The disease results from major remodelling of the airspace and changes in the lung epithelium. In contrast to asthma, which is defined by a reversible airflow obstruction, COPD is defined by a fixed obstruction, although it may be difficult to differentiate between the two diseases. The most important risk factor for the development of COPD is cigarette smoking, but only a minority of smokers will develop COPD, which implies that genetic factors may be involved. Familial aggregation of lung function as well as clustering of COPD within families supports a genetic contribution to pulmonary function. Despite tremendous efforts to understand the genetics behind COPD, most genetic factors remain obscure with the exception of α1-antitrypsin deficiency.

We have identified two families segregating irreversible obstructive lung disease. Affected family members have a moderate COPD from spirometry measures. The phenotype is associated with mutations causing reduced fibroblast growth factor (FGF) levels. Our findings show that impaired pulmonary function is associated with mutations affecting FGF levels and the results provide a direct connection between FGF signalling and COPD.

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microRNA processing is dysregulated by mutations associated with Pulmonary Arterial Hypertension. *K.M. Drake[1], S.A. Comhair[2], S.C. Erzurum[2], M.A. Aldred[1].* 1) Genomic Medicine Institute, Lerner Research Institute, Cleveland Clinic, Cleveland, OH; 2) Department of Pathobiology, Lerner Research Institute, Cleveland Clinic, Cleveland, OH.

Hereditary pulmonary arterial hypertension (PAH) is a progressive lung vascular disease caused by mutations in the bone morphogenetic protein (BMP) pathway, primarily the gene encoding the BMP type-II receptor (BMPR2), or less commonly activin receptor-like kinase 1 (ACVRL1), endoglin (ENG) or Smad-8 (gene symbol: SMAD9). microRNAs (miRs) are small, non-coding RNA molecules that negatively-regulate gene expression. Recently, BMP signaling was shown to regulate a subset of miRs, including miR-21, via a non-canonical Smad-1,5-mediated pathway. We hypothesized that mutations of BMP pathway genes in PAH patients result in the dysregulation of microRNA biogenesis. Control pulmonary artery endothelial (PAEC) and smooth muscle (PASMC) cells were treated with short-interfering RNA (siRNA) pools targeting specific genes of the BMP pathway. BMP stimulation induced a 2-3-fold increase of miR-21 in wild-type cells. This was abrogated by siRNA knockdown of BMPR2 or ACVRL1/ENG. Cells from explant lungs of two patients with heterozygous BMPR2 mutations showed no induction of miR-21, confirming that PAH mutations directly disrupt miR biogenesis. Remarkably, siRNA knockdown of SMAD9 also blocked miR-21 induction, despite only a modest blunting of canonical BMP signaling through Smad-4. These results were also confirmed in a third patient line carrying a germline SMAD9 mutation. Our data suggest that patients with BMP-pathway mutations have a primary defect in BMP-dependent microRNA processing and that this is more profound than effects on canonical BMP signaling. We are currently undertaking Illumina-GA sequencing to characterize the complete spectrum of miR dysregulation in PAH cells and to define their gene targets. To date we have identified and validated three additional miRs that are regulated in the same manner. Collectively, these miRs regulate key processes such as apoptosis, angiogenesis and response to hypoxia, suggesting that defects in their regulation may play an important role in pathogenesis of PAH.

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The regulation and the role of miR-154 microRNA family in Lung Fibrosis. *J. Milosevic[1], P. Kusum[1], M. Magister[1], A. Bais[2], P.V. Benos[2], E. Rabinovich[1], N. Kaminski[1].* 1) Dorothy P. and Richard P. Simmons Center for Interstitial Lung Disease, Division of Pulmonary, Allergy and Critical Care Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA; 2) Department of Computational Biology, University of Pittsburgh, Pittsburgh, PA.

Rationale: Idiopathic pulmonary fibrosis (IPF) is a progressive interstitial lung disease of unknown origin characterized by abnormal activation of developmental pathways. We have previously demonstrated significant and biologically relevant down-regulation of microRNAs in IPF. In this study we focus on the role and regulation of up-regulated microRNAs in IPF. Methods and Results: We analyzed the expression of microRNAs in IPF lungs and identified 46 significantly ($p<0.05$) up-regulated microRNAs in IPF samples. Impressively, 23 of the 46 increased microRNAs were localized at the chromosome 14q32 microRNA cluster. The miR-154 gene family was over represented among the up-regulated microRNAs with 11 members significantly increased. We validated changes in expression of intergenic miR-154, miR-154*, miR-134, miR-299-5p, miR-410, miR-382, miR-409-3p, miR-487b and intronic miR-127 by qRT-PCR. We ruled out differential methylation of this region using Agilent Human CpG Islands Microarray methylation arrays and EpiTYPER MassARRAY (Sequenom). Computational analysis of the upstream region of the intergenic and intronic miR cluster revealed 8 SMAD binding elements within 5kb upstream of miR-154 and 82bp upstream of miR-127. TGF-B1 stimulation of normal human lung fibroblast (NHLF) caused significant up-regulation of 11 miRNAs from chr14q32 (miR-132, miR-127-3p, miR-299, miR-154, miR-409-3p, miR-376c, miR-376b, miR-487b, miR-411, miR-323-3p) suggesting that the up-regulation of this cluster is at least in part caused by TGF-B1. Transfection of lung fibroblasts with members of miR-154 family caused increases two fold in cell proliferation and three fold in cell migration as measured by (^{3}H) thymidine incorporation and Boyden chamber assays respectively. Conclusion: The concordant up regulation of members of the miR-154 family, regulation by TGF-B1, and their role in proliferation and migration highlight this family as a potential regulator of the lung phenotype in IPF.

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The microRNA profile of Idiopathic Pulmonary Fibrosis has a developmental signature. *K.V. Pandit[1], J. Milosevic[1], P.V. Benos[2], G. Schatten[3], J. Hagood[4], N. Ambalavanan[5], N. Kaminski[1].* 1) Dorothy P. & Richard P. Simmons Center for Interstitial Lung Disease, PACCM, School of Medicine, University of Pittsburgh; 2) Department of Computational Biology, School of Medicine, University of Pittsburgh;; 3) Department of Obstetrics, Gynecology, and Reproductive Sciences, University of Pittsburgh School of Medicine, Pittsburgh; 4) Department of Pediatrics, University of California San Diego; 5) Department of Pediatric Neonatology, University of Alabama Birmingham.

INTRODUCTION: Idiopathic pulmonary fibrosis (IPF) is a chronic lethal lung disease resulting in respiratory failure and death within 3-5 years from diagnosis. Since it has been reported that IPF lungs are enriched with genes associated with lung development, we analyzed the microRNA expression patterns in IPF lungs and compared it to that of fetal lungs. METHODS AND RESULTS: RNA from 12 control lungs and 13 IPF lungs was hybridized on Agilent microRNA microarrays. 51 microRNAs were decreased and 45 increased in IPF lungs compared to controls. The expression of 34 microRNAs was confirmed by real time PCR. Among the decreased microRNAs were let-7, the miR-26, miR-29 and miR-30 families and the miR-17~92 cluster. Among increased microRNAs were members of the miR-154 and miR-199 families. Pathway analysis revealed targets of these microRNAs to be enriched with genes associated with developmental pathways. We proceeded by analyzing the microRNA profile of human fetal lungs and found it to be significantly similar to IPF lungs. To demonstrate a gradual shift in microRNA profile during the course of lung development, we analyzed the microRNA repertoire of post-natal mouse lungs at different time points since almost 90% of alveolar development in mice takes place after birth. The microRNAs decreased in IPF gradually increased in the developing mouse lung and vice versa. These observations were also supported by the analysis of their predicted and proven targets. We identified that change in microRNA patterns of expression eliminated key anti-fibrotic regulatory step and created multiple feed forward loops. CONCLUSIONS: Aberrant activation of embryological pathways regularly repressed in the adult life may explain the persistent nature of the disease. Our results indicate that the cumulative effect of the changes in microRNA expression in IPF is a global profibrotic transcriptional regulatory environment. The understanding of this regulatory environment would allow us design interventions that will disturb the pro-fibrotic balance in IPF lungs and potentially not only stop fibrosis but in some cases allow its reversal.

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Differences in average age of onset, locus heterogeneity & penetrance between Familial Pulmonary Arterial Hypertension & Familial Interstitial Pneumonia can predict the success of linkage and whole exome sequencing in discovering disease causing alleles. *J.A. Phillips[1,2], J.D. Cogan[1], E.D. Austin[1], E.K. Larkin[2], C.R. Markin[2], W.E. Lawson[2], J.H Newman[2], J.E. Loyd[2], T.S. Blackwell[2].* 1) Dept Ped; 2) Dept Med, Vanderbilt Univ Sch Med, Nashville, TN.

Familial Pulmonary Arterial Hypertension (FPAH) & Familial Interstitial Pneumonia (FIP) are AD with ages of onset (AO) of ~35 & >50yrs. In FPAH we used linkage analysis (LA) to map the BMPR2 locus which causes >80% of families. The later AO in FIP results in affected sibs with few other affected generations & 3 genes (SFTPC, TERC & TERT) cause <20% of families. Since LA is less robust in FIP, whole exomic sequencing (WES) is attractive to identify candidate loci. The number of WES variants are problematic because FIP pedigrees are limited in proving which are causal because average sharing of 50% of the genome by sibs & locus heterogeneity (LH). **Hypothesis:** Differences in AO & LH in FPAH & FIP affect the utility of LA & WES to detect disease genes. We compared numbers of affected generations; living affected & at risk relatives; informativeness of the most distant affected relatives' DNAs; & informative meioses for LA between FPAH & FIP pedigrees. We reviewed the 6 FPAH pedigrees we used to map FPAH & compared them to the 6 best of our 235 FIP families based on informativeness & available DNAs. We compared our FPAH & FIP pedigrees by 1) median & interquartile ranges, 2) exact non-parametric Wilcoxon tests of distributions of at risk relatives, affected generations, & living affecteds; 3) odds of DNAs from the 2 most distantly related, affected family members to share WES variants, & 4) the number of potentially informative meioses for LA. **Results:** Our FIP kindreds have more living at risk members (14 vs. 6, p=0.04). Potentially informative meioses were lower in FIP (2 vs. 5.5 in FPAH; p=0.002). Numbers of affected generations & DNAs from affecteds were less different in FIP vs FPAH (2 vs. 2.5; P=0.11 & 2 vs. 3.5; P=0.08, respectively). The probability DNAs from the 2 most distant affected relatives to share WES variants was 0.09 in FPAH vs. 0.25 (p=0.11) in FIP. Comparison of the numbers of affecteds/all at ≥50% risk suggests that the penetrance of FIP is ~2.4 fold less than FPAH in the pedigrees studied. **Conclusions:** Our data indicate that our best FIP pedigrees are less informative for LA & allele sharing studies to map disease loci than our FPAH kindreds due to later AO, reduced penetrance & greater potential LH. We plan to combine WES to identify candidates; analyze sharing of candidates between affected but distantly related family members, & perform surrogate phenotyping such as measuring telomere length to facilitate FIP gene discovery.

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Decreased dyskerin levels as a novel mechanism of telomere shortening in X-linked dyskeratosis congenita. *E.M. Parry[1,2], S.S. Lee[3], J.A. Phillips 3rd[4], J.E. Loyd[5], P. Duggal[6], M. Armanios[1,2].* 1) MSTP Program, Johns Hopkins University School of Medicine, Baltimore, MD; 2) Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, MD; 3) Predoctoral Training Program in Human Genetics, Johns Hopkins University School of Medicine, Baltimore, MD; 4) Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, TN; 5) Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN; 6) Department of Epidemiology, Johns Hopkins School of Public Health, Baltimore, MD.

Idiopathic pulmonary fibrosis (IPF) is progressive and fatal, yet its pathophysiology remains poorly understood. Mutations in the genes encoding the essential components of the telomerase enzyme, hTERT, the telomerase reverse transcriptase and hTR, the telomerase RNA, underlie inheritance in 8-15% of IPF families. Dyskeratosis congenita (DC) is a premature aging syndrome characterized by short telomeres. Individuals with DC suffer early mortality from organ failure in aplastic anemia, liver cirrhosis and IPF. An X-linked form of DC is caused by mutations in DKC1 which encodes dyskerin, a ribonucleoprotein that is essential for the stability and biogenesis of hTR. We identified a four generation IPF family with features of DC. Affected males showed the classic mucocutaneous features of DC and died prematurely from IPF. Although we could not find any sequence or splicing variants, genome wide analysis of 16 individuals across four generations identified significant linkage at the DKC1 locus (LOD 3.3). To examine the possibility that a regulatory sequence is mutated, we examined the known regulatory elements of DKC1 but identified no variants. However, quantitation of dyskerin protein levels identified a 50% reduction in affected males. The decrease in dyskerin levels segregated with the very short telomere phenotype, diagnostic of DC. Confirming a dyskerin defect, the decrease in dyskerin protein in affected individuals was associated with a reduction in hTR levels. This family study identifies decreased dyskerin protein levels, in the absence of coding mutations, as a novel mechanism of disease in X-linked forms of familial IPF-DC. Our data indicate that intact dyskerin levels are critical for telomerase RNA stability and for in vivo telomere maintenance. Characterizing the regulatory elements that control DKC1 transcription can elucidate genetic elements that predispose to IPF and other syndromes of telomere shortening.

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Absence of Cyclophilin B Causes Autosomal Recessive Osteogenesis Imperfecta but does not Impair Type I Collagen Peptidyl-Prolyl Isomerization. *A. Barnes[1], E. Carter[2], W. Cabral[1], M. Weis[3], W. Chang[1], E. Makareeva[4], S. Leikin[4], C. Rotimi[5], D. Eyre[3], C. Raggio[2], J. Marini[1].* 1) BEMB, NICHD/NIH, Bethesda, MD; 2) Hospital for Special Surgery, New York, NY; 3) University of Washington, Seattle, WA; 4) SPB, NICHD/NIH, Bethesda, MD; 5) NHGRI/NIH, Bethesda, MD.

Osteogenesis imperfecta, or brittle bone disease, is a genetic disorder characterized by bone fragility and growth deficiency. Recently, recessive forms of osteogenesis imperfecta have been shown to be caused by mutations in the three genes coding for components of the collagen 3-hydroxylation complex. This complex post-translationally hydroxylates a single residue in type I collagen, Pro986, as well as residues on types II and V collagen. Null mutations in *CRTAP* or *LEPRE1* lead to severe/lethal forms of OI with white sclerae and rhizomelia (types VII and VIII OI). Collagen synthesized by patient fibroblasts has minimal Pro986 hydroxylation, but is overmodified, indicating delay in collagen folding. In a pair of Senegalese siblings born to consanguineous parents, we identified a homozygous mutation in the start codon of *PPIB*, which encodes cyclophilin B (CyPB) the third member of the 3-hydroxylation complex. These children are ambulatory with moderate OI and white sclerae, but without rhizomelia or severe growth deficiency. Proband fibroblast RNA had 55% of the normal level of *PPIB* transcripts by real-time RT-PCR. On Western blot of fibroblast lysates, no CyPB protein was detectable using 3 antibodies to full protein, C-terminal half and final 15 residues or after treating proband cells with a proteasome inhibitor. Both CRTAP and P3H1 protein levels were reduced to half of normal levels, suggesting that complex stability is decreased. Immunofluorescence microscopy confirmed these findings in vivo. CyPB is a well-known peptidyl-prolyl cis-trans isomerase which is thought to be involved in the rate-limiting step of type I collagen folding. Therefore, we examined the proband's steady-state type I collagen protein as well as the levels of lysyl and 3- and 4-prolyl hydroxylation in the collagen helix. Pro986 hydroxylation was normal in proband collagen, indicating that the complex can complete its collagen modification function in the absence of CyPB. Surprisingly, steady-state collagen from our proband was not overmodified, with normal lysyl and 4-prolyl hydroxylation levels, in contrast to collagen synthesized by cells with recessive defects in *CRTAP* or *LEPRE1*, or with recessive *PPIB* mutations which allow synthesis of misfolded CyPB. Therefore, the complete absence of CyPB does not appear to delay collagen folding, strongly suggesting that there is redundancy for collagen isomerization, or CyPB may not be the major type I collagen folding isomerase.

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Clinical features and abnormal *ALK2* activity in case of fibrodysplasia ossificans progressiva (FOP) with an *ALK2* (G356D) mutation. *H. Furuya[1], H. Arahata[1], E.I. Araki[1], N. Fujii[1], Y. Fukumaki[2], T. Katagiri[3].* 1) Department of Neurology, National Oomuta Hospital, Oomuta, Fukuoka, Japan; 2) Division of Human Molecular Genetics, Research Center for Genetic Information, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan; 3) Division of Pathophysiology, Research Center for Genomic Medicine, Saitama Medical University, 1397-1 Yamane, Hidaka-shi, Saitama 350-1241, Japan.

Fibrodysplasia ossificans progressiva (FOP) is a rare autosomal dominant congenital disorder characterized by progressive heterotopic bone formation in muscle tissues. A common mutation among FOP patients has been identified in *ALK2* (R206H), which encodes a constitutively active bone morphogenetic protein (BMP) receptor. Recently, a unique mutation of *ALK2* (G356D), was identified to be a novel mutation in a Japanese FOP patient who showed rigid spine, baldness, sensorineural hearing loss, and hypodactyly accompanied by abnormal ectopic ossification. Over-expression of *ALK2* (G356D) induced phosphorylation of Smad1/5/8 and activated Id1-*luc* and alkaline phosphatase (ALP) activity in myoblasts. However, the overexpression failed to activate phosphorylation of p38, ERK1/2, and CAGA-*luc* activity. These *ALK2* (G356D) activities were weaker than those of *ALK2* (R206H), and they were suppressed by a specific inhibitor of the BMP-regulated Smad pathway. These findings suggest that *ALK2* (G356D) induces heterotopic bone formation via activation of a BMP-regulated Smad pathway. The quantitative difference between *ALK2* (G356D) and *ALK2* (R206H) activities may have caused the phenotypic differences in these patients, which may also give a clue to better understanding of the pathophysiology of FOP.

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Two novel *COL1A1* mutations in Chinese pedigrees with osteogenesis imperfecta type I. *M. Gu[1,2], W. Xu[1], X. Wu[1], X. Chen[1], Z. Wang[1,2].* 1) Dept Med Gen, Shanghai Jiao Tong Univ Sch Med, Shanghai, China; 2) E-Institutes of Shanghai Universities, Shanghai Jiao Tong Univ Sch Med, Shanghai, China.

Osteogenesis imperfecta (OI) is an autosomal dominant or recessive disorder characterized by osteoporosis, dentinogenesis, blue sclera and hearing loss. Studies have shown that the vast majority of affected individuals have mutations in either of the two genes, *COL1A1* and *COL1A2*, that encode the chains of type I procollagen. In our study, a three-generation family was ascertained through initial identification of a proband from Shanghai. Another family from Inner Mongolia was also ascertained. After informed consent was signed, all affected individuals and family members were evaluated by experienced orthopedists. The diagnosis was made based on family history, physical and radiographic examinations. The diagnostic criteria were based on Sillence's OI classification. Peripheral blood samples were collected and genomic DNA was extracted from all family members by using QIAamp DNA blood mini kit (Qiagen). Then, the members of two pedigrees were genotyped firstly with microsatellite markers at the two known loci of OI. Linkage analysis showed that the affected individuals in these families have high LOD scores within the region of 17q11.2-22, where the *COL1A1* gene is located. After screening *COL1A1* for mutations by direct sequencing, we identified two mutations. One was a TG deletion in exon 28 which leads to the frameshift mutation of *COL1A1* and converts a codon to a premature stop codon in c.1959, producing a stop codon in exon 29 and decreasing the amount of collagen I. Thus the truncated protein (p.Gly632ThrfsX21) was produced. The other one was a splicing mutation that converted the splice-acceptor site in intron 8 from AG to GG. These mutations were found in all affected individuals but unaffected relatives and 100 unrelated normal individuals were not observed. The two mutations appeared to be novel, which were neither reported in literature nor registered in Osteogenesis Imperfecta & Ehlers-Danlos syndrome variant databases (http://www.le.ac.uk/genetics/collagen). In conclusion, we identify a novel frameshift mutation (c.1896_1897 del TG) and a novel RNA-splicing mutation (c.643-2A>G) in *COL1A1* resulting in OI type I. The detailed molecular and clinical features will be useful for extending the evidence for genetic and phenotypic heterogeneity in OI type I.

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CANT1 does not account for all Desbuquois dysplasia cases but is directly involved in proteoglycan synthesis. *C. Huber[1], A. Rossi[2], R. Merrina[1], M. Fradin[1], B. Abdulibdeh[3], Y. Allanay[4], B. Albrecht[5], L. Al-Gazali[6], M.G.E.M. Ausems[7], P. Bitoun[8], D. Calvacanti[9], S. MacKay[10], G. Mortier[11], J. Morton[12], S. Robertson[13], D. Sillence[14], Y. Shafeghati[15], A. Superti-Furga[16], I. Young[17], K. Zerres[18], M. Le Merrer[1], A. Munnich[1], C. Le Goff[1], V. Cormier-Daire[1].* 1) Dept Medical Genetics, Necker Hosp, Paris, France; 2) Department of Biochemistry, University of Pavia, Pavia, Italy; 3) Molecular Genetics Lab, Makassed Hospital, Jerusalem, Israel; 4) Genetics Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara Turkey; 5) Institut fuer Humangenetik, Universitaetsklinikum, Essen, Germany; 6) Department of Paediatrics, Faculty of Medicine & Health Sciences, United Arab Emirates University, PO Box 17666, Al-Ain, United Arab Emirates; 7) Department of Medical Genetics, University Medical Center Utrecht, KC 04.084.2, PO Box 85090, 3508 AB Utrecht The Netherlands; 8) Hôpital Jean Verdier, Bondy, France; 9) Perinatal Genetic Program, Department of Medical Genetics, FCM, UNICAMP, Campinas, SP, Brazil; 10) Medical Genetics Program Health Sciences Center, 300 Prince Phillip Drive, St. John's NL, Canada; 11) Center for Medical Genetics, Antwerp University Hospital, Antwerp, Belgium; 12) Clinical Genetics Unit, Birmingham Women's Hospital Edgbaston, Birmingham, B15 2TG, UK; 13) Department of Paediatrics and Child Health, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand; 14) The Children's Hospital at Westmead, Locked Bag 4001 Westmead NSW 2145, Australia; 15) Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Teheran Iran; 16) Centre for Pediatrics and Adolescent Medicine, Freiburg University Hospital, Mathildenstr. 1,D-79106 Freiburg, Germany; 17) Molecular Genetics Laboratory, Leicester8-Division of Pediatric Orthopedics, , Leicester, UK; 18) Institute for Human Genetics 52074 Aachen, Germany.

Desbuquois dysplasia is characterized by short stature, joint laxity, scoliosis, advanced carpal ossification and hand anomalies including delta phalanx or extra ossification center. Studying 9 Desbuquois families with characteristic hand anomalies, we have identified 7 mutations in the Calcium-Activated Nucleotidase 1 gene (CANT1) which encodes a soluble UDP-preferring nucleotidase. Among the 9 families, nonsense mutations were identified in 4 and all children presented an early death due to cardio-respiratory failure. In the 5 other families, we identified 3 missense mutations located in the region encoding the 7th nucleotidase conserved region and changing Arg300. Using RT-PCR analysis, we observed a specific CANT1 expression in chondrocytes. We also found electron-dense material within distended rough endoplasmic reticulum in Desbuquois patient fibroblasts. Following this study, we have collected the samples of 16 additional Desbuquois families. Among them, 2 had characteristic hand anomalies and we identified two mutations in CANT1 (p.R300H and a novel mutation p.I374N) present at the homozygous state. The 14 other families were consanguineous and CANT1 was excluded by linkage analysis. Because Desbuquois dysplasia shares phenotypic features with Diastrophic dysplasia and recessive Larsen syndrome, which are due to SLC26A2 or CHST3 mutations, we excluded these two genes in 13/14 patients with no CANT1 mutation but we found an homozygous CHST3 mutation (p.L259P) in 1/14 patient. Remembering that SLC6A2 and CHST3 encode proteins involved in proteoglycan sulfation, we made the hypothesis that CANT1 deficiency may interfere with the availability of UDP-sugars needed for proteoglycan synthesis. Fibroblasts from two Desbuquois patients and four controls were therefore double labeled with [35S]sulfate and [3H]glucosamine. Surprisingly, in the patient cells glycosaminoglycan (GAG) synthesis was almost normal under basal conditions when compared to the controls, but significant reduced GAG synthesis was observed in presence of β-D-xyloside, a compound that increases GAG synthesis acting as a chain initiator. These data suggest that CANT1 plays a role in proteoglycan metabolism and supports its involvement in the rate of GAG synthesis. We conclude that Desbuquois dysplasia is a genetically heterogeneous condition. On going studies will hopefully lead to the identification of another disease gene presumably also involved in proteoglycan synthesis.

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Expansile panostotic high turnover bone disease with massive jaw tumor formation associated with a unique 12 bp duplication in RANK. *S. Mumm[1], A.L. Schafer[2], I.H. El-Sayed[2], M.S. Anderson[2], E.C. Hsiao[2], F.V. Schaefer[3], M.T. Collins[4], P. Andreopoulou[4], M.P. Whyte[1], D.M. Shoback[2].* 1) Washington University School of Medicine and Shriners Hospital for Children, St. Louis, MO; 2) University of California, San Francisco, CA; 3) Center for Genetic Testing at Saint Francis, Tulsa, OK; 4) National Institute of Dental and Craniofacial Research, NIH, Bethesda, MD.

A 27-year-old man was transferred to the UCSF Otolaryngology Service for management of a bleeding, basketball-size, mandibular tumor. Born in Mexico, he was deaf and mute, and was "bow-legged" but active as a child. Family history was negative for bone disease. Puberty occurred normally, but he developed difficulty straightening his limbs, multiple fractures, and a bony tumor on his chin. At age 18 yrs, all limbs were misshapen. As the mandibular mass grew, the diagnosis of McCune-Albright syndrome (MAS) was considered. The mass protruded from the oral cavity and extended to the lower ribs. Other bony defects included a maxillary mass and serpentine limbs. There were no café-au-lait spots. Laboratory results included serum Ca 7.1 mg/dL, PO4 2.4 mg/dL, albumin 1.7 g/dL, PTH 91 ng/L, 25(OH)D 11 ng/mL, and alkaline phosphatase 1760 U/L (nl 29-111). There was no clinical or biochemical hyperfunctioning endocrinopathy. Radiography of the limbs showed medullary expansion and cortical thinning with severe bowing. The patient underwent mandibular mass excision, 5 months later partial maxillectomy, and subsequently further maxillary debulking. Histopathology showed curvilinear trabeculae of woven bone on a background of hypocellular fibrous tissue. After surgery and vitamin D supplements, bone-specific alkaline phosphatase was >575 mcg/L (nl 8.4-29.3), C-telopeptide 1644 pg/mL (nl 87-1200), 1,25(OH)2D 45, and intact FGF23 60 pg/mL (nl 10-50). In samples of affected bone and leukocyte DNA, site-specific enrichment mutation analysis followed by sequencing for codon 201 of GNAS showed no mutation, making MAS and panostotic fibrous dysplasia of bone especially unlikely. We searched for mutations in the three major proteins [RANK (receptor), RANKL (ligand), and OPG (osteoprotegerin, decoy receptor)] in the "receptor activator of nuclear factor-κB" (RANK) signaling pathway. No exon or splice site mutations in the genes encoding RANKL or OPG were found, but a unique 12 bp tandem duplication in the signal peptide of RANK was detected. The elevated bone turnover, reflected in the biochemical markers and disorganized bone histology, is likely due to amplified RANK signal transduction, although this phenotype seems different from those reported previously with increased RANK signaling and in-frame duplications in the signal peptide of RANK [familial expansile osteolysis (18 bp dup), expansile skeletal hyperphosphatasia (15 bp dup), early onset Paget disease (27 bp dup)].

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MLPA analysis of *LRP5* in children with recurrent fractures. *A. Saarinen[1, 2], M. Mäyränpää[3], C. Laine[1, 3], R. Vijzelaar[4], A.E. Lehesjoki[1, 5], O. Mäkitie[1, 3].* 1) Folkhalsan Inst Genetics, Biomedicum Helsinki, Helsinki, Finland; 2) Dept of Medical Genetics, University of Helsinki, Finland; 3) Hospital for Children and Adolescents, Helsinki University Hospital, Finland; 4) MRC-Holland, Amsterdam, The Netherlands; 5) Neuroscience Center, University of Helsinki, Finland.

Background: Mutations in the low-density lipoprotein receptor-related protein 5 gene (*LRP5*) have been associated with high and low bone mass. While homozygous *LRP5* mutations cause osteoporosis-pseudoglioma syndrome (OPPG), characterized by severe osteoporosis and blindness, heterozygous mutations have been associated with reduced bone mass. LRP5 functions as a plasma membrane receptor in the Wnt signaling pathway. We previously described *LRP5* mutation analysis in 66 children with recurrent fractures. In this study we further analyzed the role of *LRP5* in these children by searching for possible exonic deletions or amplifications using multiplex ligation-dependent probe amplification (MLPA).

Methods: The novel Salsa MLPA P285 *LRP5* probemix (MRC-Holland) was used to look for possible exonic amplifications or deletions. This kit contains 38 specific probes for each of the 23 exons for *LRP5* and each of the 4 exons of *DKK1*, an inhibitory protein of Wnt signaling. Furthermore, an additional probe for the *LRP5* p.G171V alteration, associated with a high bone mass phenotype, was included.

Results: The MLPA analysis did not detect any exonic deletions in *LRP5* or *DKK1* in these patients. Several possible amplifications in *LRP5* were detected, but these could not be confirmed by PCR.

Conclusions: Genomic deletions in *DKK1* or *LRP5* are not frequent alterations underlying recurrent fractures in children. The existence of possible exonic *LRP5* amplifications will need to be verified in further studies.

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How different FGFs contribute to Apert Syndrome phenotype. *E. Yeh, R.D. Fanganiello, D.Y. Sunaga, M.R. Passos-Bueno.* Department of Genetics, Institute of Bioscience, São Paulo, São Paulo, Brazil.

Apert syndrome (AS) is characterized by craniosynostosis and limb abnormalities and is primarily caused by p.S252W mutations in FGFR2, which lead to prolonged ligand-receptor engagement and violation of FGFR2 ligand binding specificity. This mutation compromises the patient even after birth, particularly by a high rate of post-surgical cranial resynostosis. Despite a large literature on FGF-FGFR signaling, we still have unanswered questions concerning the pathways activated by each FGF, the effect of this mutation on cellular function and how the mutation ultimately cause the phenotype. By evaluation of cellular behavior in periosteal fibroblastoid cells from the coronal suture of AS p.S252W patients (n=3) and from matched controls (n=3) we observed that p.S252W mutation is associated with excessive proliferation and enhanced migration in the presence of FGF2 and FGF10. These cells also have increased osteogenic potential, which is inhibited by FGF2 and FGF10. Furthermore, we show that FGFs 2 and 10 trigger different downstream signaling pathways in AS cells and the most relevant pathways are associated with CNS development and inflammatory diseases, respectively. Since appropriate inflammation is involved in wound healing in adult humans and given that the recurrence of suture fusion after surgical repair in AS patients implies a rapid wound healing, this analysis indicates the role of FGF10 in $FGFR2^{+/S252W}$ periosteal cells as a starter and all the altered cell behavior we observed in response to FGF2 and FGF10 are very likely involved in the resynostosis process, as these ligands are expressed in the sutures even after birth. Overall, the new correlations between cellular functions and transcriptome alterations in the presence of the p.S252W mutation can partly explain the high rate of cranial resynostosis after surgical intervention.

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Craniosynostosis diagnosed by custom molecular microarray (ARRAY CGC). *P. Tavares[1], A. Lopes[1], L. Lameiras[1], L. Dias[1], L. Ramos[2], E. Galhano[3], L. Nunes[4], M. Costeira[5], P. Vidal-Ríos[6], J. Sá[1,2], P. Rendeiro[1], H.G. Santos[1], A. Palmeiro[1].* 1) Clinical Director, CGC Genetics, Porto, Portugal; 2) Hospital Pediatrico de Coimbra EPE, Coimbra, Portugal; 3) Maternidade Bissaya Barreto EPE, Coimbra, Portugal; 4) Centro Hospitalar de Lisboa Central EPE, Lisboa, Portugal; 5) Centro Hospitalar de Alto Ave EPE, Guimarães, Portugal; 6) Complejo Hospitalario de Santiago de Compostela, Santiago de Compostela, Spain.

Introduction: Craniosynostosis is a condition of variable aetiology characterized by premature closure of calvarial skull bones. With many sutures closing prematurely, the skull cannot expand to accommodate the growing brain, which leads to several consequences, including developmental delay, mental retardation and vision and hearing problems can also appear. Most cases are diagnosed during the neonatal period but many cases are detected sooner by ultrasound during the prenatal period. Because this disorder is clinically heterogeneous, some carriers are not detected until late in infancy or only detected after the birth of an affected child. The major conditions, Muenke, Pfeiffer, Apert, Crouzon, Jackson-Weiss, and Seathre-Chotzen-like Syndromes, are related with mutations located in the different genes of the Fibroblast Growth Factor Receptors (FGFR) 1, 2 and 3. Carpenter syndrome was also included, in spite of being a rare condition, to improve the differential diagnosis of craniosynostosis. The molecular characterization of the major forms is of main interest to establish an accurate diagnosis, particularly during the neonatal period and to assess the familial recurrence risk. Method: Using new custom microarray panel (Arrays CGC - Patent Pending) that contains a panel of 10 point mutations identified in 4 main genes involved on syndromic craniosynostosis, FGFR1 (Pfeiffer), FGFR2 (Apert, Crouzon Jackson-Weiss and Pfeiffer), FGFR3 (Muenke and Seathre-Chotzen-like syndrome) and RAB23 (Carpenter), it is possible to identify the molecular basis of the most frequent and severe forms. We report here our experience using this new methodology. Results: We analyzed 9 cases (8 peripheral blood and 1 amniotic fluid) and in two we detected the c.866A>C (p.GIn289Pro) mutation in heterozygozity in the FGFR2 gene and the c.755C>G (p.Ser252Trp) in the FGFR2 gene. Conclusion: This approach drastically reduces turnaround time (one week after DNA extraction), maintaining accuracy and liability independently of the sample type. Faster diagnostic is achieved, allowing early decision-making process in patient management, particularly in prenatal diagnosis.

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FAM83H Mutations Cause ADHCAI and Alter Intracellular Protein Localization. *JW. Kim[1,3], SK. Lee[1], KE. Lee[1], Z.H. Lee[1], TS. Jeong[2], YH. Hwang[1], S. Kim[2].* 1) Department of Cell and Developmental Biology & Dental Research Institute School of Dentistry, Seoul National University, Seoul, Korea; 2) Department of Pediatric dentistry Pusan National University Dental Hospital Beomeo-Ri, Mulgeum-Eup, Yangsan-Si, Gyeongsangnam-Do, Korea; 3) Department of Pediatric Dentistry & Dental Research Institute School of Dentistry, Seoul National University, Seoul, Korea.

Mutations in family with sequence similarity 83 member H (FAM83H) gene cause autosomal dominant hypocalcification amelogenesis imperfecta (ADHCAI). We performed mutational analyses on two Korean families with generalized ADHCAI and identified the disease-causing mutations. Mutations in family 1 (g.3115C>T, c.1993C>T, p.Q665X) and family 2 (g.3151C>T, c.2029C>T, p.Q677X) introduced nonsense codons in exon 5, the last coding exon. All ADHCAI-causing mutations terminate translation directly or by shifting the reading frame in the FAM83H coding region from Ser287 to Glu694, with mutations near Glu694 causing a milder, more localized phenotype. We tested the hypothesis that FAM83H truncation mutations alter the intracellular localization of the expressed protein. Wild-type FAM83H and p.E694X mutant FAM83H fused to GFP localized in the cytoplasm of HEK293T cells, but p.Q677X mutant FAM83H fused to GFP localized exclusively in the nucleus. We conclude that nuclear targeting of truncated FAM83H contributes to the severe, generalized enamel malformations in patients with ADHCAI, while truncated FAM83H localized to the cytoplasm functions partially and results in a milder, localized enamel defects. This work was supported by a grant from the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MEST) (No. R01-2008-000-10174-0(2010)) and by the Korea Research Foundation Grant funded by the Korean Government (KRF-2008-313-E00597), the Science Research Center grant to Bone Metabolism Research Center (2009-0063266) funded by the Korean Ministry of Education, Science and Technology.

2285/F

Novel mutations in the HPS1 gene among Puerto Rican patients. *C. Carmona-Rivera[1], R.A. Hess[1], K. O'Brien[2], G. Golas[2], E. Tsilou[3], J.G. White[4], W.A. Gahl[1,2], M. Huizing[1].* 1) Med Gen Branch, NIH/NHGRI, Bethesda, MD; 2) Office of Rare diseases,NIH/NHGRI, Bethesda, MD; 3) Oftal Gen Vis Func Branch, NIH/NEI, Bethsda, MD; 4) Dept of Laboratory Medicine, U. Minnesota, Minneapolis, USA.

Hermansky-Pudlak Syndrome is a disorder of oculocutaneous albinism and platelet storage pool deficiency. Eight different disease causing genes have been identified, whose gene products are thought to be involved in the biogenesis of lysosome-related organelles. HPS type 1 (HPS-1) is the most common HPS subtype in Puerto Rico, with a frequency of 1:1,800 in the northwest of the island due to a founder mutation, i.e., a 16-base pair duplication in exon 15 of the HPS1 gene (c.1472_1487dup16; p.H497QfsX90). We identified three Puerto Rican HPS-1 patients who carried compound heterozygous HPS1 mutations. One patient was heterozygous for c.937G>A, causing a missense mutation (p.G313S) at the 3' splice junction of exon 10. This mutation resulted in activation of a cryptic intronic splice site causing an aberrantly spliced HPS1 mRNA that included 144-bp of intronic sequence, producing 11 novel amino acids followed by a stop codon. The other two patients were heterozygous for the previously reported c.972delC in HPS1, resulting in a frameshift and a premature stop codon (p.M325WfsX6). These findings indicate that, among Puerto Ricans, other HPS1 mutations apart from the 16-base pair duplication should be considered in the analysis of this population.

2286/F

Case Reports of TGM1 Mutations in Iranian Patients with Lamellar Ichthyosis. *S. Ardalan Khales[1], S. Ardalan Khales[1], M. Houshmand[2], A. Farajieilanjegh[1].* 1) Medical Genetic Laboratory of Special Medical Center, Tehran, Iran; 2) National Institute for Genetic Engineering and Biotechnology, Tehran, Iran.

Autosomal Recessive Congenital Ichthyosis (ARCI) is a rare, heterogenous keratinization disorder of the skin, classically divided into two clinical subtypes, lamellar ichthyosis (LI) and nonbullous congenital ichthyosi-formis erythroderma (NCIE). Lamellar Ichtyosis is caused by mutations in the TGM1 gene that encodes the transglutaminase 1 enzyme, which is critical for the assembly of the cornified cell envelope in terminally differentiating keratinocytes. TGM1 is a complex enzyme existing as both cytosolic and membrane-bound forms. Moreover, TGM1 is proteolytically processed, and the major functionally active form consists of a membrane-bound 67/33/10-kDa complex with a myristoylated and palmitoylated amino-terminal 10-kDa membrane anchorage fragment.. In this study all 14 coding exons of TGM1 gene by using of PCR-Sequencing method in three Iranian patients with different phenotypes were investigated that a homozygote mutation (G218S) in exon 4 and three heterozygote mutations (R37K, D58N, D86N) in exon 2 were observed. The mutation (D86N) was shown in two patients simultaneously. KEY WORDS: TGM1, Lamellar, Ichthyosis, ARCI, NCIE.

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Novel *PCDH19* mutations causing EFMR. *S. Jamal[1,2], RK. Basran[1,2], S. Newton[1], Z. Wang[1,2], JM. Milunsky[1,2,3].* 1) Ctr Human Genetics, Boston Univ Sch Medicine, Boston, MA; 2) Department of Pediatrics, Boston University School of Medicine; 3) Department of Genetics and Genomics, Boston University School of Medicine.

Epilepsy and Mental Retardation Limited to Females (EFMR) [MIM 300088] was first described in 1971 (Juberg et al.) in fifteen related females with early onset grand mal seizures and mental retardation. Although EFMR demonstrates X-linked inheritance, it follows an unusual pattern by sparing transmitting males and affecting only heterozygous females. In 2008, mutations within the protocadherin 19 (*PCDH19*) gene located at Xq22 were implicated as causative of EFMR (Dibbens, et al.). The EFMR phenotype is typically characterized by seizure onset in infancy and varying degrees of intellectual impairment. Sequence analysis of *PCDH19* gene: During a 12 month period (May 2009-May 2010), the Center for Human Genetics/ Boston University School of Medicine sequenced the *PCDH19* gene of 33 female patients with developmental delay and early onset epilepsy. We identified 5 patients (15% yield) with novel *PCDH19* sequence aberrations. Amongst the mutation-positive patients, exon 1 mutations (c.78delG, c.213delA, c.434-435insG, c.729C>A (p.Y243X)), were found in 80% (4/5) of patients. Two of the exon 1 mutation-positive patients were within the extracellular cadherin (EC) domain 1, whilst the remaining two mutations were in EC domain 2 and 3. The remaining patient had a mutation (c.2490-2493delTTTT) within exon 4 of the PCDH19 gene. To date, maternal and paternal samples of 3 of the 5 positive cases were negative; thus demonstrating the de novo nature of these alterations. More patients need to be studied to establish whether genotype-phenotype correlations exist. Clinical assessment of mutation-positive patients: Ages ranged from 3 years to 19 years all of whom had seizure onset in infancy requiring the use of multiple antiepileptic drugs. They also had varying degrees of intellectual impairment along with the presence of autistic features. Clinical history of the mutation-negative patients will also be reviewed to further characterize the pertinent features of EFMR. Prior to the identification of the causative gene, EFMR was more easily identified in large pedigrees where the phenotype occurred in multiple affected females connected through unaffected males, while in smaller families, the condition may be under recognized. With the recent availability of clinical testing for *PCDH19*, we recommend testing of this gene in females with early onset epilepsy, autistic features, and intellectual impairment, irrespective of family history.

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A nonsense mutation in the FMR1 gene causing classical fragile X syndrome. *K. Gronskov[1], K. Brondum-Nielsen[1], A. Dedic[1], H. Hjalgrim[2].* 1) Kennedy Ctr, Glostrup, Denmark; 2) Danish Epilepsy Centre, Dianalund, Denmark.

Fragile X syndrome is a frequent cause of inherited mental retardation. It is caused by lack of FMR1 gene product. The most common cause is an expansion of a CGG repeat located in the 5' UTR of FMR1. If the number of CGG repeats exceeds 200, the repeat and the CpG islands in the promoter region are methylated which abolish transcription. Only a few cases of patients with intragenic point mutations in FMR1 have been reported and routine analysis of patients referred for fragile X syndrome includes solely analysis for repeat expansion. We describe here a patient with classical clinical symptoms of fragile X syndrome; Southern blot analysis showed no expansion, however Western blot analysis showed no FMR1 product. Subsequently sequencing of the FMR1 gene revealed a substitution in exon 2, c.80C>A, causing a nonsense mutation p.Ser27X. The mother was a heterozygous carrier of the mutation, and presented with mild mental retardation. This shows that in patients with typical symptoms of fragile X syndrome, in whom no expansion is detected, further studies including sequence analysis of the FMR1 gene should be performed.

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***FMR1* premutation and fibromyalgia.** *M. Mila[1,2], J. Blanch[3], I. Madrigal[1,2], D. Martinez[1], C. Badenas[1,2], A. Collado[4], J. Carbonell[3], L. Rodriguez-Revenga[1,2].* 1) Gen Service, Hosp Clinic, Barcelona, Spain; 2) CIBER de Enfermedades Raras (CIBERER), Barcelona, Spain; 3) Rheumatology Department, Hospital de Mar, Barcelona, Spain; 4) Rheumatology Department, Hospital Clinic. Barcelona, Spain.

Within the past few years, there has been a significant change in identifying and characterizing the *FMR1* premutation associated phenotypes. The premutation has been associated with elevated *FMR1* mRNA levels and slight to moderate reductions in FMRP levels. Furthermore, it has been established that ~ 20% of female premutation carriers present primary ovarian insufficiency and that FXTAS occurs in 16.5% of female premutation carriers and in 45.5% of premutated males older than 50 years. Besides primary ovarian insufficiency and FXTAS, new disorders have recently been described among individuals (especially females) with the *FMR1* premutation. Those pathologies include thyroid disease, hypertension, seizures, peripheral neuropathy, and fibromyalgia. Among fragile X families we have detected that more than 18% of females with the *FMR1* premutation present fibromyalgia. In the present study we have screened 200 DNA samples from the National DNA bank of Spain corresponding to women with a clinical diagnosis of fibromyalgia for *FMR1* CGG repeat expansion. Results showed two females carrying *FMR1* premutation alleles, giving a frequency of 1%. This frequency is much higher than the one reported in the Spanish population (1/411 females are found to be *FMR1* premutation carriers) indicating that *FMR1* gene could be one of the genes implicated in the etiology of fibromyalgia. ACKNOWLEDGMENTS This work was supported by FIS 09-00413. The CIBER de Enfermedades Raras is an initiative of the ISCIII.

2290/F

Sandhoff disease: A novel mutation in the HEXB gene. *H. Aryan[1], M. Hushmand[1,2], O. Aryani[1].* 1) Genetic, Special Medical Center, Tehran, Iran; 2) National Institute for Genetic Engineering and Biotechnology, Karaj-Tehran, Iran.

Sandhoff disease is a rare, genetic, lipid storage disorder resulting in the progressive deterioration of the central nervous system which is an autosomal recessive lysosomal storage disorder due to mutations in HEXB, the gene coding for the b-subunit of b hexosaminidases A and B. Both Hex A and Hex B activities are deficient in Sandhoff disease. The classic infantile form of the disease has the most severe symptoms. The first signs of symptoms begin before 6 months of age and the parents' notice when the child begins digressing in their development. Our case was a child with low activity of both Hex A and Hex B enzyme. We used sequencing method to detect mutations in the HEXB gene. Sequencing results showed a homozygote deletion T (c.183 delT) in our patient. Materials and Methods: Preparation of Genomic DNA: DNA of fresh blood samples prepared from patients was extracted using flexigene DNA extraction kit. DNA Amplification and Direct Sequencing: Both exonic and intron/exon junctions of Genomic DNA amplified by PCR considering that each region amplified by two pairs of primers specifically. The reaction were performed in 50 µl volume of 4µl Genomic DNA, 1µl of each primer (10nm), 1µl MgCl2, 0.5 µl dNTP and 0.2 µ Taq polymerase. Amplification was done in a Thermal Cycler (Techne) for an initial denaturation 5min at 95°C and 32cycles, that each cycle consist of a denaturation 40s at 95°C, 40s annealing 50-60°C and extension 30s at 72°C, which followed by a final extension 10min at 72°C. These amplified PCR samples sequenced using ABI3370 (MacroGen, Korea). Result: Our case was a 2 year- old- male child with progressive cerebella ataxia and psychomotor retardation. Biochemistry analyses of hexosaminidase A and B in this patient showed a low activity in hexosaminidase B. Patient admitted with blindness and cherry-red macular spots, seizures, and myoclonus. In this study we used direct sequencing for detection of mutation.Direct sequencing of PCR products of exons and splicing sites for Hexb gene showed that the patient was homozygote for c.183del T (n.513 del T) in exon 4 (leading to stop codon in codon 206, exon 5). His parent blood was used to confirm this mutation. Sequencing results for his parent showed heterozygote state for the mentioned mutation.

2291/F

ACVRL1 germinal mosaicism with two mutant alleles in a family with Hereditary Haemorrhagic Telangiectasia associated with PAH. *M. EYRIES[1,4], F. COULET[1,4], F. PIRES[1], M.C. WAILL[1], B. GIRERD[2], D. MONTANI[2], M. HUMBERT[2], P. LACOMBE[3], J. ROUME[3], F. SOUBRIER[1,4].* 1) Laboratoire d'Oncogénétique et d'Angiogénétique moléculaire , Groupe Hospitalier Pitie-Salpetriere, Paris, France; 2) Centre National de Référence de l'hypertension pulmonaire sévère, Service de pneumologie, Hôpital Antoine Béclère, Université Paris-Sud 11, INSERM U999, Clamart, France; 3) Service de Génétique, Hôpital Ambroise Paré, Boulogne, France; 4) Université Pierre et Marie Curie (UPMC), Paris, France.

Pulmonary Arterial Hypertension (PAH) and Hereditary Hemorrhagic Telangiectasia (HHT) are distinct clinical entities caused by germline mutations in genes encoding members of the TGF-β/BMP superfamily: BMPR2 in PAH and ACVRL1, ENG, or SMAD4 in HHT. When PAH and HHT co-exist within the same family, ACVRL1 mutations predominate. We report a remarkable ACVRL1 germinal mosaicism characterized by the presence of two mutant alleles in a woman initially diagnosed with PAH at age 21. She also met the Curaçao diagnostic criteria for HHT based on additional findings of telangiectases and epistaxis. Mutation analysis of ACVRL1 identified two closely located heterozygous deleterious mutations within exon 10: c.1388delG (p.Gly463fsX464) and c.1390del (p.Leu464X). DNA of the index case's parents was not available because they were deceased. However, they did not have any sign of the disease, like the four sisters of the index case, suggesting that mutations found are both neomutations. We performed allele-specific PCR analysis to quantify the two mutant alleles in lymphocytes of the index case demonstrating that c.1388delG is the predominant mutation suggesting that the mutational event leading to this mutation occurs earlier than the one leading to the c.1390del. Both mutant alleles were detected by direct sequencing in lymphocytes, buccal swab, urine sediments and hair roots of the patient indicating a mosaicism affecting ectoderm and mesoderm derivatives. The two mutations were transmitted independently to the three sons of the index case. One son received the c.1388delG and two sons received the c.1390del. They all present symptoms of HHT with various intensity but genotype/phenotype correlation is not currently interpretable because of their young age. The presence of these two distinct alterations results probably from two successive mutational events that occurred very early during embryogenesis. The proximity of the two mutation sites might be explained by their localisation within a palindromic region of 6 bp. These regions are known to form conformational structures that favour deletion of DNA. This is the first known report of ACVRL1 germinal mosaicism characterized by the presence of two mutant alleles responsible for HHT.

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Common splicing mutation in *CCM2* in the Ashkenazi Jewish population. *C.J. Gallione[1], A. Solatycki[2], D.A. McDonald[1], I.A. Awad[3], J.L. Weber[2], D.A. Marchuk[1].* 1) Dept Genetics, Duke Univ Medical Ctr, Durham, NC; 2) PreventionGenetics, Marshfield, WI; 3) Section of Neurosurgery, University of Chicago Pritzker School of Medicine, Chicago, IL.

Cerebral Cavernous Malformations (CCM) are vascular malformations of the central nervous system consisting of clusters of dilated vessels, leading to stroke, seizures and focal neurological deficits. CCM occurs sporadically and as an autosomal dominant trait. Three genes have been identified for inherited CCM, and mutations in one of these three genes are identified in the majority of CCM families. Routine genetic testing for CCM in six unrelated patients self-reporting as Ashkenazi Jewish revealed the same 2-base pair change in *CCM2*, c.30+5_6delinsTT, however the significance of this variant was unclear since it does not occur within the canonical splice donor site. Furthermore, aberrant splicing of the first exon of a gene can be difficult to predict, precluding simple assay design for experimental validation of the putative splicing defect. We have confirmed this difficulty by showing that RT-PCR from patient blood with this mutation does not exhibit an aberrantly spliced variant. However, using a common polymorphic SNP in exon 2 to track the fate of the two alleles, we show that the RT-PCR product consists of only one allele. Both alleles are present in RT-PCR products from normal individuals harboring the same common SNP, confirming that the loss of one allele is due to the *CCM2*, c.30+5_6delinsTT variant. These data provide proof that this sequence variant is the causative mutation in these families. We are currently examining SNP haplotypes surrounding *CCM2* exon 1 to determine whether this mutation has arisen from a founder in the Ashkenazi Jewish population. The results of this study could lead to a rapid genetic test for CCM in individuals of this population.

2293/F

Isolated ectopia lentis: report of a new deletion in the *ADAMTSL4* gene and evidence for genetic heterogeneity of the autosomal recessive form of the disease. *N. Hanna[1,2], G. Sultan[3], C. Muti[2], B. Grandchamp[2], L. Gouya[1,2], S. Funtowiez[2], D. Lacombe[4], H. Dollfus[5], C. Baudouin[3,6], G. Jondeau[2], C. Boileau[1,2,7].* 1) Laboratoire de Génétique moléculaire, Hôpital Ambroise Paré, AP-HP, Université Versailles-Saint Quentin en Yvelines, Boulogne, France; 2) Centre de Référence Marfan, Hôpital Bichat, AP-HP, Paris, France; 3) Service d'Ophtalmologie, Hôpital Ambroise Paré, AP-HP, Boulogne, France; 4) Service de Génétique Médicale, Hôpital Pellegrin, CHU de Bordeaux, Université Bordeaux II, France; 5) Centre de Référence pour les Affections Rares en Génétique Ophtalmologique (CARGO) et Service de Génétique Médicale, Hôpitaux Universitaires de Strasbourg, Strasbourg, France; 6) Centre Hospitalier National d'Ophtalmologie des Quinze-Vingts, Paris, France; 7) Inserm U781, Hôpital Necker-Enfants Malades, Paris, France.

Ectopia lentis (EL) is characterized by partial or complete displacement of the lens. Often associated with systemic diseases (such as homocystinuria, Marfan syndrome or Weill-Marchesani syndrome) it can also appear as an isolated condition with autosomal dominant (ADEL, OMIM#1296000) or autosomal recessive (AREL, OMIM#225100) inheritance. ADEL has been associated with mutations within the *FBN1* gene, while only two mutations have been identified to date in the *ADAMTSL4* gene in families with AREL (Ahram *et al*., 2009; Greene *et al*., 2010). To evaluate the contribution of *ADAMTSL4* gene mutations to isolated EL, we studied 12 French probands negative for homocystinuria and with no mutation within the *FBN1* gene. Bidirectionnal sequencing of the 17 coding exons of the gene was performed. When available, family studies were performed and regional haplotypes were constructed with 9 microsatellite makers flanking the gene at 1q21.3. An unreported and identical homozygous frameshift deletion was found in 2 unrelated probands: c.767_786del, p.Gln256ProfsX38. Family analysis showed that the mutation was carried on two different haplotypes, one proband carrying the two haplotypes. In the 10 remaining EL probands, no mutation was identified in the *ADAMTSL4* gene to explain the EL phenotype. Interestingly, one of these probands belonged to a small French family of Gypsy origin with 4 affected children. None of the affected children were haplo-identical thus demonstrating exclusion of the gene locus. In the same way, linkage was also excluded to the *FBN1* gene. In conclusion, in a small sample of EL probands, only 2/12 (16 %) of EL cases were related to a mutation in the coding sequence of the *ADAMTSL4* gene. Furthermore, we report a family with AREL unlinked to either of the known genes associated with EL. This family demonstrates the existence of further genetic heterogeneity in isolated ectopia lentis.

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2294/F

The maternal pattern of X-chromosome inactivation as a parameter for evaluating the contribution of X-chromosome mutations to mental retardation in males. *A.M. Vianna-Morgante, K.N. Coqueti, P.A. Otto.* Departamento de Genética e Biologia Evolutiva, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil.

Nearly a third of obligate carriers of mutations causative of X-linked mental retardation (XLMR) have been reported to have extreme X-inactivation skewing (≥ 90:10) in peripheral blood cells, compared to about 2% of their non-carrier relatives (Plenge et al., Am J Hum Genet 71:168, 2002). Selective advantage of cells with the non-mutated allele on the active X chromosome would explain this skewing. Based on these findings, we used the pattern of X-inactivation in mothers of mentally retarded boys as a parameter to evaluate the frequency of XLMR among non-familial cases. To determine the X-inactivation pattern in these women, we investigated the methylation status of the *AR* alleles in blood cells. We selected 100 boys with moderate to severe mental retardation of unknown cause, who had normal karyotypes and tested negative for fragile X syndrome; their mothers were heterozygous for the polymorphic CAG repeat of the *AR* gene, a requisite of the X-inactivation assay. Ten women (10%) had completely skewed X-inactivation (100:0), a frequency significantly higher (P = 0.0001; Fisher exact test) than the frequency reported for adult women from the general population (1.7%; 95% CI = 0.0068 - 0.0345; Amos-Landgraf et al., Am J Hum Genet 79:493, 2006). Assuming that every mother with completely skewed X-inactivation is a carrier of an X-chromosome mutation that causes mental retardation in her son, the frequency of XLMR in our sample of 100 boys is 10% (95% CI = 0.0490 - 0.1762), the fragile X syndrome being excluded. Although these figures are quite in agreement with previous estimations of the frequency of XLMR among mentally retarded men, they might be an underestimation, when it is taken into account that only about a third of obligate carriers of XLMR mutations have highly skewed X inactivation. Financial Support: FAPESP (CEPID 9814254-2) and CNPq.

2295/F

The Greek mitochondrial genome does not harbor deafness-causing mutations. *H. Kokotas[1], M. Grigoriadou[1], G.S. Korres[1], E. Ferekidou[1], D. Kandiloros[2], S. Korres[3], M.B. Petersen[1].* 1) Department of Genetics, Institute of Child Health, 'Aghia Sophia' Children's Hospital, Athens, Greece; 2) B' Department of Otorhinolaryngology - Head and Neck Surgery, Athens University Medical School, Attikon Hospital, Athens, Greece; 3) A' Department of Otorhinolaryngology - Head and Neck Surgery, Athens University Medical School, Hippokration Hospital, Athens, Greece.

Hearing impairment is one of the most common sensory handicaps with a frequency of at least 1/1,000, whereas at the age of 80 years more than 50% of the elderly have developed hearing loss severe enough to impair communication. Mitochondria harbor their own DNA, known as mtDNA, encoding certain essential components of the mitochondrial respiratory chain and protein synthesis apparatus. MtDNA mutations have an impact on cellular ATP production and many of them are undoubtedly a factor that contributes to sensorineural deafness, including both syndromic and non-syndromic forms. Mitochondrial mutations are present in less than 1% of the children with prelingual deafness, but are more frequent at a later age. In the Caucasian population at least 5% of postlingual, non-syndromic hearing impairment is due to known mtDNA mutations in some studies, representing the most frequent cause of hearing loss after the 35delG mutation in the GJB2 gene encoding connexin 26 in some populations. The use of aminoglycoside antibiotics can cause hearing loss in genetically susceptible individuals. Hot spot mutation regions for deafness mutations are the MTRNR1 gene, encoding the 12S rRNA, the MTTS1 gene, encoding the tRNA for Ser(UCN), and the MTTL1 gene, encoding the tRNA for Leu(UUR). Specific mtDNA mutations are usually involved in either non-syndromic or syndromic cases; however there are mtDNA mutations that have been reported to cause deafness in both syndromic and non-syndromic cases. The fact that scientists usually perform tests in either a cohort of patients with syndromic or a cohort of non-syndromic patients but rarely in a combined cohort means that there is a chance of mtDNA mutations to go undetected in several deafness cases. For this reason, we investigated the MTRNR1 A1555G mutation, the MTTL1 A3243G mutation, and the MTTS1 A7445G, 7472insC, and T7510C mutations in the Greek population, by testing 513 unrelated cases suffering either from prelingual or postlingual, bilateral, sensorineural, syndromic or non-syndromic deafness using PCR-RFLP protocols. The homoplasmic A1555G mutation was detected in two familial cases, one of which was found negative and the other heterozygous for the common GJB2 35delG mutation. None of the cases presented with the A3243G, A7445G, 7472insC, or T7510C mutations. We conclude that the A1555G mutation is rather uncommon in the Greek population and that in general, the Greek mtDNA is void of deafness-causing mutations.

2296/F

Gene expression profiling in facioscapulohumeral muscular dystrophy muscles. *F. Rahimov[1], O.D. King[2], L.C. Warsing[3,4], J. Chen[2], K. Hanger[2], R.J. Bloch[5], J.B. Miller[2], C.P. Emerson, Jr.[2], K.R. Wagner[3,4], L.M. Kunkel[1,6].* 1) Division of Genetics, Children's Hospital Boston, Boston, MA; 2) Boston Biomedical Research Institute, Watertown, MA; 3) The Johns Hopkins University, School of Medicine, Baltimore, MD; 4) Kennedy Krieger Institute, Baltimore, MD; 5) University of Maryland School of Medicine, Baltimore, MD; 6) Howard Hughes Medical Institute.

Facioscapulohumeral muscular dystrophy (FSHD) is a progressive myopathy caused by deletions of integral number of repeat elements within the subtelomeric macrosatellite element D4Z4 on chromosome 4q35. However, the underlying mechanism by which FSHD arises is poorly understood. We performed global transcriptome profiling of mature muscle tissues collected from affected and unaffected individuals from 14 cohorts using the Affymetrix GeneChip® 1.0 ST array and RNA sequencing approaches. We identified a number of differentially regulated genes between affected and unaffected muscle tissues. In addition, we performed high-throughput quantitative real-time RT-PCR analysis on 46 growth and differentiation marker genes and several candidate genes residing on chromosome 4q35 in muscle tissues and primary cell lines derived from these tissues using the Fluidigm Biomark System. The putative biomarker genes identified in this study will be valuable for understanding the molecular etiology of FSHD and assessing the success of clinical trials for this disease.

2297/F

Rescue from the lethal phenotype of Herlitz junctional epidermolysis bullosa by spontaneous read-through of a nonsense mutation. *F. Pacho, H. Schneider.* Department of Pediatrics, University of Erlangen-Nuremberg, Erlangen, Bavaria, Germany.

Mutations in the laminin-332 genes, LAMA3, LAMB3 and LAMC2, are known to cause junctional epidermolysis bullosa (JEB). Patients carrying a nonsense mutation in both alleles of any of these genes normally die in infancy due to widespread skin wounds, loss of fluid, cells and proteins, and severe infections. In a child with JEB and compound-heterozygous LAMA3 nonsense mutations as typically seen in the lethal Herlitz type of JEB, we observed clinical and molecular rescue due to spontaneous premature termination codon (PTC) read-through. RNA from keratinocytes of this patient was shown to include a full-length transcript with the PTC, which partially escaped nonsense-mediated mRNA decay. Translation of the mRNA is expected to result in a truncated laminin α3 protein lacking approximately 40 amino acids of the laminin globular domain 1 and the entire globular domains 2 to 5. The truncated α3 chain may be able to associate with laminin chains β3 and γ2 to laminin-332, but would lack binding sites for integrins responsible for tight adhesion of the basal keratinocytes to the underlying dermis. Analysis of skin biopsies and a continuous improvement of the patient's condition indicated, however, that translational read-through allowed deposition and accumulation of laminin-332 molecules with a full-length α3 chain in the epidermal basement membrane, outweighing laminin-332 turnover. To elucidate this phenomenon, various PTCs surrounded by their natural neighbouring codons were cloned between an ATG codon and an EGFP reporter gene and introduced into 293 cells by retroviral gene transfer. FACS analysis revealed significant reporter gene expression despite the PTC mutation only for the genetic context of our patient. Gene expression could be reduced to background levels by replacing the base before, or the 1st or 2nd base behind the UGA. Site-directed mutagenesis was used to identify genotypes allowing PTC read-through. The genetic context of the naturally occurring mutation of our patient is close to a hypothetical consensus sequence for maximum PTC read-through. These findings provide clues for the prediction of PTC read-through in human genetic disease.

2298/F

Different mutations in three Jewish families with Darier's disease. *L. Peleg[1], M. Karpati[1], B. Amichai[2].* 1) Danek Gertner Human Genetic Inst, Sheba Medical Ctr, Ramat Gan, Israel; 2) Dept Dermatology, Huzot Clinic, Clalit health Service, Ashkelon, Israel.

Darier's disease (DD) is an autosomal dominant skin disorder characterized by loss of adhesion between epidermal cells and abnormal keratinization. The disease has a world wide distribution with a varied prevalence. Defects in the sarco/endoplasmic reticulum Ca2+ ATPase type 2 underlie DD. The encoding gene ATP2A2 is positioned on chromosome 12q23-24. To date about 150 mutations had been identified in familial and sporadic cases. The aim of this study was to identify the genetic defect in patients from three unrelated Jewish families with DD. DNA was extracted and PCR was carried out to amplify the exons and flanking intron region of the ATP2A2 gene. The amplified fragments were then sequenced using ABI 3130 XL Genetic Analyzer. Two novel mutations were identified in two families: a C®T substitution at position 391, altering arginine 131 to stop codon was identified in three members of an Ashkenazi family. An A to C change at position 530 altering glutamine 177 to proline was found in four patients of an unrelated Ashkenazi family. These mutations were not found in 50 healthy individuals of the same ethnic origin. Codons 131 and 177 are located in a highly conserved cytoplasmic β-strand domain and thus are candidates to actually be disease causing. Two other mutations in codon 131 were already reported in non-Jewish patients. In the third family of a Tunisian extraction, Asparagine 767 was change to Serine (A2300G). That mutation was already reported as a disease causing mutation in European and Japanese patients. The existence of different mutations in three Jewish families verify the previously published reports that most mutations in that gene are private.

2299/F

Identification of recombinant alleles using Quantitative Real-time PCR: Implication for Gaucher disease. *A. Velayati, M.A. Knight, B.K. Stubblefield, E. Sidransky, N. Tayebi.* Section on Molecular Neurogenetics, Medical Genetics Branch/ NHGRI, NIH, Bethesda, MD.

Background: Gaucher disease is an autosomal recessive disorder caused by the deficiency of glucocerebrosidase. The glucocerebrosidase gene (GBA) is located in a very gene-rich region on chromosome 1q 21. The presence of contiguous, highly homologous pseudogenes for both GBA and metaxin 1 at this locus increases the likelihood of DNA rearrangement. We describe an easy method to identify and analyze recombinant alleles in patients with Gaucher disease. Methods: Genomic DNA from twenty patients with Gaucher disease known to carry recombinant GBA alleles and five controls were studied. Six different probes for either the GBA gene or pseudogene were designed to identify DNA rearrangements, as well as copy number variation within the GBA locus. Quantitative real-time PCR using TaqMan probes was performed on genomic DNA, and β-globin was co-amplified as an internal control. Southern blot analyses using the restriction enzyme HincII and direct sequencing were performed to confirm the real-time results. Results: GBA fusions and duplications could be detected in all the cases, corresponding to the Southern blot results. Different sites of recombination could also be distinguished. Conclusion: Quantitative real-time PCR is a sensitive and rapid method to detect fusions and duplications in patients with recombinant GBA alleles. Since this technique is faster and cheaper than Southern blotting, it can be applied as a suitable method in diagnostic laboratories. Keywords: Gaucher disease, glucocerebrosidase, recombinant alleles.

2300/F

KCNQ2 and KCNQ3 mutations in familial and sporadic cases of Benign Familial Neonatal Convulsions. *N.E. Verbeek[1], M. Poot[1], F.E. Jansen[2], D. Lindhout[1], M.J.A. van Kempen[1].* 1) Medical Genetics Dept; 2) Neuropediatrics Dept, University Medical Center Utrecht, Utrecht, Netherlands.

Background: Benign neonatal epilepsy (EBN) is an autosomal dominantly inherited epilepsy syndrome, characterized by seizure onset in the first week postnatally, spontaneously resolving within a few months. In the vast majority of patients, psychomotor development is normal, but seizures may recur in up to 15% of patients later in life. EBN is caused by mutations in the voltage-gated potassium channel subunit gene KCNQ2 (20q13.3) or KCNQ3 (8q24). The majority of reported mutations was found in KCNQ2 (92%). This is possibly due to a lower number of patients being analysed for KCNQ3 mutations. Purpose of this study is to determine the frequency of KCNQ2 and KCNQ3 mutations in EBN families and sporadic cases.

Methods: MLPA- and sequence analysis of the KCNQ2 and KCNQ3 gene was performed in patients with neonatal seizures. Larger deletions were analysed with Infinium humanhap300 SNP-array (Illumina, San Diego, USA).

Results: In total 62 probands were referred to our laboratory with the indication EBN. In this cohort 42 patients were confirmed clinically to have (possible) benign neonatal seizures (26 were familial cases and 16 were sporadic). In 16 families and 9 sporadic cases mutations in the KCNQ2 gene were detected, consisting of missense, splice-site, frameshift or nonsense mutations, and large deletions (ranging from 49 to 479 kbp containing one to 14 annotated genes). Additional analysis in 10 KCNQ2-negative probands (3 familial and 7 sporadic) revealed missense mutations in the KCNQ3 gene in 2 families and 2 sporadic cases.

Conclusions: KCNQ2 or KCNQ3 mutations were found in 69% of familial or sporadic cases. Mutation detection in neonates with (possible) benign neonatal seizures can prevent superfluous diagnostic procedures, provided DNA diagnostics has a short turn-around-time. In sporadic patients 82% were KCNQ2 and 18% were KCNQ3 mutations, which is higher than previously reported. Besides missense, splice-site, frameshift and nonsense mutations, large deletions comprise a substantial portion (16%) of KCNQ2-mutations, in KCNQ3 only missense mutations are found suggesting distinct mechanisms of etiopathogenesis.

2301/F

"Pseudoheterozygosity " in Autosomal Recessive Diseases. *H. Reznik-Wolf[1], A. Abu-Horowitz[1], Y. Bar-Joseph[1], M. Dushnitzki[1], B. Davidov[3], M. Frydman[1,2], H. Yonath[1,2], E. Pras[1,2].* 1) D Gertner Inst of Hum Genetics, Sheba Medical Ctr, Ramat-Gan, Israel; 2) Sackler Faculty of Medicine, Tel Aviv University, Israel; 3) Institute of Medical Genetics, Rabin Medical Center, Israel.

In most autosomal recessive diseases the mutations can be identified in both genomic DNA (gDNA) and coding DNA (cDNA). Rarely, a single mutation is identified in gDNA in heterozygous state, while in the cDNA the mutation is found in a hemizygous state. A baby boy with severe lactic acidosis due to complex I deficiency, passed away at 5 months of age. cDNA, extracted from his fibroblasts, revealed a homozygous mutation (R228Q) in *NDUFS2*. The genomic DNA from the parents was studied to assure their carrier state. Surprisingly, the mother did not carry the mutation, and was found to be homozygous for the wild type allele. Study of gDNA from the deceased child identified the mutation in heterozygous state. Comparing the gDNA and cDNA sequences revealed that the maternal allele was not expressed in the child's fibroblasts. The mother was found to be homozygous for all polymorphic markers and SNPs in the gene. Subsequent prenatal diagnosis performed with flanking and intra-genic polymorphic markers, identified that the fetus carries the same maternal allele as the deceased child, but a different paternal allele. A baby girl was born two years ago and she is healthy and developing well. We are still trying to identify the presumed regulatory maternal mutation. When only one mutation is found in an individual affected by an autosomal recessive disorder, the possibility of a mutation that affects expression should be considered.

2302/F

The Variant Spectrum of the Wilson Disease Gene, *ATP7B*, in Costa Rica. *D. Bugbee[1], C. Centeno-Cerdas[2], M. Sandi[3], A. Leal[3], D.W. Cox[1].* 1) Department of Medical Genetics, University of Alberta, Edmonton, Canada; 2) Department of Biochemistry School of Medicine, University of Costa Rica, San Pedro, Costa Rica; 3) Human Genetics Section School of Biology, University of Costa Rica, San Pedro, Costa Rica.

Wilson disease (WND) is an autosomal recessive copper deposition disorder that presents with hepatic and/or neurological signs anywhere from 3 to 70 years of age. The gene causing Wilson disease was identified in 1993 as *ATP7B*. A P-type ATPase, ATP7B excretes excess copper from the liver into the bile. Variation of the *ATP7B* gene can lead to protein products with decreased copper transport and trafficking activity. Decreased protein function results in a toxic copper buildup within several tissues that is fatal if untreated. To date, 644 gene variants have been identified, of which, over 500 are possibly disease causing as summarized within the Wilson Disease Database (http://www.wilsondisease.med.ualberta.ca/database.asp). Identification and classification of this variation is important to better understand its impact on protein function, copper accumulation, and in turn, Wilson disease. We have examined the specific variations of *ATP7B* found in Costa Rican patients. We partially/fully sequenced the *ATP7B* gene in a series of 26 WND patients from 18 different families. Possible disease causing variants (DV) M1I, M645R, M665I, L708P, I1184T, H1207R, and N1270S were all identified. M645R, M665I, L708P, and N1270S have Mediterranean origins. N1270S is the most frequent DV being 57.5% of all DVs found in the patient set. M1I and I1184T are novel to this study. I1184T is a polar to nonpolar amino acid change in the ATP loop which is predicted to be disease causing by the algorithm SIFT (Sorting Intolerant From Tolerant). The effect of the start site M1I variant is unknown, but might alter the translational initiation site in favour of an available downstream methionine located at c.97-c.99 (M33). Since this start codon is in-frame it is possible that a shorter ATP7B translation product is produced. The identification of these variants will help disease diagnosis in the Costa Rican population, and further adds to the variation spectrum of *ATP7B*.

2303/F

An Analysis of DNA Sequence Variation in the C2ORF71 Gene. *P.I. Sergouniotis[1,2], Z. Li[1], D.S. Mackay[1], L.A. Ocaka[1], G.A. Wright[2], S.R. Devery[2], A.T. Moore[1,2], A.R. Webster[1,2].* 1) UCL Institute of Ophthalmology, London, EC1V 9EL, United Kingdom; 2) Moorfields Eye Hospital, London EC1V 2PD, United Kingdom.

Mutations of *C2ORF71* have been recently reported to be associated with autosomal recessive (AR) retinitis pigmentosa (RP) in humans and defects in vision in zebrafish. The C2ORF71 gene is located on 2p23.2, is conserved in euteleostomi but has no evident paralogues. In humans, it is a 2-exon gene, predicted to encode a 1288 amino acid protein of unknown function that is expressed mainly in the photoreceptor cells of the retina. We sought to determine the prevalence of mutations in *C2ORF71* in cohorts of probands with progressive AR retinal dystrophy.

A combination of High Resolution DNA melting analysis (HRM) and automated DNA sequencing was used to screen *C2ORF71* in 286 affected unrelated individuals. Among them, 95 are affected with AR early onset retinal dystrophy and 191 are affected with AR adult onset retinal disease. 151 European and 40 South Asian control DNAs were also screened in a similar fashion.

Overall, 40 DNA sequence variants were detected. Among them, 28 were novel with 17 changes found in controls (8 missense, 7 synonymous, 2 other). Importantly, 11 novel variants (6 missense, 5 synonymous) in 20 alleles were detected in our cohort of patients but not in controls. Only one proband was compound heterozygote but segregation analysis revealed her unaffected father to be homozygous for one of the putative mutations. Average heterozygosity of the *C2ORF71* coding region, a measure of the probability of non identity of two randomly chosen chromosomal sites, was found to be 2.12×10^{-3}. This value is 12 to 700 times greater than that of three other retina disease genes examined in the similar fashion (*ABCA4, VMD2* and *EFEMP1*).

HRM is an economical, fast and sensitive PCR-based method for detecting DNA sequence variants. However, on two occasions, misincorporation of nucleotides during the PCR process prior to HRM was observed, reducing the specificity of the method. *C2ORF71* is a highly polymorphic gene with many rare variants, and this confounds mutation detection. Further analysis will determine the spectrum of retinal disease caused by mutations in *C2ORF71* and distinguish true pathogenic alleles from the high background of polymorphism elucidating the role of this rare cause of RP in the visual process.

2304/T

Tafazzin mRNAs of Barth Syndrome Individuals. *I. Gonzalez, A. Manolakos, V. Funanage, S. Kirwin.* Biomedical Research, Nemours/AI duPont Hospital for Children, Wilmington, DE.

Barth syndrome (BTHS, OMIM 302060) is due to mutations in the tafazzin (TAZ) gene located on Xq28. The tafazzin protein is an acyltransferase involved in the remodeling of cardiolipin, a component of the inner mitochondrial membrane. The phenotype includes dilated cardiomyopathy or left ventricular noncompaction, 3-methylglutaconic aciduria, neutropenia, skeletal myopathy, and growth delay. Biochemically, tetralinoleoyl cardiolipin is dramatically reduced and monolysocardiolipin accumulates. This study sought to determine how mutations affect TAZ mRNA quantities and alternative splice variants. To accomplish this, we used PAXgene tubes to collect blood samples from Barth syndrome individuals at the 2008 Barth Syndrome International Conference. Several studies were carried out: correlation of tafazzin splice variant distributions with the particular mutation of each subject; comparison of the proportions of splice variants between BTHS and healthy subjects; comparison of the mRNAs from bloods collected in PAX tubes with those of bloods collected in EDTA tubes; comparison of the splice variant distributions in EDTA bloods under simulated shipping delay conditions with those processed on the day of collection. For several samples we were able to compare tafazzin mRNAs from blood samples collected in PAX tubes with the mRNAs of blood cells that were cultured for 3 days in a previous study. We used FAM-labeled PCR primers to study splice variant distributions by capillary electrophoresis fragment analysis. As in our earlier study, where variants were cloned and sequenced, we found that besides the four main splice variants there is a large number of fragments that (by size) correspond to previously identified alternatively and incompletely spliced mRNA forms. PAXgene tube collection is claimed to yield mRNAs that represent the living condition as the mRNAs are stabilized from the moment of blood draw, and we found differences between this set of mRNAs and those from EDTA bloods and from cultured cells. We also found dramatic changes in mRNAs from EDTA bloods processed on the day of collection and those processed on the 3rd day, which would represent the effect of shipping delays. This study shows that TAZ mRNA requires immediate stabilization after blood draw in order to yield the most reliable results; this result is likely applicable to other mRNAs.

2305/T

Digenic inheritance in Arrhythmogenic Right Ventricular Dysplasia (ARVD) identified by Multiplex Targeted High Throughput Sequencing. *J.L. Blouin[1], S. Nikolaev[2], A. Munoz[2], S.E. Antonarakis[1,2], S. Fokstuen[1].* 1) Dept Genetic Medicine, Univ Hosps Geneva, Switzerland; 2) Genetic Medicine and Development, University of Geneva School of Medicine; Switzerland.

Cardiomyopathies and arrhythmias are common, seemingly monogenic autosomal dominant cardiac disorders. They are considered as primary cause of sudden cardiac death in young adults. These diseases are characterized by a remarkable genetic and allelic heterogeneity, which makes it difficult to unravel the causative mutation in a diagnostic laboratory. To circumvent these limitations, we used the new molecular diagnostic possibilities based on targeted high throughput sequencing of multiplexed barcoded samples. Five patients suffering from different monogenic cardiac disorders were analysed: 1 ARVD with a known pathogenic mutation (c.148_151delACAG) in exon 1 of Plakophilin-2 (*PKP2*) as a positive control, 2 long-QT syndromes and 2 hypertrophic cardiomyopathies (HCM) without known mutations. We designed a capture microarray that includes all exons of 132 genes involved in cardiovascular mendelian disorders (1 Mbp genomic region). Genomic DNA of the 5 patients were barcoded and mixed in equimolar amounts. Sequencing with paired-end 38 bp reads was performed in a single lane of a Illumina GAII, and data analysis was performed by MAQ software. Small coding indels, nonsense and non-synonymous variants probably damaging by Polyphen were scored and subjected to validation by Sanger sequencing. Potentially pathogenic novel variants were confirmed in 4 patients. First, in the patient known to have a causative mutation in *PKP2* gene, an additional nonsense variant (p.Lys991X) was found in Voltage-Gated Sodium Channel type V (*SCN5A*) gene. Mutations in *SCN5A* have mainly been described in long-QT and Brugada syndromes but have so far not been associated with ARVD. To our knowledge, this is the first report of a case of non-desmosomal digenic inheritance in ARVD. Second, a missense variant (p.Arg281Gln) in Acid Alpha-Glucosidase (*GAA*) gene (glycogen storage disease) was found in a patient diagnosed with HCM. Third, a missense variant (p.Met1384Ile) was detected in the Fibrillin (*FBN1*) gene (Marfan syndrome) in a 8 year old patient with long-QT syndrome. Finally, one patient with long-QT has a missense variant (p.Arg1860Ser) in *SCN5A* gene. Our results demonstrate that multiplex targeted high throughput sequencing holds considerable promises for molecular diagnosis of highly heterogeneous monogenic disorders in clinical practice, and allows a better understanding of the complexity of mendelian disorders.

2306/T

Initial presentations of genotype-positive individuals with MYBPC3 gene mutation prior to left ventricular hypertrophy. *H. Wang[1, 2, 3], B. Xin[1], L. Nye[1], C. Troyer[1], S. De[4], A. Borowski[4], J. Thomas[4], W. Tang[4, 5].* 1) DDC Clinic for Special Needs Children, Middlefield, OH; 2) Department of Pediatrics, Rainbow Babies & Children's Hospital, Cleveland, OH; 3) Department of Molecular Cardiology, Cleveland Clinic, Cleveland, OH; 4) Department of Cardiovascular Medicine, Heart and Vascular Institute, Cleveland Clinic, Cleveland, OH; 5) Lerner Research Institute, Cleveland Clinic, Cleveland, OH.

Hypertrophic cardiomyopathy (HCM) is typically inherited in an autosomal dominant pattern with a variable age of onset and prognosis. We have recently reported over 20 Old Order Amish children with severe neonatal HCM caused by a novel homozygous mutation c.3330+2T>G in the cardiac myosin-binding protein C (MYBPC3) gene in the Ohio Amish population. This mutation in the splice-donor site of MYBPC3 intron 30 results in a frame-shift which leads to production of a truncated protein. It is estimated that approximately 1500 individuals carry this HCM-causative mutation in the community, which represents a very unique opportunity for us in further understanding of the condition, its development, diagnosis and prevention. In this study, we focused on the initial presentations of the genotype-positive individuals with MYBPC3 gene mutation, before they developed left ventricular hypertrophy (LVH). One hundred and three genotype-positive individuals were identified from high-risk families, and 57 individuals, twelve years-old or older, completed a questionnaire. They demonstrated various clinical complaints, including dizziness or lightheadedness (30%), heart racing or fluttering (35%), skipped heart beat (26%), easily tired (30%), shortness of breath (25%) and chest pain (30%). However, there were no significant differences in cardiac-specific biomarkers (including B-type natriuretic peptide levels and cardiac troponin I levels) as well as inflammatory biomarkers (including high-sensitivity C-reactive protein and myeloperoxidase levels), when we compared 55 genotype-positive individuals with 67 genotype-negative individuals. Echocardiogram was performed in 41 consecutive genotype-positive individuals. Of these, 6 were felt to have possible phenotype expression, with 4 having echocardiographic features of HCM and 2 having concentric LVH. The remaining 35 individuals' echocardiograms were compared with 30 healthy controls. There were minimal differences in conventional 2D and Doppler derived parameters of systolic and diastolic function between the genotype-positive carriers and healthy controls, although the regional reductions in basal septal strain were observed the genotype-positive carriers. We concluded that the genotype-positive individuals with MYBPC3 gene mutation may have significant clinical manifestations before the left ventricular hypertrophy is developed, and we need to develop strategies to effectively follow up this cohort in our future study.

2307/T

Molecular genetic spectrum in Korean patients with Long QT syndrome. *SY. Kim[1], EJ. Bae[2], CI. Noh[2], YJ. Hyun[1], SI. Cho[1], SW. Seo[1], H. Park[1], SH. Song[3], SS. Park[1].* 1) Department of Laboratory Medicine, Seoul National University Hospital, Seoul, Korea; 2) Department of Pediatrics, Seoul National University Children's Hospital, Seoul, Korea; 3) Department of Laboratory Medicine, Seoul National University Bundang Hospital, Seongnam, Korea.

The Long QT syndrome (LQTS) is an inherited arrhythmic disorder causing serious cardiovascular mortality. To date, at least 12 genes are known to cause the LQTS which principally encode cardiac ion channels. This study aimed to identify the mutation spectrum of Korean LQTS patients in the major causative genes *KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2*, and *CACNA1C*.

We enrolled 39 unrelated Korean LQTS patients showing QTc interval of 470 ms or more prolongation, and their 46 familial members. Each gene was analyzed using comprehensive molecular genetic methods: direct sequencing for point mutations, multiplex ligation-dependent probe amplification for exonal deletion or duplication, and RT-PCR for the splicing site mutations. The significance of novel variants was assessed by familial segregation analysis, allelic frequency in normal control subjects, in silico prediction of the effect on protein function, and interspecies conservation of the amino acid.

Seventeen Korean probands (43.6%, 17/39) carried mutations in one of the genes: nine (52.9%, 9/17) with *KCNQ1* mutations, four (23.5%, 4/17) with *SCN5A* mutations, three (17.6%, 3/17) with *KCNH2* mutations, and one with *CACNA1C* mutation. Two de novo mutations were identified in the *KCNQ1* and *SCN5A*, respectively. Two rare syndromic patients were molecularly confirmed through this study: one proband was diagnosed with the autosomal recessive, Jervell and Lange-Nielsen syndrome. One was diagnosed with Timothy syndrome. Seven novel causative mutations were identified: p.Gln359_Lys362dup, p.Gly48SerfsX36, and splicing mutation p.V307V in the *KCNQ1*; p.Q1476K, p.W1345C, and p.R121W in the *SCN5A*; p.R20P in the *KCNH2*. Mutations were identified in 20 family members (43.5%), therefore appropriate management was provided for them.

This study identified molecular genetic characteristics of LQTS in Korean population previously unexplored. The *KCNQ1* was the most important gene in Korean patients. The *SCN5A* mutations had higher proportion in Korea than in western countries. No mutational hotspot region was observed. The genetic characteristics of Korean LQTS will be helpful for the diagnosis, appropriate medical therapies, and genetic counseling for the patients and their family members. This study also provides better understanding the worldwide spectrum of LQTS.

2308/T

A new locus for syndromic Thoracic Aortic Aneurysms and Dissections maps to chromosome 15q. *I.M.B.H. van de Laar[1], R.A. Oldenburg[1], J. Roos-Hesselink[2], B. de Graaf[1], I. Frohn-Mulder[3], Y. Hoedemaekers[1], M. Kros[4], P.M. Pattynama[5], S.M. Bierma-Zeinstra[6], E.H.G. Oei[5], B.A. Oostra[1], M.W. Wessels[1], A.M. Bertoli-Avella[1].* 1) Clinical Genetics, Erasmus Medical Centre, Rotterdam, Netherlands; 2) Department of Cardiology, Erasmus Medical Center, Rotterdam, the Netherlands; 3) Department of Pediatric Cardiology, Erasmus Medical Center- Sophia, Rotterdam, the Netherlands; 4) Department of Pathology, Erasmus Medical Center, Rotterdam, the Netherlands; 5) Department of Radiology, Erasmus Medical Center, Rotterdam, the Netherlands; 6) Department of General Practice, Erasmus Medical Center, Rotterdam, the Netherlands.

Background: Aortic aneurysms represent a leading cause of cardiovascular morbidity and mortality. Thoracic aortic aneurysms and dissections (TAAD) are a main feature of connective tissue disorders such as Marfan syndrome, Loeys-Dietz syndrome and vascular Ehlers-Danlos syndrome. Methods: We characterized the clinical spectrum of a large Dutch family with syndromic TAAD. Thirty family members had an extensive physical and cardiologic examination. Histological examinations of aneurismal aortas were performed. We carried out a genome-wide linkage analysis using Affymetrix 250k SNP arrays to localize the disease gene. Mutation analysis in functional candidate genes in the linkage interval is ongoing. Results: We describe a new syndromic form of TAAD with tortuosity throughout the arterial tree, skeletal, connective tissue anomalies and mild craniofacial features. Twenty-two patients were identified from whom 12 had aneurysms of the thoracic aorta (9 patients) or other arteries involving the pulmonary, splenic, common iliac, mesenteric, renal and vertebral artery. Aortic dissections and/or rupture occurred in mildly dilated arteries. Associated (congenital) heart defects were found as well. All patients had striking joint abnormalities, including early-onset intervertebral disc degeneration, osteochondritis dissecans and osteoarthritis (OA). We therefore name this syndrome TAAD/OA. Subsequently, we mapped its genetic locus to chromosome 15q with a significant multipoint LOD score of 3.6. All 12 patients with arterial aneurysms and 10 patients with a spectrum of skeletal and connective tissue anomalies were sharing the 15q21-q24.2 disease haplotype. Conclusions: We characterized the clinical spectrum of a new TAAD syndrome, TAAD/OA. The clinical phenotype overlaps with known syndromes such as Marfan syndrome, Loeys-Dietz syndrome and vascular type Ehlers-Danlos syndrome. Our data provide evidence for a new TAAD locus. Identification of the disease gene will give insight into the pathogenesis of arterial aneurysms and osteoarthritis.

2309/T

The Genetic Contribution to Hypothyroidism in a South Tyrolean Population. *C.B. Volpato[1], A. De Grandi[1], M. Facheris[1,2], C. Minelli[1], C. Pattaro[1], M. Gögele[1], A.A. Hicks[1], P.P. Pramstaller[1,2,3].* 1) Institute of Genetic Medicine, European Academy Bozen/Bolzano (EURAC), Bolzano, Italy Affiliated Institute of the University Lübeck, Germany; 2) Department of Neurology, Central Hospital, Bolzano, Italy; 3) Department of Neurology, University of Lübeck, Germany.

Thyroid hormones (THs) have important roles in growth and development. The key regulator of thyroid function is thyroid-stimulating hormone (TSH). Serum TSH is a indicator of thyroid function and the best assay to check thyroid function and clinically define hypothyroidism. In order to look for genes contributing to hypothyroidism we have used linkage followed by association under the linkage peaks. We used a South Tyrolean population (Italy) in whom the founder-population structure can simplify genetic analysis of complex traits. Samples from 1336 individuals were collected and genotyped with 1000 microsatellites and Illumina HumanHap300 SNP microarrays. To define individuals with hypothyroidism we used two accepted cutoff criteria (from the literature): TSH values over 3.0 mU/l for sub-physiological hypothyroidism, and over 4.60 mU/l for clinically-defined hypothyroidism. Merlin software was used for non-parametric genome wide linkage analysis. Two set of pedigrees were built, with 46 families (2 to 11 affected) using TSH >3.0 mU/l and 18 families (2 to 5 affected) using TSH >4.6 mU/l respectively. Linkage analysis with both criteria revealed overlapping linkage peaks in two regions, most notably at 6q26-27 where SNP association under the linkage peak identifies PDE10A as a gene of interest. Therefore, using a combination of microsatellite linkage and SNP association we have identified novel association to a gene that is involved in hypothyroidism in our South Tyrolean sample. The presence of linkage over PDE10A in families with clinical hypothyroidism supports the argument that functional variants within this gene contribute to the pathogenesis of abnormal thyroid function.

2310/T

Kallmann Syndrome in Finland. *J. Tommiska[1,2], K. Vaaralahti[1], E.-M. Laitinen[2], M. Tervaniemi[1], L. Valanne[3], T. Raivio[1,2].* 1) Institute of Biomedicine/Physiology, University of Helsinki, Helsinki, Finland; 2) Children's Hospital, Helsinki University Central Hospital, Helsinki, Finland; 3) Department of Radiology, Helsinki University Central Hospital, Helsinki, Finland.

Kallmann syndrome (KS) is a clinically and genetically heterogeneous disorder comprised of isolated hypogonadotropic hypogonadism (IHH) and absent sense of smell (anosmia). KS results from disturbed intrauterine migration of gonadotropin-releasing hormone (GnRH) neurons to the hypothalamus. The exact incidence of KS is currently unknown. In familial KS (~40% of KS), autosomal dominant, autosomal recessive, and X-chromosomal recessive modes of inheritance have been described. Defects in 7 genes (*KAL1*, *FGFR1*, *FGF8*, *PROKR2*, *PROK2*, *NELF*, *CHD7*) have been shown to underlie KS. A heterozygous mutation in *FGFR1* is found in ~10% of cases. However, causative gene defect(s) can be identified in only ~30% of patients. We examined the nationwide incidence of KS in Finland. In addition, 30 probands (25 males; 5 females) were phenotyped in detail and analyzed for mutations in the coding regions of *KAL1*, *FGFR1*, *FGF8*, *PROKR2*, *PROK2*, and *NELF*. Three patients were also screened for mutations in *CHD7* due to phenotypic features associated with CHARGE syndrome. Between 1976 and 1987, among 767 778 newborns, 15 KS subjects were retrospectively identified giving a KS incidence of 1 in 51 000. A difference existed between the incidences in males (12/392 900) and females (3/374 878) (P=0.04). The phenotypic spectrum of the 30 probands ranged from severe hypogonadism with complete absence of puberty to reversal of IHH following androgen exposure. Importantly, all 5 females carried an *FGFR1* mutation and had severe IHH. Overall, 3 mutations in *KAL1* (C157CfsX46, R262X, g.2357_2360delAgta) and 9 in *FGFR1* (W4X, G48S, T82TfsX28, R209H, K321RfsX13, S436YfsX3, R609X, E670A) were detected. Mutant receptors G48S and E670A displayed impaired MAPK signaling *in vitro*, and were associated with variable olfactory and reproductive phenotypes in the families. No mutations were found in *PROKR2*, *PROK2*, *FGF8*, *NELF*, or *CHD7*, which may reflect the special genetic features of the Finnish population. Eighteen of 25 male probands (72%) remained without identified mutations, and 7 of them had severe IHH. This, together with the lack of mutations in any of the known KS genes apart from *FGFR1* and *KAL1*, implies the existence of previously undescribed gene(s) underlying KS in Finnish men. Indeed, sex-dependent penetrance of the mutations in KS genes yet-to-be discovered could explain the higher incidence of KS in males, as well as the *FGFR1* mutations as the sole cause of KS in Finnish females.

2311/T

Proline-rich domain of MYO15A is necessarily for hearing and stereocilia maintenance. *Q. Fang[1], J. Bird[2], A.A. Indzhykulian[3], M. Mustapha[1,4], S.M. Jones[5], I.A. Belayantseva[2], D.F. Dolan[1], T.B. Friedman[2], G. Frolenkov[3], S.A. Camper[1].* 1) University of Michigan Medical School, Ann Arbor , MI; 2) National Institute of Deafness and Communication Disorders, Rockville, MD; 3) University of Kentucky, Lexington, KY; 4) Stanford University, Palo Alto, CA; 5) East Carolina University, Greenville, NC.

Mutations in unconventional myosin *Myo15a* are responsible for profound congenital deafness and vestibular dysfunction in shaker 2 (*sh2*) and shaker 2J (*sh2J*) mice (Probst et al, 1998; Anderson et al, 2000). The *sh2* mutation likely inactivates the motor ATPase, while the *sh2J* deletion truncates the C-terminal PDZ ligand that is important for binding whirlin and transporting it to stereocilia tips (Belyantseva et al., 2005). As a result, stereocilia are abnormally short in both *sh2* and *sh2J* mutants. Mutations in the human ortholog, *MYO15A*, cause deafness *DFNB3* (Wang et al, 1998). Alternatively spliced transcripts predict multiple isoforms of MYO15A that include a large, evolutionarily conserved proline-rich N-terminal domain (Liang et al, 1999). The N-terminal domain is encoded solely by exon 2, and mutations in this exon have been linked to deafness in humans. Elegant studies in cochlear explants have shown that isoforms lacking the proline-rich N-terminal domain are sufficient to rescue stereocilia elongation and whirlin transport (Belyantseva et al, 2005). To test whether the proline-rich region has a distinct function, we generated a mouse model that recapitulates a human mutation in the proline-rich domain using knock-in technology. Knock-in mutants were profoundly deaf, but differ from *sh2* and *sh2J* mice in both hair bundle morphology and the absence of circling behavior. Vestibular evoked response testing did reveal subtle vestibular abnormalities. Cochlear stereocilia appear to develop normally with whirlin localized at their tips, but the stereocilia are not maintained, implicating the proline-rich region in preservation of the hair bundle. Classic genetic analysis of compound heterozygous mice containing different combinations of *Myo15a* mutant alleles revealed no evidence of allelic complementation for hearing or hair bundle maintenance, consistent with the functional importance of full length MYO15A isoforms containing the proline-rich domain for normal mammalian hearing. In conclusion, this new isoform-specific *Myo15a* mutant mouse suggests a novel function for the proline-rich N-terminus that is more critical for auditory function than for vestibular function.

2312/T

Examination of Gene Expression Patterns Associated with PMP22-related Auditory Dysfunction. *M.J. Kovach, T.A. Carver, T. Walker.* Biological & Environmental Sci, Univ Tennessee, Chattanooga, TN.

Characterization of genes responsible for deafness, and their expression patterns, can help dissect the molecular events underlying both deafness and normal hearing. A variant of Charcot-Marie-Tooth (CMT1E) disease associated with deafness, has been linked to point mutations in the PMP22 gene. Although PMP22 is largely expressed in Schwann cells responsible for nerve myelination, PMP22 mRNA has also been detected in a variety of non-neural tissues, including the cochlea and lungs, at critical times of tissue development. This widespread expression suggests a more universal biological function for the PMP22 protein. The mouse homolog of PMP22, Gas3, was first isolated as a Growth Arrest Specific gene. In non-neural tissues, Gas genes are induced during periods of growth arrest and downregulated at terminal differentiation. Gas genes are thought to function during the intervening period where cells committed to a particular lineage continue to divide at a reduced rate. Not surprisingly, Gas genes are known to regulate gene expression, apoptosis and timing of cell division. Thus, PMP22 appears to play a role in nerve myelination and cell growth regulation of non-neural tissues. This dual expression is consistent with both neural and cochlear hearing loss in CMT1E patients. This study examines the involvement of PMP22 in cochlear development, where we propose it acts as a transcriptional regulator of genes important to normal ear functions. We hypothesize that a mutation in PMP22 leads to abnormal gene expression patterns that characterize the deafness phenotype. The Trembler-J mouse was chosen as a model for PMP22-associated auditory dysfunction to study gene expression patterns at critical developmental time points. Lung tissue was used as a non-neural surrogate for the cochlear labyrinth. Tissues were collected from normal and TrJ mice throughout development and gene expression profiles were characterized by differential display and qRT-PCR. Thus far, ~100 differentially expressed transcripts have been identified and classified according to cellular function, 55% of which are homologs or family members of genes in the human cochlear library. Divergent patterns of PMP22 cochlear localization and expression are seen at the D7/D14 developmental transition. In the TrJ mouse, this transition is associated with an upregulation of genes involved in signal transduction and membrane/cytoskeletal processes, and downregulation of genes involved in cell cycling.

2313/T

Disruption of the histone H3 acetylation gene, MYST4, leads to Noonan syndrome and hyperactivated MAPK signaling. *C. Thiel[1], M. Kraft[1], A.K. Voss[2, 3], T. Thomas[2, 3], I. Goehring[1], B.N. Sheikh[2, 3], L. Gordon[2], H.S. Scott[2, 3], G.K. Symth[2, 3], I.C. Cirstea[4], M.R. Ahmadian[4], U. Trautmann[1], M. Zenker[1], M. Tartaglia[5], A.B. Ekici[1], A. Reis[1], H.-G. Doerr[6], A. Rauch[1, 7].* 1) Institute of Human Genetics, University of Erlangen-Nuremberg, Erlangen, Germany; 2) Walter and Eliza Hall Institute of Medical Research, Parkville, Melbourne, Australia; 3) Department of Medical Biology, University of Melbourne, Parkville, Australia; 4) Institute of Biochemistry and Molecular Biology II, Heinrich-Heine University Medical Center, Düsseldorf, Germany; 5) Dipartimento di Ematologia, Oncologia e Medicina Molecolare, Istituto Superiore di Sanità, Rome, Italy; 6) Division of Paediatric Endocrinology, University Hospital for Children and Adolescents, Erlangen; 7) Institute of Medical Genetics, University of Zurich, Schwerzenbach-Zurich, Switzerland.

Only recently awareness is rising that the genetic information of an individual is not only encoded by its nucleotide composition, but also based on the so called epigenome by means of epigenetic modifications of DNA and histones. By chromosomal breakpoint mapping in a patient with a Noonan syndrome phenotype encompassing short stature, blepharoptosis and attention deficit hyperactivity disorder, we identified haploinsufficiency of the histone acetyltransferase MYST4 as the underlying cause. Using acetylation, whole genome expression and chromatin immunoprecipitation studies in the patient, MYST4 siRNA cell lines and the querkopf mouse model we find that H3 acetylation is important for neural, craniofacial and skeletal morphogenesis, mainly by specifically regulating the MAPK signaling pathway. This further elucidates the complex role of histone modifications in mammalian development and adds a new pathomechanism to the phenotypes resulting from misregulation of the Ras signaling pathway.

2314/T

Expression profiles of SPG11 and SPG15 proteins, involved in hereditary spastic paraplegia with thin corpus callosum. *R.P. Murmu[1], E. Martin[1], P.S. Denora[1], M.P. Muriel[1], K.H. El Hachimi[1,2], T. Esteves[1], J. Fernandez[1], A. Dauphin[1], C. Duyckearts[1], A. Brice[1], G. Stevanin[1,2].* 1) CR-icm, INSERM / UPMC UMR_S975, NEB, Paris, France; 2) EPHE, Paris, France.

Hereditary spastic paraplegias (HSP) are genetically inherited and clinically diverse neurodegenerative disorders which are due to degeneration of the corticospinal tract and of additional syndrome-specific structures. Truncating mutations in the SPG11 and SPG15 genes cause complicated spastic paraplegia due to loss of the functions of spatacsin and spastizin, respectively. We explored the intracellular and tissue localisations of these proteins by using specific polyclonal anti-spatacsin (SPG11) and anti-spastizin (SPG15) antisera that we developed. We observed expression of both proteins in human and rat nervous systems, particularly strong in cortical and spinal motor neurons as well as in sensory neurons and retina. Both proteins were also expressed ubiquitously in embryos. In cultured cells, these two proteins had similar diffuse punctate, cytoplasmic and sometimes nuclear (spastizin) distributions. They partially co-localized with protein-trafficking vesicles, mitochondria or cytoskeleton. This study of the endogenous expression of spatacsin and spastizin shows similarities in their expression patterns that could account for their overlapping clinical phenotypes. The findings of our study are expected to shed light into the functions of these proteins which may provide insight into mechanisms underlying the degeneration of corticospinal tract in hereditary spastic paraplegias.

2315/T

Is 8860 variation a rare polymorphism or associate as a secondary effect of HCM disease? *M. Houshmand[1,2], M. Montazeri[2], N. Kuchekian[1].* 1) Medical Genetic, Special Medical Center, Tehran, Iran; 2) National Institute for Genetic Engineering and Biotechnology, Tehran, Iran.

mtDNA defects, both deletions and point mutations, have been associated with hypertrophic cardiomyopathies (HCM). The aim of this study was to establish a spectrum for mtDNA mutations in Hypertrophic cardiomyopathy (HCM) Iranian patients. HCM is widely accepted as a pluricausal or ultifactorial disease. Because of the linkage between energy metabolism in the mitochondria and cardiac muscle contraction, it is reasonable to assume that mitochondrial abnormalities may be responsible for some forms of HCM. Point mutations and deletions in the two hot spot regions of mtDNA were investigate by PCR and Sequencing methods. Some unreported point mutations have been founded in this study but no deletion was detected. Meanwhile some of these point mutations have been investigated among HCM patients for the first time. A8860G transition was presented in high proportion that resulted to the doubt that if this rare polymorphism associate as a secondary effect of HCM disease.

2316/T

Sorting defects of mutant CLC-Kb in Bartter syndrome. *E.J. Kamsteeg[1], R.D.A. Weren[1], J. Schoots[1], J.H. Robben[2], N.V.A.M. Knoers[1], H. Scheffer[1], P.M.T. Deen[2], L.H. Hoefsloot[1].* 1) Human Genetics, Radboud University Medical Center, Nijmegen, Netherlands; 2) Physiology, Radboud University Medical Center, Nijmegen, Netherlands.

Bartter syndrome (BS) is an autosomal recessive disorder characterized by renal salt wasting and hypokalaemic metabolic alkalosis. The primary defect is a reduced NaCl reabsorption in the thick ascending limbs of Henle's loop. Bartter syndrome type 3 (BS3) is caused by mutations in CLCNKB, encoding the renal chloride channel CLC-Kb. This chloride channel functions in the basolateral plasma membrane and transports chloride from the cell's interior to the interstitium. CLCNKB has many non-pathogenic variants, making detected missense variants difficult to interpret. Defects of these variants may include a disturbed subcellular sorting or defective gating. Analysis of these variants in polarized epithelial cells is a valuable tool to assess their sorting properties. Here, we present the analysis of the subcellular distribution of eleven CLC-Kb missense variants in such cells. CLC-Kb needs the scaffolding protein barttin to reach the basolateral plasma membrane. Therefore, barttin was stably-expressed in the polarized renal epithelial MDCK type II cells. These cells were then transiently transfected with GFP-tagged CLC-Kb encoding constructs and subjected to imunnofluorescence studies and confocal laser-scanning microscopy. Of the eleven variants, six (S113T, V170M, A204T, M427V, G465R and R595Q) were transported to the basolateral plasma membrane, like wt-CLC-Kb. Three others (P124L, G164R en L656P) were retained intracellularly, and colocalized to a major extend with an endoplasmic reticulum (ER) marker. The last two variants (G433E and C626Y) showed a unexpected staining of the nucleus, although the latter also is expressed in the basolateral plasma membrane. These data indicate that a subset of the CLC-Kb variants is properly sorted to the basolateral plasma membrane. These variants may reflect non-pathogenic variants, or pathogenic variants that are functionally impaired (i.e. defective gating). However, another subset is retained in the ER, as is common with misfolded membrane proteins, such as CFTR or AQP2. Finally, two other variants were missorted to the nucleus. The mechanism causing nuclear missorting remains to be determined. Altogether, studying the subcellular distribution of CLC-Kb variants in polarized cells is a valuable additive tool to test their pathogenicity.

2317/T

Insulin like growth factor-1 receptor gene (IGF1R) mutations in patients with intrauterine growth retardation (IUGR) and postnatal catch-up growth failure. *M.J. Kang[1], K.J. Kim[1], M. Hong[1], E.J. Seo[1,2], J.Y. Park[1], G.H. Kim[1,2], H.Y. Jin[3], B.H. Lee[1,2,3], S.M. Lee[4], H.W. Yoo[1,2,3].* 1) Genome Research Center for Birth Defects, University of Ulsan College of Medicine, Asan Medical Center Children's Hospital, Seoul, Korea; 2) Medical Genetics Clinic and Laboratory,University of Ulsan College of Medicine, Asan Medical Center Children's Hospital, Seoul, Korea; 3) Department of Pediatrics,University of Ulsan College of Medicine, Asan Medical Center Children's Hospital, Seoul, Korea; 4) Department of Pediatrics, Hanyang University Guri Hospital, Seoul, Korea.

The mutations in the *IGF1R* genes are associated with intrauterine growth retardation (IUGR) and postnatal growth failure. Here we report three patients from two unrelated Korean families with *IGF1R* mutation and their clinical and molecular genetic characteristics. All three patients showed unexplained IUGR (birth weight <-1.5 SDS) and persistent short stature (<-2.0 SDS). *IGF1R* gene analysis identified a novel heterozygous mutation of c.420delC (p.Ala110fsX20) in exon 2, resulting in a frame-shift with premature termination of IGF1R protein in family 1 with autosomal-dominant inheritance pattern. The expression of IGF1Rβ protein is reduced in patients' fibroblasts compared to that in control cell lines. Both IGF-1-induced autophosphorylation of P-Tyr of IGF1R and phosphorylation of downstream signaling protein, Akt were enhanced in a dose-dependent manner, but less efficiently in patients' fibroblasts than in control fibroblasts. Array CGH of chromosome 15 also identified a heterozygous deletion of 15q26.2 to 15qter, a region including *IGF1R*, in a sporadic case in family 2. This study demonstrates functional perturbation of the signaling pathway associated with cell growth in patients with *IGF1R* mutation, which should be included in differential diagnosis in familial IUGR patients without catch-up growth.

2318/T

SMC1A MUTATIONS CAUSING CORNELIA DE LANGE SYNDROME AFFECT THE RECRUITMENT OF TRANSCRIPTION FACTORS. *A. Musio, S. Menga, L. Mannini.* Inst Tecnologie Biomed, CNR, Pisa, Italy.

Cornelia de Lange syndrome (CdLS, MIM#s 122470, 300590, 610759) is a rare disease characterised by mental and growth retardation, facial dysmorphism and limb abnormalities. It has been shown that CdLS is caused by mutations in cohesin and cohesin regulatory genes. Mutations in NIPBL, SMC1A and SMC3 genes have been identified in about 60% of CdLS patients. NIPBL is involved in the loading of cohesin onto chromatin, whereas SMC1A and SMC3 code for proteins belonging to the core cohesin complex. NIPBL mutations include point mutations, deletions and insertions, causing a null allele. SMC1A and SMC3 mutations are missense or small in frame deletions. Beyond its structural function in maintaining sister chromatid cohesion, cohesin plays additional roles distinct from its basic role. Depletion of cohesin genes leads to chromosome aberrations, whereas mutations are associated to colorectal cancer, suggesting that cohesin is a genome stability caretaker. In addition, experimental evidence obtained in Drosophila, Saccharomyces cerevisiae, zebrafish and mammalian cells highlight a key role of cohesin in transcription regulation.At this point, mounting evidence indicates that CdLS is owed to gene expression dysregulation by a cohesin-dependent mechanism. However, the underlying molecular mechanism is still poorly understood. Taking these facts into consideration, it was particularly fascinating to explore whether the gene transcription machinery was somehow affected by SMC1A mutations. We report here that SMC1A mutations impair the assembly of transcription complex initiation in the promoter regions and that the recruitment of RNA polymerase II, TATA box Binding Protein and TFIIB transcription factor is deficient in CdLS cell lines, therefore affecting the key step in the transcription process. Supported by Tuscany Region grant (A.M.).

2319/T

Exploring the role of miR-590 in Williams Beuren syndrome. *G. Merla[1], V.A. Gennarino[2], C. Fusco[1], L. Micale[1], B. Augello[1], M.G Turturo[1], E.V. D'Addetta[1], S. Banfi[1], A. Calcagni[1], M.N. Loviglio[1].* 1) Laboratory of Medical Genetics, IRCCS "Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy; 2) TIGEM, Telethon Institute of Genetics and Medicine, Naples, Italy.

Williams Beuren syndrome (WBS) is a recurrent genomic disorder caused by hemyzygosity of 28 contiguous genes at 7q11.23. Patients with WBS have a characteristic constellation of medical and cognitive findings. Despite a significant advance in the last years still much has to be learnt in order to further translate the knowledge into a better care of patients. We investigated the role of miR-590, the only miRNA located within the WBS deleted region. Preliminary results showed that both miR-590-5p and miR-590-3p miRNA strands are downregulated in cell lines of WBS patients. Because individual miRNAs regulate the expression of multiple target genes, we speculated that the downexpression of miR-590 in WBS has strong influences on the generation of a number of WBS phenotypes because of an altered control of its target genes. This project moved from the idea that if we can modulate the action of such miRNA, we will find a possible "large spectrum" therapeutic target for WBS. In order to asses the biological pathways controlled by miR-590, and to identify the set of its putative target (PTs) genes, we exploited global transcriptome analysis in cells following overexpression and inactivation of miR-590. Preliminary, by qPCR, luciferase assays, and western blot analysis, we have validated a set of predicted miR-590 PTs identified by querying HOCTAR a recently developed software for miRNA target prediction. We are now performing functional assays on some selected PTs to assess their biological relevance on WBS. The results of these comprehensive analyses on miR-590 will be presented.

2320/T

Calvarial Osteoblasts from Infants with Nonsyndromic Craniosynostosis Exhibit Increased Osteogenic Potential. *S.A. Boyadjiev Boyd[1], M.L. Decaris[2], C. Horner[2], A. Bhat[2], E. Cherkez[1], G. Yagnik[1], J. Liu[1], G.J. Boggan[3], T. Tollefson[4], C. Senders[4], M.L. Cunningham[5], J.K. Leach[2].* 1) Dept Pediatrics Gen, SOM, UCD Med Ctr, Sacramento, CA; 2) Dept. of Biomedical Engineering, University of California Davis, Davis, CA; 3) Dept. of Neurosurgery, University of California Davis, Sacramento, CA; 4) 4Dept. of Otorhinolaryngology, University of California Davis, Sacramento, CA; 5) Dept. of Pediatrics, Division of Craniofacial Medicine, University of Washington, Seattle, WA.

Nonsyndromic craniosynostosis (NSC), the premature fusion of one or more cranial sutures, is a clinical condition with accelerated ossification. Its causes remain unknown and it is unclear if NCS occur due to innate osteoblast defect or due to abnormal signaling from the surrounding tissues. We hypothesized that sutural and perisutural osteoblasts from infants with NSC have greater osteogenic potential as compared with control calvarial osteoblasts. Control osteoblasts and osteoblasts from infants with sagittal, metopic or coronal NSC were seeded on tissue culture plastic and cultured in media containing osteogenic supplements in 1% (hypoxic), 5% (normoxic), or 21% O2 (ambient air) for up to 14 days. Osteogenic potential was determined by quantifying intracellular alkaline phosphatase activity (ALP), cellular proliferation by DNA content, mineral deposition by von Kossa staining, and changes in gene expression by qPCR. For all oxygen tensions examined, NSC osteoblasts exhibited significantly higher levels of osteogenic differentiation ($p<0.05$). We detected significantly more mineralized nodules in NSC osteoblasts as compared to control osteoblasts at all oxygen tensions, with cells cultured in 5% O2 exhibiting the largest nodules. Osteoblasts from NSC patients demonstrated ALP levels that were significantly higher than control cells for all culture conditions. A markedly increased expression of osteogenic genes was also observed for cells from NSC patients. Our data demonstrate that NSC osteoblasts exhibit increased osteogenic potential compared to control osteoblasts under a variety of oxygen tensions. We suggest that NSC osteoblasts harbor genetic variants that determine more robust osteogenic maturation program as compared to control osteoblasts.

2321/T

Further characterization of cell lines harbouring an unmethylated full mutation of the *FMR1* gene. *E. Tabolacci[1], L. Borrelli[1], S. Lanni[1], U. Moscato[2], P. Chiurazzi[1], G. Neri[1].* 1) Inst Medical Genetics, Catholic Univ, Rome, Italy; 2) Institute of Public Helath, Catholic Univ, Rome,Italy.

Fragile X syndrome (FXS, OMIM #300624) is the commonest cause of inherited mental retardation, due to mutation of *FMR1*, an X-linked gene containing a CGG repeat in its promoter region. Expansion of this sequence beyond 200 repeats (full mutation) and consequent epigenetic changes cause the transcriptional inactivation of *FMR1* and lack of the FMRP protein. Rare individuals of normal intelligence, carrying an unmethylated full mutation (UFM), have been observed and the epigenetic modifications in their *FMR1* gene were characterized [Pietrobono et al., 2005; Tabolacci et al., 2008]. These cell lines are very rare, possibly representing the status of FXS cells before epigenetic silencing. CTCF (CCCCTC-binding factor), a zinc-finger protein which binds to the chromatin insulators, is an important regulator of the transcription of genes harbouring trinucleotide repeats, playing an important role in the barrier activity of insulators. We started to investigate the role of CTCF in regulating *FMR1* gene expression and observed that the amount of CTCF bound to *FMR1* was slightly higher in the UFM cell lines, compared to normal controls, while in the FXS cells this amount was low. On the other hand, knock-down experiments with anti-CTCF siRNA, transfected into normal and UFM fibroblasts demonstrated a 50% reduction of *FMR1* transcript, both sense and antisense (AS-FMR1), with respect to untreated controls. After CTCF knock-down, the epigenetic analysis of the *FMR1* promoter region demonstrated a reduction of H3-K4 methylation and an increase of H3-K9 methylation. These results suggest that CTCF is a key modulator of the *FMR1* transcription, given that its depletion causes *FMR1* transcript reduction and the transition to a heterochromatin organization. Further experiments are in progress to study the effect of CTCF knock-down on DNA methylation, including the boundary region recently described by Naumann et al. (2009), and on FMRP level. We have also identified antisense FMR1 RNAs (AS-FMR1) in different regions of the *FMR1* promoter both in controls and in UFM cell lines, but not in FXS cell lines. The AS-FMR1 levels were approximately 7-fold overexpressed in UFM cells relative to normal controls and absent in FXS cell lines. The elucidation of the mechanism sparing UFM males from inactivating their full mutation is important for planning therapeutic attempts at converting methylated into unmethylated full mutations, restoring the expression of the *FMR1* gene.

2322/T

Genetic structure and subcellular targeting of the hereditary spastic paraplegia gene REEP1. *J. Price[1], J. Huang[1], G. Montenegro[1], T. Deconinck[2,3], M. Pericak-Vance[1], G. Wang[1], P. de Jonghe[1], S. Züchner[1].* 1) Hussman Institute for Human Genomics, Miller School of Medicine, University of Miami; 2) Molecular Genetics Department, Flanders Interuniversity Institute for Biotechnology, University of Antwerp, Antwerp, Belgium; 3) Division of Neurology, University Hospital Antwerp, Antwerp, Belgium.

Previously, we have shown that mutations in REEP1 cause hereditary spastic paraplegia (HSP) type 31. REEP1 is now considered the third most common HSP gene and genetic testing is frequently carried out according to the reported gene annotation. REEP1 is a protein of unknown function, but a controversy has emerged whether it is localized in mitochondria or the endoplasmic reticulum. We studied the genetic structure of REEP1 and identified additional exons and alternative isoforms. Sequencing analysis was carried-out at these novel exons in a large sample of HSP index patients. In-vitro experiments were performed at transiently transfected cell lines. We identified two novel missense mutations in two of the new REEP1 exons, which would have been missed by conventional testing approaches. Interestingly, at least one new isoform is missing the mitochondrial targeting signal at the N-terminal end of REEP1. In cell culture experiment we found differential subcellular targeting of REEP1 with isoform2a localizing to mitochondria and isoforms1 and 2b localizing to the ER. The identification of additional coding exons inREEP1 has important consequences for genetic testing in SPG31. Future genetic tests should include these novel coding exons. The subcellular localization of REEP1 is significantly different in the expressed isoforms. How this relates to the pathology of SPG31 will be an important part of future functional studies.

2323/T

Cloning of Copper binding protein from Indian Childhood Cirrhosis liver revealed Arg 72 variant of p53: New insight in pathophysiology. *R. Prasad[1], N. Sharma[1], G. Kaur[2], B.R. Thapa[3].* 1) Department of Biochemistry, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India; 2) Department of Physiology, Government Medical College and Hospital (GMCH), Chandigarh, India; 3) Division of Pediatric Gastroenterology, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India.

Indian Childhood Cirrhosis (ICC) is a disease of abnormal copper metabolism which is commonly characterized by swelling and degeneration of liver cells along with the presence of orcein staining deposits of copper. Erstwhile, we identified and characterized a ~ 50KDa copper binding protein from ICC liver (Prasad R, Kaur G, Mond R, Walia BNS. Pediatr.Res.44:673-681, 1998). We now report the identification of p53 cDNA encoding the 50 kDa copper binding protein by isolating a cloned DNA complementary to copper binding protein mRNA. A basic local alignment research tool (BLAST) search of cDNA revealed that it belongs to Arg 72 variant of p53. Genetic analysis of p53 gene in 14 ICC patients divulged Arginine form of p53 at codon 72 in both homozygous and heterozygous forms. Immunofluorescence and Immunohistochemistry analysis documented a marked expression of p53 protein in ICC liver. p53 antibodies were also detected in the plasma of ICC patients. On functional characterization, expression of p53 cDNA transfected in Hep G2 cell lines was significantly augmented with increasing extra cellular copper concentration. On Immunohistochemistry, p53 was localized in both cytoplasm and nucleus of transfected Hep G2 cells. Marked apoptotic potential of Arg 72 p53 was demonstrated by increased expression of CD95 in ICC liver. Taken together, the findings of the present study deduce that Arg form of p53 at codon 72 is found in ICC patients and it gains marked apoptotic potential when copper concentration is increased which could be prominent predisposing genetic factor in the pathogenesis of ICC.

2324/T

Clinical, molecular and functional characterisation of autosomal recessive bestrophinopathy (ARB). *A.E. Davidson[1,2], I.D. Millar[3], P.D. Brown[3], A.T. Moore[1,4], G.C.M. Black[2], A.R. Webster[1,4], F.D.C. Manson[2].* 1) Genetics, Institute of Ophthalmology, UCL, London, United Kingdom; 2) Genetic Medicine, The University of Manchester, Manchester, United Kingdom; 3) Faculty of Life Sciences, The University of Manchester, Manchester, United Kingdom; 4) Moorfields Eye Hospital, London, UK.

We aim to characterise the clinical presentation and molecular pathophysiology of ARB.

BEST1 mutations were sought in patients with a diagnosis of ARB. Probands underwent ophthalmic examination, ISCEV-standard electrophysiology, autofluorescence imaging and spectral domain optical coherence tomography. Cellular localisation and protein stability of pathogenic bestrophin-1 isoforms was determined by immunofluorescence and the addition of proteasomal and lysosomal protease inhibitors followed by western blotting in a transiently transfected epithelial model system. Chloride channel function was measured by whole-cell patch-clamping in transiently transfected HEK293 cells.

All ARB patients had biallelic *BEST1* mutations. The ocular phenotype associated with ARB includes central visual loss, hyperautofluorescent confluent subretinal lesions, abnormal EOG light rise, abnormal full-field ERGs, and hyperopic refraction. This disorder is also often associated with angle closure glaucoma and cystoid macular oedema. Using an epithelial model system we demonstrate that ARB missense isoforms are mis-localised away from the basolateral cell membrane, displaying a perinuclear reticular pattern typical of the endoplasmic reticulum (ER). Mutant ARB isoforms also had reduced stability compared to wildtype bestrophin-1 and were rapidly degraded via a proteasomal dependant route. Whole-cell patch-clamping demonstrated that all ARB mutant isoforms investigated had a significantly reduced chloride conductance compared to wildtype bestrophin-1.

Patients with ARB have a distinct but variable autosomal recessive ocular phenotype. The majority of ARB bestrophin-1 isoforms are not correctly trafficked to the cell surface and are retained within the ER where they are degraded via a proteasomal dependant route, thus providing an explanation for the lack of chloride-specific currents. This work is the first comprehensive functional characterisation of missense bestrophin-1 isoforms that occur in either the homozygous or compound heterozygous states. The localisation and proteasomal data presented here suggests that ER associated degradation (ERAD) of mutant bestrophin-1 isoforms is the disease mechanism underlying most of the ARB genotypes, and supports our previous hypothesis that ARB represents the null bestrophin-1 phenotype in humans.

2325/T

A mutation in the 3'UTR of the HDAC6 gene abolishing the post-transcriptional regulation mediated by hsa-miR-433 is linked to a new form of dominant X-linked chondrodysplasia. *B. Arveiler[1,2], D. Simon[1], M. Barillot[1], V. Godard[1], N. Dagoneau-Blanchard[1], C. Blanchard[1], C. Rooryck[1,2], T. Barnetche[1], M. Marche[1], I. Burgelin[1], I. Coupry[1], B. Laloo[3], A. Tingaud-Sequeira[4], M. André[4], A. Knoll-Gellida[4], N. Chassaing[5,6], B. Gilbert-Dussardier[7], P. Babin[4], K. Massé[8], C. Grosset[3], D. Lacombe[1,2].* 1) Human Genetics Laboratory, Univ Victor Segalen Bordeaux 2, Bordeaux, France; 2) CHU de Bordeaux, Bordeaux, France; 3) INSERM U889, Bordeaux, France; 4) Laboratoire de Physiologie des Poissons, Bordeaux, France; 5) INSERM U563, Toulouse, France; 6) CHU de Toulouse, Toulouse, France; 7) CHU de Poitiers, Poitiers, France; 8) CNRS UMR 5164, Bordeaux, France.

A family with dominant X-linked chondrodysplasia was previously described. The disease locus was ascribed to a 24 Mb interval in Xp11.3-q13.1. We have identified a variant (c.*281A>T) in the 3'UTR of the HDAC6 gene that totally segregates with the disease. The variant is located in the seed sequence of hsa-miR-433. Our data showed that, in MG63 osteosarcoma cells, hsa-miR-433 down-regulated both the expression of endogenous HDAC6 and that of an eGFP-reporter mRNA bearing the wild-type 3'UTR of HDAC6. This effect was totally abrogated when the reporter mRNA bore the mutated HDAC6 3'UTR. The HDAC6 protein was found to be overexpressed in thymus from an affected male foetus. Concomitantly, the level of total alpha-tubulin, a target of HDAC6, was found to be increased in the affected foetal thymus, whereas the level of acetylated alpha-tubulin was found to be profoundly decreased. Skin biopsies were obtained from a female patient who presented a striking body asymmetry with hypotrophy of the left limbs. The mutated HDAC6 allele was expressed in 31% of left arm-derived fibroblasts, whereas it was not expressed in the right arm. Overexpression of HDAC6 was observed in left arm-derived fibroblasts. Altogether these results strongly suggest that this HDAC6 3'UTR variant suppressed hsa-miR-433-mediated post-transcriptional regulation causing the overexpression of HDAC6. This variant is likely to constitute the molecular cause of this new form of X-linked chondrodysplasia. This represents to our knowledge the first example of a skeletal disease caused by the loss of a miRNA-mediated post-transcriptional regulation on its target mRNA. We shall present our current work heading towards deciphering the physiopathology of the disease. Data presented will concentrate on the phenotype of fibroblasts expressing the mutated HDAC6 allele, on the expression pattern of HDAC6 during development in X. laevis, D. rerio and the mouse, and on the overexpression of HDAC6 in these models.

2326/T

Identification and functional consequences of a recurrent NLRP12 missense mutation in periodic fever syndromes. *I. Jéru[1, 2, 3], G. Le Borgne[1, 2], E. Cochet[3], H. Hayrapetyan[4], P. Duquesnoy[1], G. Grateau[5], A. Morali[6, 7], T. Sarkisian[4], S. Amselem[1, 2, 3].* 1) U.933, INSERM, Paris, France; 2) Université Pierre et Marie Curie-Paris6, UMR S_933, Paris, F-75012 France; 3) AP-HP, Hôpital Trousseau, Service de Génétique et d'Embryologie médicales, Paris, F-75012 France; 4) Center of Medical Genetics and Primary Health Care, Yerevan, 375010 Armenia; 5) AP-HP, Hôpital Tenon, Centre de référence Amyloses d'origine inflammatoire et Fièvre méditerranéenne familiale, Service de Médecine Interne, Paris, F-75020 France; 6) Centre Hospitalier-Universitaire Nancy-Brabois, Hôpital d'Enfants, Centre de compétence des maladies digestives rares et inflammatoires de l'enfant, Vandoeuvre-les-Nancy, F-54500 France; 7) INSERM, U954, Vandoeuvre-Les-Nancy, F-54505 France.

Introduction. Recently, two first disease causing-mutations (a nonsense and a splice site mutations) have been identified in the NLRP12 gene in patients with a clinical manifestations evocative of cryopyrinopathies, which correspond to a subgroup of periodic fever syndromes (PFS). The diagnosis of PFS remains often difficult since there is no pathognomonic sign of these disorders, and since the majority of patients corresponds to sporadic cases precluding intrafamilial segregation analyses. The majority of mutations identified in PFS genes corresponds to missense variations, whose deleterious effect remain often questionable. In addition, since PFS genes are expressed at low levels in peripheral blood mononuclear cells, the effect of sequence variants can hardly be assessed by the study of endogenous proteins. To further test the involvement of NLRP12 in PFSs, we screened this gene in patients with symptoms of genetically-unexplained cryopyrinopathies. The functional consequences of the identified missense variation was subsequently assessed. Methods. NLRP12 was screened for mutations by direct sequencing. Functional assays were performed in HEK293T cells stably expressing the pro-apoptotic protein ASC and pro-caspase 1, in order to determine the consequences of normal and mutated NLRP12 proteins on speck formation, caspase 1 signaling, and NFKB activation. Results. A heterozygous NLRP12 missense mutation involving a CpG site (c.1054C/T, p.Arg352Cys) was identified in exon 3, which encodes the nucleotide-binding site (NBS) of the protein, in two patients originating from different countries and carrying different NLRP12 haplotypes. The mutation, which does not alter the inhibitory properties of NLRP12 on NFKB activation, increases speck formation and activates caspase 1 signaling. Conclusions. Given the rarity of known NLRP12-associated disorders, the identification of this new NLRP12 molecular defect contributes to the delineation of the clinical spectrum associated with mutations in this gene and underlines the importance to screen NLRP12 in patients presenting with unexplained PFSs. This study also demonstrates by means of functional assays the deleterious effect of this recurrent missense mutation; in this regard, the gain of function associated with this NBS mutant on speck formation and caspase 1 signaling is consistent with the patients' inflammatory phenotype.

2327/T

Defining the phenotypic specificity of Schimke immuno-osseous dysplasia: A hypothesis. *M. Morimoto[1,2], K.S. Cho[3,4], C. Myung[1], K. Beirnes[1], D. Leung[1], A. Fam[1], K. Choi[1,2], Y. Huang[1], C. Huang[3], S. Lou[3], A.K. Gormley[5], Z. Yu[6], D. Parham[6], B. Najafian[7], C.F. Boerkoel[1,2].* 1) Child and Family Research Institute, Department of Medical Genetics, University of British Columbia, Vancouver, BC Canada; 2) Rare Disease Foundation, Vancouver, BC Canada; 3) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 4) Department of Biological Sciences, Konkuk University, Seoul, Republic of Korea; 5) Department of Pediatrics, University of Oklahoma, Oklahoma City, OK; 6) Department of Pathology, University of Oklahoma, Oklahoma City, OK; 7) Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN.

Objective: Schimke immuno-osseous dysplasia (SIOD), an autosomal recessive multisystem disorder, is caused by mutations in the *SMARCAL1* gene. Although the SMARCAL1 enzyme binds open chromatin diffusely and affects transcription globally, we hypothesize that its deficiency gives rise to the pathognomic features of renal failure, skeletal dysplasia, immunodeficiency, and vascular disease by differential effects on gene expression.

Methods: We assessed this using an F_1 genetic screen in *Drosophila melanogaster* to measure suppression or enhancement of ectopic wing veins induced by the overexpression of SMARCAL1. Furthermore, using RT-PCR and immunoblotting, we assessed expression of key pathways in SIOD autopsy tissue and following knock down of SMARCAL1 in cultured primary cells.

Results: In the *Drosophila* model, mutations in the wingless and epidermal growth factor pathways strongly suppressed, mutations of the notch pathway strongly enhanced, and mutations in the decapentaplegic and hedgehog pathways had no effect or weakly suppressed the ectopic wing veins providing evidence that SMARCAL1 interacts differentially with developmental pathways. Furthermore, gene expression analysis in flies and SIOD patient tissues identified differentially altered gene expression. Specifically, expression of elastin in the arterial wall and lung was significantly altered and correlated with arterial and pulmonary pathology.

Conclusion: Similar to mutations of *RNA polymerase II* and general transcription factors, dysfunction of SMARCAL1 alters the expression of some genes more than others and in turn affects tissue development and maintenance. We show for the first time that alterations in gene expression cause SIOD. We hypothesize that this arises because some regions of the genome are more sensitive to SMARCAL1 dysfunction than are others.

2328/T

Defects of embryonic myosin in Freeman-Sheldon syndrome reduce force and prolong relaxation of skeletal myofibers. *A.E. Beck[1,2], A.W. Ward[3], M.J. McMillin[1,2], F.S. Korte[3], M. Regnier[3], M.J. Bamshad[1,2,4].* 1) Dept Pediatrics, Univ Washington, Seattle, WA; 2) Seattle Children's Hospital, Seattle, WA; 3) Dept Bioengineering, Univ Washington, Seattle, WA; 4) Dept Genome Sciences, Univ Washington, Seattle, WA.

Congenital contractures such as clubfoot affect ~1 of every 200-500 live births in the United States and are caused by a combination of genetic and environmental risk factors. To understand the genetic and biophysical mechanisms that cause congenital contractures, we studied single skeletal myofibers from a person with distal arthrogryposis (DA). The DAs are a group of ten autosomal dominant disorders characterized by congenital contractures of the hands and feet, most notably clubfoot and camptodactyly. We and others have discovered that DA syndromes can be caused by mutations in each of at least nine genes that encode proteins of the skeletal muscle contractile complex including: troponin I, troponin T, tropomyosin, myosin binding proteins and several myosin heavy chains. Here we report the first functional studies of skeletal myofibers from a person with congenital contractures caused by a defect of embryonic myosin. Specifically, we measured the contractile properties of individual myofibers sampled from the gastrocnemius muscle of an individual with Freeman-Sheldon syndrome (FSS) caused by a R672C *MYH3* mutation. Maximal force per cross-sectional area was reduced by more than 70% in FSS myofibers, but maximal force per myofiber was maintained as FSS myofibers were larger than control fibers. Neither cooperativity of activation nor calcium sensitivity of contraction was significantly affected. Following calcium-induced activation, time to 90% relaxation was ten-fold longer in FSS myofibers, and this effect was independent of myofiber diameter or fiber type. Relaxation time could be restored toward normal by chemically disrupting myosin-actin interactions. These observations can be collectively explained by slower turnover of actin-myosin crossbridges in FSS myofibers. Furthermore, while the expression of *MYH3* has been thought to be limited to fetal development and shortly thereafter, we found that *MYH3* mRNA and embryonic myosin protein are present in both normal fetal and adult skeletal muscle. This finding suggests that mutations in *MYH3* could affect contractility in both prenatal and adult skeletal muscle. Elucidating the mechanism of aberrant relaxation could provide important information leading to development of novel therapeutics that either mitigate severe outcomes or potentially prevent the development of contractures.

2329/T

***SLC2A13* loss of function associated with failure to thrive and developmental delay.** *C. Dias[1,2,3], J.L. Huang[1,2,3], A. Lehman[1,2,3], C. du Souich[1,2,3], S. Bhatt[4], D. Chai[1,2], P. Eydoux[1,2], P. Stankiewicz[4], C.F. Boerkoel[1,2,3].* 1) Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia; 2) Child and Family Research Institute, Children's and Women's Health Centre of British Columbia, Vancouver, British Columbia, Canada; 3) Rare Disease Foundation, Vancouver British Columbia, Canada; 4) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

The combination of profound developmental delay, failure to thrive, and epilepsy is a diagnostically challenging clinical presentation. Causes for this triad uncover insights into the biology of human growth and cognition. We present a 3 y. o boy with hypotonia, severe failure to thrive, severe developmental delay, nonspecific dysmorphic features, and seizures causing hemiparesis. He had normal metabolic and endocrine studies. Brain MRI showed normal anatomy and delayed myelination. Chromosome analysis and array comparative genomic hybridization revealed a de novo balanced translocation t(6;12)(q25.1;q12). Subsequent, analyses refined the breakpoint to intron 4 of the SLC2A13 gene, encoding a membrane channel in the Golgi apparatus, and intron 6 of LOC729178, which expresses a noncoding mRNA. Further RT-PCR studies showed nearly complete loss of mRNA transcription from the untranslocated allele of SLC2A13. Current studies focus on defining the basis for loss of expression from the normal allele. Based on its expression pattern and predicted function, we propose that deficiency of SLC2A13 causes growth restriction and delayed neurological development by affecting vesicular trafficking or protein modification. This is the first description of SLC2A13 deficiency in humans.

2330/T

ZNF750 is a nuclear protein with novel variants in its promoter & 5' UTR in psoriasis patients. *I. Cohen[1], R.Y. Birnbaum[1], A.M. Bowcock[2], K. Leibson[1], S. Sivan[1], O.S. Birk[1,3].* 1) Morris Kahn Laboratory of Human Genetics, National Institute for Biotechnology in the Negev, Ben Gurion University, Beer-Sheva, Israel; 2) Department of Genetics, Washington University School of Medicine, Saint Louis, MO, United States; 3) Genetics Institute, Soroka Medical Center, Beer-Sheva, Israel.

We previously showed that a dominant mutation in the novel zinc finger protein, ZNF750, that is located within PSORS2 locus, causes seborrhea-like dermatosis with psoriasiform elements. Furthermore, ZNF750 c.-625A>C promoter variant has been linked with familial psoriasis in Chinese patients. We now sequenced DNA samples of 250 bone-fide psoriasis patients. No mutations were found in the ZNF750 coding sequence. However, sequence variants in the ZNF750 5'-UTR were found in 3 of 250 unrelated psoriasis patients and not in 300 controls. It is yet unclear how this variation affects ZNF750 expression. To begin to understand ZNF750 function, we determined ZNF750 sub-cellular localization: HEK293 and HaCaT cells were transiently transfected with EGFP-ZNF750 constructs of full-length or of partially truncated protein, abrogating the ZNF750 NLS. Both confocal fluorescence microscopy and cellular fractionation followed by western blot analysis demonstrated that ZNF750 is expressed in the cell nucleus both in HEK293 and HaCaT cells. Removal of the nuclear localization signal of ZNF750 abrogated its nuclear localization. Our data suggest that ZNF750 5'-UTR variants might be associated with bone fide psoriasis. Although ZNF750 has a single zinc finger C2H2-like motif, its nuclear localization suggests that it might act as a transcription factor.

2331/T

Haploinsufficiency for the erythroid transcription factor KLF1 causes Hereditary Persistence of Fetal Hemoglobin. *M.A. GEORGITSI[4], J. BORG[1, 2], P. PAPADOPOULOS[3], L. GUTIERREZ[3], G. GRECH[1, 2], P. FANIS[3], M. PHYLACTIDES[5], A.J.M.H. VERKERK[6], P.J. VAN DER SPEK[6], C.A. SCERRI[1, 2], W. CASSAR[1, 2], R. GALDIES[1, 2], W. VAN IJCKEN[7], Z. OZGUR[7], N. GILLEMANS[3], J. HOU[3, 8], M. BUGEJA[1, 2], F.G. GROSVELD[3, 8, 9], M. VON LINDERN[10], A.E. FELICE[1, 2], G.P. PATRINOS[4], S. PHILIPSEN[3, 8].* 1) Laboratory of Molecular Genetics, Department of Physiology & Biochemistry, University of Malta, Msida, MSD 2080, Malta; 2) Thalassaemia Clinic, Section of Pathology, Mater Dei Hospital, Msida, MSD 2080, Malta; 3) Erasmus Medical Center, Department of Cell Biology, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands; 4) University of Patras, Department of Pharmacy, University Campus, Rio 26504, Patras, Greece; 5) Cyprus Institute of Neurology and Genetics, P.O. Box 23462, 1683 Nicosia, Cyprus; 6) Erasmus Medical Center, Department of Bioinformatics, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands; 7) Erasmus Medical Center, Center for Biomics, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands; 8) Netherlands Consortium for Systems Biology, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands; 9) Center for Biomedical Genetics, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands; 10) Erasmus Medical Center, Department of Hematology, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands.

Hereditary Persistence of Fetal Hemoglobin (HPFH) is characterized by persistence of fetal hemoglobin (HbF, $\alpha_2\gamma_2$) above 2% in adults. A few genetic loci account for the variable expression of total HbF levels, including *HBB*, *HBS1L-MYB*, and the recently identified γ-globin genes repressor *BCL11A*, however they account for <50% of cases. Ten of 29 members from a unique Maltese family presented with HPFH (HbF: 3%-20%). Genome-wide SNP array and linkage analysis data, assuming autosomal dominant inheritance, were combined with expression profile data of HPFH and normal primary erythroid progenitors (HEPs). A candidate region was mapped on chromosome 19p13.12-13. Sequencing analysis of candidate genes identified a nonsense mutation in the *KLF1* gene, p.K288X, ablating the DNA binding domain of this key erythroid transcriptional regulator. Only HPFH family members were p.K288X carriers; p.K288X was absent in the general Maltese population. Expression profiling of HPFH HEPs revealed downregulation of KLF1 target genes, including *BCL11A*, as previously seen in *Klf1 null* mouse erythroid progenitors. Lentiviral shRNA-mediated knockdown of *KLF1* in normal HEPs, followed by quantitative S1 nuclease protection assays and qPCR, showed a marked increase of *HBG1*/*HBG2* and downregulation of *BCL11A* expression, similar to the observations in HPFH carriers. Lentiviral-mediated transduction of normal HEPs with mutant (p.K288X) *KLF1* did not affect *HBG1*/*HBG2* expression level, excluding a dominant-negative role. Transduction of HPFH HEPs with full-length *KLF1* resulted in a marked increase of BCL11A protein and decrease of *HBG1*/*HBG2* mRNA levels. Chromatin immunoprecipitation assays on fetal liver HEPs and adult peripheral blood HEPs revealed consistent binding of KLF1 on one of the potential CACC sites on the proximal *BCL11A* promoter only in the adult stage. In conclusion, in addition to its established role in adult globin expression via the activation of *HBB*, KLF1 is a critical activator of the *BCL11A* gene, which represses fetal *HBG1*/*HBG2* expression in adults. These findings provide a rationale for the observed effects of *KLF1* haploinsufficiency on HbF levels and support the notion that KLF1 is a regulator of human fetal-to-adult globin switching. Since HbF mitigates the severity of both β-thalassemia and sickle-cell disease, the reactivation of *HBG1*/*HBG2* genes in adults, via attenuation of KLF1 activity, provides novel lead for molecular therapeutics of β-type hemoglobinopathies.

2332/T

Investigation of the effects of SLC4A11 (CHED2) gene depletion on corneal endothelial cell growth and proliferation. *J. Liu[1], L.W. Koh[1], R. Mohan[2], T. Aung[1,3,4], E.N. Vithana[1,3].* 1) Singapore Eye Research Institute, Singapore; 2) Mason Eye Institute and College of Veterinary Medicine, University of Missouri-Columbia, Missouri, USA; 3) Dept of Ophthalmology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; 4) Singapore National Eye Centre, Singapore.

NaBC1 protein, encoded by SLC4A11 gene, has been shown to function as an electrogenic Na/Borate co-transporter in the presence of borate in mammalian cells. In 2006, Vithana et al identified the SLC4A11 gene as the cause of a blinding cornea dystrophy, Congenital Hereditary Endothelial Dystrophy (CHED2). Herein, we aimed to identify the cellular characteristics and the gene regulation events involved in CHED2 pathology by depleting SLC4A11 in immortalized human corneal endothelial cell line (HCEC). In 2004 Park et al found reduced cell growth and proliferation in HeLa cells after 48 and 72 hours' treatment of transient siRNA specific for SLC4A11. In contrast, we found that SLC4A11 gene could not be depleted at the protein level in HCECs by transient treatment with siRNAs. Gene depletion could only be achieved by long-term and stable expression of siRNA via short-hairpin RNA (shRNA). Real-time assessment of cell growth using the XCELLigence system (Roche) consistently indicated the reduced cell number with time in SLC4A11 gene-depleted cells compared to the control. However, Ki-67 immunostaining indicated that cell proliferation is not significantly different between cells that stably express SLC4A11 targeted siRNAs and the negative control. After further analysis, we conclude that the reduction of cell number with time in SLC4A11-depleted HCECs is mainly due to the increase in cell death. We also investigated the apoptotic signaling pathways that were affected by depletion of SLC4A11 in HCECs. And we studied the role of borate on growth and proliferation in HCECs. Park et al previously demonstrated the importance of borate on growth of HeLa cells through the rescue of cell growth and proliferation by incubating siRNA treated cells with 0.5mM borate. In our study, the HCEC phenotypes due to SLC4A11 gene depletion could not be rescued by treatment with 0.5mM borate. Thus our data do not support a critical role for either borate or NaBC1 as a Na/Borate cotransporter in HCECs. NaBC1/SLC4A11 may have a different transporter role in HCECs. The Knowledge gained from this study may possibly lead to the identification of therapeutic targets and reagents applicable for CHED as well as a wide group of corneal diseases that essentially involve the maintenance/restoration of corneal clarity.

2333/T

Bioinformatics and Cellular Approaches Confirm ER-Associated Protein Degradation (ERAD) as a Major Mechanism Underlying Numerous Monogenic Disorders. *B.R. Ali[1], N.A. Akawi[1], A. John[1], L. Al-Gazali[2].* 1) Department of Pathology, Faculty of Medicine and Health Sciences, UAE University, Al-Ain, Abu Dhabi, United Arab Emirates; 2) Department of Pediatrics, Faculty of Medicine and Health Sciences, UAE University, Al-Ain, Abu Dhabi, United Arab Emirates.

More than a third of all cellular proteins are targeted to the endoplasmic reticulum (ER) as a first step in their trafficking routes along the secretory pathway to their final cellular destinations. The processes of protein folding, assembly into multi-subunit complexes and export out of the ER are subjected to a stringent quality control system to ensure that only properly folded and assembled proteins exit the ER. Malfolded proteins and unassembled subunits of protein complexes are rejected by this ER quality control system and re-translocated to the cytosol for degradation by the ubiquitin/proteasome. This process has been named ERAD (ER-Associated protein Degradation) and has been implicated in the cellular mechanisms of at least 50 human monogenic diseases including cystic fibrosis. Due to the high stringency of ERAD and the large number of cellular proteins that has to pass through the secretory pathway (~8,000), we reasoned that ERAD should be implicated in the mechanisms of many more human monogenic conditions. We therefore, utilized bioinformatics and data mining approaches and found that at least 45% of all known human disease genes have an ER-targeting signal and consequently we identified many ERAD disease candidates. We experimentally validated our predictive approach experimentally by establishing that ERAD is indeed responsible for most of the loss-of-function missense mutations in the genes involved in a number of the identified ERAD disease candidates including Robinow syndrome, Acromesomelic Dysplasia type Maroteaux and SMED-SL.

2334/T

Genotypic and phenotypic diversities in Wilson's disease. *B.H. Lee[1,2,3], J.H. Kim[2], K.J. Kim[2], J.Y. Park[2], G.H. Kim[2,3], H.W. Yoo[1,2,3].* 1) Pediatrics, University of Ulsan College of Medicine, Asan Medical Center Children's Hospital, Seoul, Korea; 2) Genome Research Center for Birth Defects and Genetic Disorders, University of Ulsan College of Medicine, Asan Medical Center Children's Hospital, Seoul, Korea; 3) Medical Genetics Clinic and Laboratory, University of Ulsan College of Medicine, Asan Medical Center Children's Hospital, Seoul, Korea.

Wilson's disease (WD) is an autosomal recessive disorder of copper metabolism. Wide genotypic-phenotypic heterogeneities have been reported, hampering the study on their correlations. This study was performed to identify factors related to genotypic-phenotypic diversities of WD in 237 unrelated Korean WD families. Presenting phenotypes were classified as hepatic (H1, H2), neurological (N1, N2, NX), and asymptomatic (ASx), modifying the guidelines by Ferenci et al *(Liver Int 2003;23:139-42)*. Presenting phenotypes in our cohort were H1 (12.2%), H2 (42.4%), N1 (21.6%), N2 (0.4%), NX (0.4%), ASx (22.4%), and other (0.4%). Onset age was youngest in patients with ASx presentation, but similar among H1, H2 and N1 patients. Liver cirrhosis was associated in 63% of N1 patients, similar as in H1+H2 patients (49%). On follow-up (average, 8.2±5.8 years), liver cirrhosis was rarest in ASx patients (4%). Decompensated cirrhosis was highest in H1 (48%) but similar between N1 (2%) and H2 (5%) patients. Favorable outcome was rarest in N1 patients (8%). Forty-seven *ATP7B* mutations including 11 novel mutations were identified in 85% of the 474 alleles. MLPA assay in *ATP7B* and analyses of *ATOX1* and *COMMD1* identified no additional mutations. Yeast complementation and growth curve assays demonstrated functional perturbation of the seven novel missense mutants. Five major mutations, p.Arg778Leu (36.5%), p.Ala874Val (9.9%), p.Asn1270Ser (8.0%), p.Lys838SerfsX35 (4.2%), and p.Leu1083Phe (4.0%), accounted for 63% of the alleles. H1 was more common (26.8% vs. 8.5%, $P = 0.006$), onset age was younger (12 vs. 15 years, $P = 0.009$), and N1+N2+NX tended to be less common (12.2% vs. 27.0%, $P = 0.06$), in patients with nonsense, frame-shifting or splicing mutations than in those with missense mutations alone. No other genotype-phenotype correlations were identified among common mutations or genotypes. In addition, widely heterogeneous presenting phenotypes were noted in patients with both mutations in a single domain of ATP7B. In conclusion, the presenting phenotype strongly affects the clinical outcome of WD, and is related, in part, to mutation type. Several common mutations unique to those of particular ethnicity should be identified to allow rapid and easy diagnosis of WD without the need for invasive intervention. More effort is needed to identify genetic defects in those without *ATP7B* mutation and the factors related to genotypic-phenotypic heterogeneity.

2335/T

Hirschsprung's disease and common variants in genes involved in enteric neural crest cell proliferation and migration. *T. Carter[1], J. Troendle[1], D. Kay[2], M. Browne[3,4], P. Romitti[5], M. Conley[1], D. Kuehn[1], M. Caggana[2], C. Druschel[3,4], L. Brody[6], J. Mills[1].* 1) Division of Epidemiology, Statistics, and Prevention Research, Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH, Bethesda, MD; 2) Division of Genetics, Wadsworth Center, New York State Dept of Health, Albany, NY; 3) Dept of Epidemiology and Biostatistics, School of Public Health, University at Albany - State University of New York, Albany, NY; 4) Congenital Malformations Registry, New York State Dept of Health, Troy, NY; 5) Dept of Epidemiology, The University of Iowa College of Public Health, Iowa City, IA; 6) Genome Technology Branch, National Human Genome Research Institute, NIH, Bethesda, MD.

INTRODUCTION: Hirschsprung's disease results from failure of neural crest cells to colonize the embryonic gut. The differentiation and survival of neural crest-derived enteric neurons require RET signaling. Rare mutations in RET are present in familial (50%) and non-familial (15%) cases but common RET variants have also been associated with Hirschsprung's disease. The aim of this study was to determine if common variants in other genes that regulate enteric neural crest cell proliferation and migration also play a role in Hirschsprung's disease. We examined single nucleotide polymorphisms (SNPs) in candidate genes (ASCL1, HOXB5, PHOX2B, PROK1, PROKR1) which, when mutated in animals, block embryonic enteric neural crest cell proliferation and migration. METHODS: We conducted a nested case-control study of 305 Hirschsprung's disease cases 1,216 controls selected from among all New York State live births between 1998 and 2005. Cases were identified from the New York State Congenital Malformations Registry and had no other major birth defects. Controls without major malformations were randomly selected and frequency-matched to cases by race/ethnicity. DNA was genotyped for 33 haplotype tagging SNPs in the five candidate genes and for five SNPs in RET. Logistic regression was used to calculate odds ratios (OR) and 95% confidence intervals (CI). RESULTS: PROKR1 rs4854479 was associated with being a case in non-Hispanic whites (168 cases, 668 controls) (OR: 2.08; 95% CI: 1.1, 4.1). Among Hispanic subjects (50 cases, 198 controls), associations were found for HOXB5 rs4793943 (OR: 5.6; 1.2, 26.1) and PHOX2B rs6826373 (OR: 2.6; 1.1-6.1). RET rs1864410 was strongly associated with Hirschsprung's disease among non-Hispanic white (OR: 11.9; 7.2, 19.7), Hispanic (OR: 21.0; 6.1, 72.2), and Asian (23 cases, 92 controls) (OR: 13.1; 2.7, 63.4) subgroups. These ORs represent effect estimates for subjects who were homozygous for the minor allele compared with those homozygous for the major allele. CONCLUSIONS: In addition to RET, common variants in genes regulating enteric neural crest cell proliferation and migration were associated with Hirschsprung's disease. Gene expression data in animals and humans suggest that the proteins encoded by these genes could modulate RET signaling. Our findings require confirmation but they suggest that other genes affecting enteric neural crest cell proliferation should be investigated as contributors to Hirschsprung's disease.

2336/T

Identification of a deletion of the entire *CTRC* gene in a patient with familial chronic pancreatitis. *E. Masson[1,2,4], J.M. Chen[1,3,4], P. Hammel[5], C. Le Maréchal[1,2,3,4], C. Férec[1,2,3,4].* 1) Institut National de la Santé et de la Recherche Médicale (INSERM), U613 "Génétique moléculaire et Génétique épidémiologique", Brest, France; 2) Laboratoire de Génétique Moléculaire et d'Histocompatibilité, Centre Hospitalier Régionale Universitaire (CHRU) Brest, Hôpital Morvan, Brest, France; 3) Etablissement Français du Sang (EFS) - Bretagne, Brest, France; 4) Université de Bretagne Occidentale (UBO), Faculté de Médecine et des Sciences de la Santé, Brest, France; 5) Pôle des Maladies de l'Appareil Digestif, Service de Gastroentérologie-Pancréatologie, Assistance Publique-Hôpitaux de Paris, Hôpital Beaujon, Clichy, France.

Introduction: Chronic pancreatitis (CP) is a relapsing inflammatory disease of the pancreas. More than a century ago, pancreatitis was thought to be an autodigestive disease. This hypothesis was given the strongest support in 1996, when a "gain of function" mutation in the cationic trypsinogen gene (*PRSS1*) was reported to cause hereditary pancreatitis. Thereafter, "loss of function" mutations in the trypsin's inhibitor gene (*SPINK1*) and, more recently in the *CTRC* (Chymotrypsin C) gene, have also been found to be associated with the disease. These mutations are thought to disrupt the trypsin-inhibitor balance, leading to trypsin-driven premature digestive zymogene activation cascade and pancreatic autodigestion. **Aim of the study**: Copy number variations (CNVs) in the *SPINK1* and *PRSS1* genes (large deletions and gain of copy number respectively), have been previously found to cause pancreatitis. In this study, we attempted to identify this kind of mutation in the *CTRC* gene. **Materials and methods**: The eight exons of the *CTRC* gene were screened by means of quantitative fluorescent multiplex PCR. A total of 223 unrelated CP patients including 37 patients with a positive family history and 186 young patients with idiopathic CP, participated in this study. All were recruited after our previously study published in 2008 and no *PRSS1*, *SPINK1* or *CTRC* clearly disease-causing mutations were found among them. **Results**: We have identified a large heterozygous deletion encompassing the entire *CTRC* gene in one patient with familial CP. Direct sequencing of the aberrant chromosomal junction characterized the deletion as being ~54 kb in length, with the insertion of a short Alu sequence of ~ 30 bp. Moreover, in this patient, the *CTRC* deletion was found to be co-inherited with the p.N34S variant in the unlinked *SPINK1* gene. In the context of this particular family, the *CTRC* deletion may constitute a clearly disease-causing factor, whereas the *SPINK1* variant probably acts as a disease modifier. Cascade screening of the other family members and further investigation of the genotype/phenotype relationship may shed some light on this question. **Conclusion**: This is the first time that a genomic event involving the *CTRC* gene was found to cause chronic pancreatitis.

2337/T

A linkage study for Gallbladder Disease in a South Tyrolean Population. *A. De Grandi[1], C.B. Volpato[1], C. Pattaro[1], M. Felder[4], A.A. Hicks[1], P.P. Pramstaller[1,2,3].* 1) Institute of Genetic Medicine, European Academy Bolzano, Bolzano, BZ, Italy; Affiliated Institute of the University Lübeck, Germany; 2) Department of Neurology, Central Hospital, Bolzano, Italy; 3) Department of Neurology, University of Lübeck, Germany; 4) Department of Gastroenterology, Central Hospital, Bolzano, Italy.

Gallbladder disease (GBD) is one of the major digestive diseases. The significant risk factors associated with GBD are age, gender (female), obesity, lipids, diet, parity, type 2 diabetes, medications, and ethnicity. GBD appears to be strongly related to the metabolic syndrome. Most people with gallstones, however, remain asymptomatic through their lifetimes. Inhabitants of an isolated village in South Tyrol (North Italy) were identified through a first screening phase as individuals putatively affected by cholelitiasis. They and their first-degree relatives were called for ultrasonic examination by a gastroenterology specialist and 45% of participants were diagnosed as having of gallstones, despite the majority being asymptomatic. We have reconstructed the pedigrees starting from the affected individuals through the collection in the parish archive of genealogical data of 15 generations. Different pedigree splitting parameters were used to generate either two sets of large pedigrees (444 persons in 8 pedigrees with 59 affected, and 349 persons in 17 pedigrees with 61 affected) for Simwalk 2 analysis and those of more modest sizes (320 persons in 16 pedigrees with 100 affected) for Merlin analysis. Despite changes in signal levels due to different power in the tree sets of pedigrees, overlapping linked regions on chromosomes 3, 5, 6 and 8 were observed between analyses. The maximum significant linkage values were reached using the largest pedigrees. We are currently investigating association signals within the linked regions to identify genes of interest. One such compelling candidate in one of the linked regions is one of the organic solute transporters (OST alpha). Expression of OST alpha has been reported to be significantly reduced (3.3-fold, P = 0.006) in normal-weight but not overweight gallstone carriers compared with controls, leading us to speculate that underlying mutations in this gene might contribute to GBD in this population.

2338/T

Ablation of Sbds in the murine pancreas leads to Shwachman-Diamond syndrome phenotypes. *M.E. Tourlakis[1,2], J. Zhong[2], L.L. Chen[1,2], S. Zhang[1,2], P.R. Durie[3,4], J.M. Rommens[1,2].* 1) Department of Molecular Genetics, University of Toronto, Toronto, ON; 2) Program in Genetics and Genomic Biology, Hospital for Sick Children, 101 College St. East Tower, Toronto, ON, Canada M5G 1L7; 3) Department of Pediatrics, University of Toronto, Toronto, ON; 4) Program in Physiology and Experimental Medicine, Hospital for Sick Children, Toronto, ON.

The autosomal recessive disorder Shwachman-Diamond syndrome (SDS) involves multiple systems, with exocrine pancreatic dysfunction being a major feature. More than 95% of SDS patients exhibit loss of function mutations in the essential *SBDS* gene.

We modeled SDS in the murine pancreas to understand the consequences of loss of Sbds. Mice with null or disease-associated missense (*R126T*) *Sbds* alleles in combination with a conditional allele (CKO)were investigated for pancreatic dysfunction during development. Conditional inactivation was achieved with *Cre* recombinase under control of the Ptf1a early pancreatic progenitor transcription factor. $Sbds^{CKO/R126T}$;$Ptf1a^{Cre/+}$ and $Sbds^{CKO/-}$;$Ptf1a^{Cre/+}$ pancreata displayed progressive dysplasia postnatally. Trichrome staining of mutant pancreata indicated prominent connective tissue at pre-weaning periods, with fewer total acinar cell numbers and fat infiltration at post-weaning periods, resembling presentation in SDS patients. The pancreas remained small at all time points in both models; at P30, pancreata of $Sbds^{CKO/-}$;$Ptf1a^{Cre/+}$ mice were 0.31% of total body mass compared to 0.58% of control littermates. Acinar components appeared disorganized with tubular complexes. Notably, amylase-negative cells were observed but apoptotic bodies were not evident. Consistent with clinical findings, endocrine components were spared based on insulin and glucagon immunohistochemical staining, as well as glucose tolerance testing at 6 months of age. Mutant animals did show a modest reduction in total body size suggesting nutritional deprivation.

We show that Sbds is required for normal postnatal development of the pancreas and that its loss is sufficient to recapitulate the dysfunction seen in this organ in SDS. Moreover the clear genotype-phenotype correlation of the two models confirms that the *R126T* allele is hypomorphic in nature. A building body of evidence suggests that SBDS has a role in ribosome biogenesis and/or translation. Given the level of specialization of the exocrine pancreatic proteome, we propose that acinar cells are acutely sensitive to translation disruption in the absence of functional SBDS/Sbds.

2339/T

Founder effect of glucose-galactose malabsorption in Old Order Amish. *B. Xin, A. Bright, H. Wang.* DDC Clinic for Special Needs Children, Middlefield, OH.

Glucose-galactose malabsorption (GGM) is a rare autosomal recessive disease caused by a defect in glucose and galactose transport across the intestinal brush border. Clinically, GGM is manifested by neonatal onset of watery and acidic severe diarrhea, which is fatal unless these sugars are removed from the diet. Mutations in the Na+/glucose cotransporter gene SLC5A1 have been linked with the disease. Here, we report thirty-three patients with congenital GGM from a large Old Order Amish pedigree and the associated mutations in SLC5A1 gene. Besides previously reported clinical features such as classic watery diarrhea and dehydration, we found that increased bowel sounds, distended abdomen, vigorous nursing regardless of their illness, and irritability and apathy were also part of the initial presentation in all affected individuals. The patients underwent a dramatic turnaround with an immediate cease of the diarrhea and a quick rehydration if they were correctly diagnosed and adequately managed, followed by a normal growth and development pattern afterwards; whereas a prolonged clinical course would follow if the disease was not recognized. Sequence analysis of SLC5A1 gene revealed four homozygous missense mutations, c.152A>G (p.N51S), c.1231G>A (p.A411T), c.1673G>A (p.R558H), and c.1845C>G (p.H615Q), that co-segregate with the GGM phenotype in all of the affected individuals. Neither unaffected individuals in the pedigree nor 100 ethnically matched healthy controls were homozygous for any of these mutations. These findings suggest founder effect of the SLC5A1 mutations associated with the disease in Amish and a population specific genetic testing is in need to pursue an early diagnosis which is critical for a favorable outcome.

2340/T

Identification of the causative gene for Congenital Short Bowel Syndrome. *C.S. van der Werf[1], J. Paredes[2], N. Hsiao[3], T.D. Wabbersen[4], H.C. Etchevers[5], P.M. Kroisel[6], D. Tibboel[7], R.A. Schreiber[8], E.J. Hoffenberg[9], I. Ceccherini[10], S. Lyonnet[5], R. Seruca[2], G.J. te Meerman[1], S.C.D. van Ijzendoorn[3], I.T. Shepherd[4], J.B.G.M. Verheij[1], R.M.W. Hofstra[1].* 1) Genetics, University Medical Center Groningen, Groningen, Groningen; 2) IPATIMUP - Institute of Molecular Pathology and Immunology of the University of Porto, Portugal; 3) Department of Cell Biology, University Medical Center Groningen, The Netherlands; 4) Department of Biology, Emory University, Atlanta, USA; 5) INSERM, U781, Hôpital Necker-Enfants Malades, Paris, France; 6) Institute of Human Genetics, Medical University of Graz, Graz, Austria; 7) Department of Pediatric Surgery, Erasmus MC-Sophia Children's Hospital, PO Box 2060, 3000 CB, Rotterdam, The Netherlands; 8) Division of Gastroenterology, BC Children's Hospital, Rm K4-200, 4480 Oak Street, Vancouver, BC V6H 3V4; 9) Department of Pediatrics, Section of Pediatric Gastroenterology, Hepatology, and Nutrition, University of Colorado, Denver, CO, USA; 10) Laboratorio di Genetica Molecolare, Istituto Giannina Gaslini - 16148 Genova, Italy.

Congenital Short Bowel Syndrome is a rare gastrointestinal disorder characterized by an inherited markedly diminished short small intestine length at birth and malrotation. 60% of the cases are familial, as siblings are affected. Because boys and girls are both affected and in 25% of the cases the parents are consanguineous, an autosomal recessive pattern of inheritance has been suggested. The aim of our study is to identify and characterize the causative gene. Homozygosity mapping was performed using 610K SNP arrays of Illumina on 5 patients of 4 different families, including one consanguineous family with 2 affected siblings and one unaffected child. We found an overlapping homozygous region in 4 of the 5 patients. In this region a homozygous deletion concerning one exon of one specific gene was detected in one of the patients. Furthermore, a homozygous deletion in the first intron was detected in the affected siblings of the consanguineous family, this deletion co-segregates with the disease phenotype in this family. Sequencing of this gene in 3 other patients resulted in the identification of additional mutations: one patient proved to have a heterozygous frameshift mutation and a heterozygous splice site mutation, whereas 2 other patients were homozygous for a nonsense mutation and a missense mutation, respectively. To further analyze the effect of the splice site mutation, we performed an exon trapping experiment that showed that the splice site mutation indeed affects the splicing of the exon. Transfection of CHO-K1 cells with the pIRES-EGFP gene-expressing vector containing the mutant gene (missense mutation) showed abnormal localization of the mutant protein compared to the wildtype protein. Furthermore, we determined the spatial and temporal expression pattern of the gene in human embryos and fetal tissues as well as of the orthologues of the gene in zebrafish. Currently zebrafish knock down experiments are being performed and the function of the gene is being studied in a gastrointestinal epithelial cell model. Although the pathophysiology is not yet fully understood, all our findings indicate that we identified the causative gene for Congenital Short Bowel Syndrome.

2341/T

Conventional mutations are associated with a different phenotype than polyglutamine expansions in spinocerebellar ataxias. *A. Durr[1,2], S. Forlani[1], C. Cazeneuve[2], C. Cagnoli[3], K.P. Figueroa[4], D. Lorenzo[5], J. Johnson[6], J. van de Leemput[6], M. Viemont[2], A. Camuzat[1], S. Benaich[1,2], A. Singleton[6], L. Ranum[5], S. Pulst[4], A. Brusco[3], E. Leguern[2], A. Brice[1,2], G. Stevanin[1,2,7].* 1) CR-icm, INSERM / UPMC UMR_S975, NEB, Paris, France; 2) APHP, Departement de Genetique et Cytogenetique, Groupe Hospitalier Pitie-Salpetriere, 75013 Paris, France; 3) Department of Genetics, Biology and Biochemistry, University of Torino, Italy; 4) Cedars-Sinai Medical Center, Los Angeles, CA, USA; 5) University of Minnesota, Minneapolis, MN, USA; 6) National Institute on Aging, NIH, Bethesda, MD, USA; 7) EPHE, Paris, France.

Autosomal dominant cerebellar ataxias comprise a wide spectrum of diseases with different clinical/neuropathological profiles. At least 30 responsible loci (SCA) have been mapped. Nucleotide repeat expansions have been identified as responsible for the disease in 10 genes including those caused by polyglutamine-coding (CAG)n repeat expansions in the SCA1-3,6,7 and 17 genes and rare forms caused by non-coding repeats in the SCA8,10, 12 and 31 genes. More recently, conventional mutations were reported in SPTBN2/SCA5, TTBK2/SCA11, KCNC3/SCA13, PRKCG/SCA14, ITPR1/SCA15/16, FGF14/SCA27 and AFG3L2/SCA28. The relative prevalence of the SCA genes and their associated phenotype was investigated in 826 index patients from families with a dominant transmission of the disease collected from 1990 to 2008 in the Pitie-Salpetriere university-hospital in Paris or through the SPATAX network (coordinator: A Durr) using standardized clinical charts. The most frequently mutated genes were SCA3 (20.4%), SCA2 (9.7%), SCA1 (7.7%), SCA7 (5.7%) and SCA6 (1.8%). Missense mutations in SCA14 (1.8%), SCA28 (1.6%), SCA13 (1.2%) and SCA5 (0.8%) were less frequent. We found SCA17 (0.2%) and SCA12 (0.2%) to be very rare, while no cases of SCA10 and SCA27 were identified. In subclinical selections, heterozygous deletions in SCA15/16 (4/76) and a non-sense mutation in SCA11 (1/77) were also detected. Genotype-phenotype correlations showed that CAG repeat expansion diseases shared a rapidly progressive and severe disease course with onset in the thirties. On the contrary, the clinical picture associated with conventional mutations in the recently identified genes was milder despite the frequent presence of marked cerebellar atrophy on MRI.

2342/T

Noncoding mutations of HGF cause dysregulation of HGF and HGF/MET signalling pathways. *R.J. Morell[1], J.M. Schultz[1], S. Riazuddin[2], T.B. Friedman[1].* 1) Lab Molecular Genetics, NIDCD, Rockville, MD; 2) National Centre of Excellence in Molecular Biology, Punjab University, Lahore, Pakistan.

Hepatocyte Growth Factor (HGF) mediates a multitude of cellular pathways through a receptor tyrosine kinase encoded by the proto-oncogene MET. Expression of HGF and up-regulation of MET are characteristics of many solid tumors. Previously, we reported three noncoding germline mutations of the HGF gene associated with autosomal recessive nonsyndromic hearing loss (DFNB39). We have investigated the effects of these noncoding mutations on HGF isoform expression and on expression levels of genes in the HGF/MET signaling pathway. We established eight lymphoblastoid cell lines from individuals in four families segregating the most common DFNB39 mutation: a 3 bp deletion in a highly conserved region of intron 4 that is also transcribed as part of the 3'UTR of a novel HGF transcript (isoform 6). From each family we created a cell line from a homozygous affected individual and a heterozygous parent or sibling. We used a combination of Taqman probes that distinguish alternate splice transcripts of HGF, and SYBR Green assays of genes in various signaling pathways, in real-time PCR experiments comparing relative levels of cDNA between the homozygotes and heterozygotes. We found significant up- and down-regulation of several genes related to the MET mediated MAPK signaling pathway. Most prominently, the MAPK inhibitor SPRY2, a known regulator of cochlear development, is significantly up-regulated in homozygous cell lines. HGF isoform 6 was also significantly up-regulated in homozygous cell lines. This finding is consistent with the results of our luciferase assay which compared the effects of mutant versus wild-type 3'UTRs of isoform 6 on Renilla luciferase transcript stability. In transient transfections of HEK293 cells or NIH3T3 cells, Renilla luciferase signals persisted at significantly higher levels in cells transfected with plasmids bearing the 3 bp deletion versus those bearing a wild-type 3'UTR. We hypothesize that the 3 bp deletion affects the binding of an unknown factor and allows HGF isoform 6 to escape regulation. Overall, our experiments indicate that dysregulation of a novel short isoform of HGF leads to disruption of many genes related to the MAPK pathway. These findings will guide our evaluation of a mouse model of DFNB39 deafness.

2343/T

DFNA8/12 hearing loss in the US Population- Report of nine novel mutations in the *TECTA* gene. *M.S. Hildebrand[1], N.C. Meyer[1], C.J. Nishimura[1], H. Workmann[2], A.P. DeLuca[3,4], K.R. Taylor[3,4], B. Tompkins[3,4], C.W. Goodman[3,4], A.E. Shearer[1], P.L.M. Huygen[5], T.L. Casavant[3,4,6,7], R.J.H. Smith[1,8].* 1) Dept Otolaryngology, Univ Iowa, Iowa City, IA; 2) Department of Genetics, The Children's Medical Center of Dayton, Dayton, Ohio, USA; 3) Department of Biomedical Engineering, The University of Iowa, Iowa City, Iowa, USA; 4) Center for Bioinformatics and Computational Biology, The University of Iowa, Iowa City, Iowa, USA; 5) Department of Otorhinolaryngology, Radboud University Nijmegen Medical Centre, 6500 HB Nijmegen, The Netherlands; 6) Department of Electrical and Computer Engineering, The University of Iowa, Iowa City, Iowa, USA; 7) Department of Ophthalmology and Visual Sciences, The University of Iowa, Iowa City, Iowa, USA; 8) Interdepartmental PhD Program in Genetics, Department of Otolaryngology, University of Iowa, Iowa City, Iowa City, IA, USA.

The prevalence of DFNA8/12 hearing loss is unknown as comprehensive genetic screening in populations has not been conducted. To address this issue, we used a biased approach to determine the prevalence of DFNA8/12 mutations in autosomal dominant non-syndromic hearing loss (ADNSHL) families in the American population. In a cohort of 834 American ADNSHL families or patients, biased pre-selection was performed based on visual inspection of audiometric data. Of 72 selected families or patients, 63 (87.5%) had a DFNA8/12 audioprofile predicted by computational audioprofiling analysis. The *TECTA* gene was screened in all 72 families or patients. Nine probands were found to carry *TECTA* mutations, for a positive predictive value of 12.7%. Eight mutations were novel; one mutation (p.Arg1890Cys) has been reported in the Dutch population. The results of this study demonstrate that biased methods are effective for identification of DFNA8/12 hearing loss cases. Additionally, we have confirmed observed genotype-phenotype correlations in DFNA8/12 hearing loss and introduced new correlations that will be important in clinical management of patients. Finally, our results indicate that mutations at the DFNA8/12 locus are a common cause of ADNSHL in the American population, and that the vast majority of alleles at this locus are private mutations.

2344/T

Severe inner ear malformation and profound sensorineural hearing loss in a girl with a monoallelic *SLC26A4* mutation. *J. Samanich[1], J. Chobot-Rodd[2], J. Bent[3].* 1) Center for Congenital Disorders, Department of Pediatrics, Montefiore Medical Center, Bronx, NY; 2) Children's Evaluation and Rehabilitation Center, Albert Einstein College of Medicine, Bronx, NY; 3) Department of Otolaryngology, Montefiore Medical Center, Bronx, NY.

Background: Pendred syndrome (OMIM #274600) is an autosomal recessive condition caused by mutations in *SLC26A4*, and characterized by enlarged vestibular aqueduct (EVA), hearing impairment (HI) and goiter. However, monoallelic mutations in this gene have been associated with EVA or Mondini's dysplasia and HI, without thyroid involvement. More severe cochlear anomalies are not generally associated with *SLC26A4* mutations. Herein, we report a young girl with bilateral profound sensorineural HI and severe inner ear malformation. **Case Report**: Our patient was born full-term via cesarean section in the Dominican Republic with an unremarkable perinatal course. Prenatal history was non-contributory. Newborn hearing screening was not performed. Hearing impairment was suspected at one month of age, and was confirmed with formal testing at 26 months of age. CT scan of the temporal bones revealed marked abnormality of the inner ear structures bilaterally. She had amorphous cochlea bilaterally, with amorphous and enlarged vestibules and semicircular canals bilaterally. She had normal middle ear structures as well as external auditory canals bilaterally. At the age of three years, gross and fine motor skills were age appropriate, and speech was delayed. Family history was significant for a maternal second cousin with congenital deafness of unknown etiology. Physical examination was unremarkable, with no sign of goiter. DNA sequencing of the entire coding sequence and intron-exon borders of *SLC26A4* was performed at the Center for Genetic Testing at Saint Francis, and revealed a monoallelic mutation, c.565G>T in exon 5 (A189S). This mutation has been found in association with EVA, but not with the more severe inner ear anomalies seen in our patient. **Conclusions**: Biallelic mutations in *SLC26A4* are associated with Pendred syndrome, and monoallelic mutations have been associated with EVA and HI. We report a child with a monoallelic mutation, A189S, causing bilateral profound sensorineural HI and severe inner ear anomalies. It is possible that she has a mutation in another gene modifying her phenotype, or that there was an unidentified environmental teratogen adding to the severity of her phenotype. Additionally, it is possible that other children with similar severe inner ear anomalies have mutations in *SLC26A4*, but are not routinely tested. This report adds to the knowledge of inner ear phenotypes associated with mutations in this gene.

2345/T

A Novel FLNA Mutation (c. 3686A>G/Y1229C) in Exon 22 Results in Melnick-Needles Syndrome. *G.A. Bellus[1], J. March[1], T. Futch[1], R.S. Lachman[2].* 1) Genetic & Metabolic Services, Department of Pediatrics, University of Colorado School of Medicine, Aurora, CO; 2) Ahmanson Department of Pediatrics, Cedars-Sinai Research Institute, UCLA School of Medicine, Los Angeles, CA.

Melnick-Needles syndrome (MNS)(OMIM 309350) is a rare, X-linked dominant skeletal dysplasia that is one of eight genetic disorders known to be caused by mutations in the filamin A gene (FLNA) located at Xp28. Other FLNA related disorders include: periventricular heterotopia (PVH); PVH-Ehlers-Danlos variant; otopalatodigital syndrome (OPD) type 1; OPD type 2; OPD spectrum disorder; frontometaphyseal dysplasia, chronic idiopathic intestinal pseudo-obstruction and FG syndrome. Clinical features of MNS include characteristic facial appearance (small face, full cheeks, micrognathia , prominent supraorbital ridge, exophthalmos) and skeletal features (short clavicles and scapulae, irregular ribbon-like ribs, bowing of long bones). MNS is lethal in males who inherit the condition from their mother but the phenotype is variable in females and males with spontaneous mutations. Thus far, mutational analyses of FLNA in individuals with MNS have revealed a total of 4 recurrent missense mutations that all localize to exon 22 (D1184E, A1188T, S1199L & G1176D) indicating that the MNS phenotype may be specific to mutations that alter the repeat 10 domain of the filamin A protein. We report a case of a 20 year old female with average stature who first came to medical attention at age 13 with back pain, scoilosis and mild leg length discrepancy. She was eventually referred to genetics clinic where she was noted to have mild dysmorphic facial features and radiographic skeletal findings that were typical of MNS. DNA sequencing of FLNA in this patient revealed the presence of a novel FLNA variant (c. 3686A>G/Y1229C) that was not found in either of her unaffected parents and is therefore likely to be a new mutation. Analysis of the X-inactivation pattern in blood leukocytes revealed skewing at a ratio of greater than 97:3 suggesting that skewing of X-inactivation may play a significant role in the severity of the MNS phenotype in this patient and possibly other females with MNS. Our findings support the hypothesis that the MNS phenotype is due to specific sequence changes in the filamin A repeat 10 domain.

2346/T

A Brazilian family with IBMPFD caused by p.R93C mutation in the VCP gene and literature review for genotype-phenotype correlations. *R. Fanganiello[1], V. Kimonis[2], C. Corte[3], R. Nitrini[4], M.R. Passos-Bueno[1].* 1) Dept Genetics & Evolution, Univ Sao Paulo, Sao Paulo, Sao Paulo, Brazil; 2) Division of Genetics and Metabolism, Department of Pediatrics, University of California, Irvine, CA 92868, USA; 3) Clinost, Fortaleza, Ceará, Brazil; 4) Department of Neurology, Reference Center for Cognitive Disorders, Hospital das Clínicas, University of São Paulo, São Paulo, SP, Brazil.

Inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia (IBMPFD) is a progressive, rare and usually misdiagnosed autosomal dominant disorder clinically characterized by a triad of features: proximal and distal muscle myopathy, early onset Paget disease of bone (PDB) and frontotemporal dementia (FTD). It is molecularly homogeneous, caused by missense mutations in the valosin containing protein gene (VCP). We described the clinical and molecular findings of the first Brazilian family identified with IBMPFD. The proband presented with the classical clinical features. Progressive myopathy affecting the limb girdles was detected by clinical examination followed by muscle biopsy and creatine kinase measurement. PDB was suggested after bone anatomopathological examination and FTD, with features of semantic dementia, was diagnosed through clinical, neuropsychological and language evaluations. Brain magnetic resonance imaging revealed severe atrophy of the anterior temporal lobes, including the hippocampi. R93C mutation in VCP was detected by direct sequencing gene mutation screening in subject W. and in his mother, subject N, who died at 81 years of age, with a 20 years history of progressive dementia. We were not able to evaluate other members of the family but 4 more individuals diagnosed with “dementia” were reported in this family. We also present a comprehensive genotype-phenotype correlation analysis of mutations in VCP in 182 patients from 29 different families described in the literature and show that while IBM is a conspicuously penetrant symptom, independently of the protein domain harboring the mutation, PDB has a lower penetrance when associated with mutations in the AAAD1 domain and FTD has a lower penetrance when associated with mutations in the Junction (L1-D1) domain. Furthermore, the R93C mutation is likely to be associated with the penetrance of all the clinical symptons of the triad. We would like to point out that IBMPFD should be considered when the patient has at least 2 of the symptoms of the triad and mutation screening on VCP encoding gene should be suggested for confirmation of the diagnosis.

2347/T

A novel SEC23A missense mutation causing Cranio-Lenticulo-Sutural Dysplasia is involved in the MAPK signaling pathway. *S. Kim, J. Liu, E. Cherkez, S. Boyadjiev, J. Kim.* Pediatrics, UCDMC Mind Inst, Sacramento, CA.

Cranio-lenticulo-sutural dysplasia (CLSD) is an autosomal recessive disease with late-closing fontanels, cataracts, facial dysmorphisms and skeletal defects. The extracellular matrix (ECM) is a complex structural entity surrounding and supporting cells that are found within tissues. Collagens are the most abundant proteins of ECM. We have identified a patient with CLSD due to M702V mutation in SEC23A. Cultured skin fibroblasts from this new patient showed secretion defect of collagen and enlarged endoplasmic reticulum. In addition, phosphorylation of MAPK/Erk and cyclin D1 protein expression in the patient’s cells were significantly decreased, while AKT phosphorylation was significantly increased as compared to control cells. G0/G1 arrest was also shown in the patient cells. In corroboration, phosphorylation of MAPK/Erk and cyclin D1 were significantly decreased when wild type fibroblasts were transfected with sec23A 702V plasmid. Our data suggest that SEC23A exports secretory proteins involved in MAPK/Erk -related cell proliferation and cell cycle signaling pathway.

2348/T

New FKBP10 patient expanding the phenotypic spectrum of this rare and severe recessive form of Osteogenesis imperfecta. *M. Le Merrer, G. Baujat, S. Dos Santos, G. Goudefroye, G. Pinto, G. Finidori, J.P. Bonnefont, A. Munnich, V. Cormier-Daire, A.S. Lebre.* Université Paris Descartes, AP-HP Hôpital Necker-Enfants Malades et Inserm U781, Départements de Génétique et d’Orthopédie et traumatologie pédiatriques, Paris F-75015 France.

Osteogenesis imperfecta (OI) is a heritable brittle bone disease presenting with clinical heterogeneity (severity from mild to neonatal lethal) and genetic heterogeneity (autosomal- dominant and -recessive forms). OI involves type I procollagen (designed as proα1(I)), a heterotrimer composed of two α1(I) and one α2(I) chains assembled in the lumen of the endoplasmic reticulum (ER). The proα1(I) triple helical domain, which is characterized by a repeating Gly-X-Y triplet, undergoes a series of posttranslational modifications in the ER. They include 3-hydroxylation of the Pro-986 residue, which is accomplished by a complex comprising cartilage-associated protein (CRTAP), prolyl 3-hydroxylase (P3H1) and cyclophilin B (CyPB), encoded by the CRTAP, LEPRE1, and PPIB genes, respectively. Chaperones, including HSP47 and the ER-resident cis-trans isomerase FKBP65 (encoded by the SERPINH1 and FKBP10 genes, respectively) assist with proper chain registration and folding of the molecules into the collagen triple helix. The majority of OI patients have dominant forms with mutations in COL1A1 or COL1A2 genes. Recently, mutations in the CRTAP, LEPRE1, PPIB, SERPINH1 and FKBP10 genes have been described in OI patients with recessive forms. In this study, we described the clinical, radiologic, and genetic features of a new FKBP10 patient originated from Turkey with a homozygous mutation in exon 6 (c.950_951dupTC, p.Gly318fs). This patient presented with progressive long bone deformities including an unusual aspect of the iliac bone with severe acetabular protrusion, narrowness of the iliac wing, no platyspondily, no dentinogenesis imperfecta and white sclera. Interestingly, bisphosphonate treatment was inefficient. We performed a meta-analysis of the literature and compared his phenotype with the other reported patients with recessive forms of OI to identify a possible clinical or radiological pattern. This report further describes the phenotypic spectrum of FKBP10 mutations and will hopefully contribute to define the hallmark of FKBP10 defect.

2349/T

***MKS1* mutations are a new cause for Joubert syndrome, expanding the genetic overlap with Meckel syndrome.** *D. Doherty[1], E.A. Otto[2], N.T. Gorden[1], J. Adkins[1], J.C. Dempsey[1], D. Knutzen[3], S. Aysun[4], M.A. Parisi[5], F. Hildebrandt[2], I.A. Glass[1].* 1) Dept Pediatrics, Univ Washington, Seattle, WA; 2) Dept of Pediatrics, University of Michigan, Ann Arbor, MI; 3) Dept Obstetrics and Gynecology, Madigan Army Medical Center, Tacoma, WA; 4) Dept of Pediatric Neurology, Hacettepe University, Ankara, Turkey; 5) National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD.

Mutations in *TMEM67/MKS3*, *CC2D2A*, *CEP290*, and *RPGRIP1L* cause both Joubert (JS) and Meckel syndromes, while *MKS1* mutations have only been reported in patients with Meckel syndrome. To determine whether *MKS1* mutations can cause the JS phenotype, we sequenced the exons and intron-exon boundaries in our large cohort of patients with JS, ascertained by the molar tooth sign on brain MRI and clinical features. We identified *MKS1* mutations in 6/190 families. In three families with known consanguinity and homozygosity at the *MKS1* locus, we identified 1) a homozygous missense mutation (c.55G>T, p.D19Y) that is predicted to be damaging, 2) a homozygous single basepair insertion (c.1528-1529insC, p.R510PfsX81) near the 3' end of the gene resulting in a frameshift that replaces the C-terminus of the protein, and 3) a homozygous mutation that removes the branch splice site in intron 3, previously described in Meckel syndrome. In three families without known consanguinity, we found 1) compound heterozygosity for a splice mutation previously reported in a fetus with Meckel syndrome (c.417G>A) and a missense mutation (c.1208C>T, p.S403L), 2) compound heterozygosity for a single basepair deletion leading to a frameshift and truncation (c.381delC, p.T127Tfs17X) and a 3-base pair deletion (c.1115_1117delCCT, p.S372del) that removes a single serine residue and is predicted to be damaging, and 3) homozygosity for the same 3-base pair deletion (c.1115_1117delCCT, p.S372del). Additional clinical features at 2-21 years of age include: kidney disease (1/6), liver disease (1/6) and abnormal ERG (1/6) but no encephalocele, polydactyly, or coloboma (optic pit in 1/6). These are the first *MKS1* mutations reported in patients with JS, confirming genetic overlap with Meckel syndrome. The mutations associated with JS may have less impact on MKS1 function, since all Meckel patients have combinations of truncating and splice mutations while most JS patients have single amino acid changes (2 patients) or combinations of single amino acid changes with truncating/splice mutations (2 patients). Based on our data, we estimate that *MKS1* mutations account for ~3% of JS and propose that *MKS1* testing should be considered in all JS patients, not just those with features of Meckel syndrome.

2350/T

Genetic variation of serotonin receptor 2A gene may influence age at onset but not cognitive performance in patients with temporal lobe epilepsy. *I. Manna[1], A. Labate[1,2], U. Aguglia[3], G. Palamara[2], L. Mumoli[1,2], A. Fratto[1,2], F. Condino[1], G. Di Palma[1], A. Quattrone[2], A. Gambardella[1,2].* 1) Institute of Neurological Sciences, National Research Council,Cosenza, Italy; 2) Institute of Neurology, University Magna Graecia, Catanzaro, Italy; 3) Regional Epilepsy Centre, Hospital of Reggio Calabria, Italy.

Serotonin (5-hydroxytryptamine or 5-HT) and its receptors, including the 5-HTR2A receptor, are important for learning and memory. There is also evidence that impairment of the 5-HT neurotransmission may be involved in the pathophysiology of temporal lobe epilepsy (TLE). In this study, we wished to explore the association of a functional single nucleotide polymorphism (SNP) at position 1354C/T of the 5-HTR2A receptor with the memory decline. We conducted a detailed neuropsychological and molecular study in a group of patients with mild nonlesional TLE. A total of 138 consecutive patients (78 female, mean age 50.2 years, SD ± 17.9; range 14 to 87) with mild non-lesional TLE were entered into the study. All patients received a diagnosis of TLE after comprehensive clinical, neuropsychological, EEG, and MR evaluations. All patients were Caucasian and were born in Italy. All gave written informed consent prior to participation in the genetic studies. Genomic DNA was isolated from each sample. The target fragment of 5-HTR2A gene was amplified by PCR. The SNPs in 5-HTR2A gene were detected by denature high performance liquid chromatography (DHPLC) combined with DNA sequencing. which predicts an amino acid substitution (His to Tyr) at residue 452. All patients had undergone a standardized neuropsychological examination, twenty-five percent (34/138) had test scores indicating verbal memory deficit (VMD), and they where divided in two groups: cognitively impaired (34) and preserved (104). We did not observe a significant differences of the 1354 C/T polymorphism between the two TLE groups, both in the comparison of genotype distribution (p= 0.177) and allele frequencies (p= 0.065). We also investigated the association between the 1354 C/T polymorphism and age at onset of epilepsy, and found that patients carrying the 1354T allele had a significant earlier onset of the disease (p= 0.006). The results of the present study demonstrate that the T allele of 5-HTR2A variant at position 1354 may influence an earlier age of onset of TLE. Nonetheless, this polymorphism has not major impact on memory functions in patients with mild TLE.

2351/T

ATP7B mutation analysis of nine Taiwanese families with Wilson disease. *S. Chang[1,4], G. Ma[1], W. Lin[1], M. Chen[1,2,3].* 1) Dept Medical Genetics, Changhua Christian Hosp, Changhua City, Taiwan; 2) Department of Obstetrics and Gynecology, National Taiwan University, Taipei, Taiwan; 3) Department of Obstetrics and Gynecology, Changhua Christian Hospital, Changhua, Taiwan; 4) Department of Life Science, National Chung Hsing University, Taichung, Taiwan.

Wilson disease (WD) is a genetically and phenotypically heterogeneous disorder of copper transport with hepatic and/or neurological symptoms. It is caused by mutations in ATP7B that result in copper storage in liver and brain. At present more than 400 ATP7B mutations have been reported in WD patients. In this study, we screened for mutations in all the coding sequences and exon-intron boundaries of the ATP7B gene in nine unrelated WD families, and the results were compared with the oneline reference sequence and normal control data. A total of 12 mutations (including 11 misssense mutaions and one nonsense muation) that were not found in the normal controls and the SNP database were found in our patinets. Almost all of these mutations have been reported in literature as disease-causing mutations, except for two novel mutations, p.D1047V and p.V1297I. All of the missense mutations lay in critical functional domains of the ATP7B protein (e.g. the transmembrane region, the transduction domain, the phosphorylation domain, the ATP-hinge region and the ATP-binding domain). Of the mutations we identified, the p.R778L mutation was found repetitively, with a carrier rate of 77.8% (in 7/9 families) and a mutant allele frequency of 38.9%, suggesting a hotspot of Taiwanese population. The mutations of p.Q1142H and p.I1148T are alos common, with frequency of 11.1%. Our results add the short list of ATP7B mutations in Chinese/Taiwanese population, and are helpful for the diagnosis and counseling of WD.

2352/T

A genome-wide association and linkage study (GWALS) identifies two loci modifying lung disease severity in Cystic Fibrosis: North American CF Modifier Consortium. *G. Cutting[1], C.F. Modifier Consortium[2].* 1) Inst Gen Med, Johns Hopkins Sch Med, Baltimore, MD; 2) North American CF Modifier Consortium (Univ of N Carolina, Case Western U, Hosp for Sick Children, Toronto, Johns Hopkins).

Cystic Fibrosis (CF) is a multi-system life-limiting disorder due to dysfunction of the CFTR gene. Progressive obstructive lung disease, the major cause of mortality in CF, is highly variable and poorly correlated with CFTR genotype. Affected twin and sibling analysis demonstrates that variation in forced expiratory volume in 1 second (FEV1), a lung function measure predictive of survival, is heritable (h2 = 0.5-0.8). To identify the genetic modifiers underlying variation in CF lung function, 3 independent studies (Genetic Modifier Study (GMS: case-control), CF Twin and Sibling Study (CFTSS: family-based) and Canadian Consortium for Genetic Studies (CCGS: population-based)) combined to form the CF Modifier Consortium. The Consortium developed a CF-specific lung function measure based on FEV1 using survival-corrected centile scores. 3,467 CF patients stratified by lung function were genotyped using the Illumina 610K SNP array. 570,748 SNPs were validated with call concordance rate of 0.9987. Association analysis of 2494 patients from the GMS and CCGS identified seven regions of suggestive significance ($p<1.8 \times 10^{-6}$). When the analysis was confined to 1978 patients with identical CFTR genotypes (F508del /F508del), a cluster of five SNPs in moderate LD ($r2>0.6$) within a suggestive region on chromosome 11p13 exceeded genome-wide significance ($p<8.8 \times 10^{-8}$). Linkage analysis of 486 sibling pairs from the CFTSS was performed using 19,566 SNPs with minor allele frequency>0.4 and $r2 <0.01$ between adjacent SNPs. A genome-wide significant peak with multipoint LOD score of 5.03 occurred on chromosome 20q13.2 and a region of suggestive linkage (LOD score= 2.6) was observed on 1q11. As the 20q13.2 region had been previously linked to a nutritional phenotype, and nutritional status is correlated with CF lung function, analysis was repeated with BMI as a covariate, resulting in an increase in LOD to 5.72 for the same region. Regional association analysis was performed using 2494 unrelated patients from the GMS and CCGS samples and 306 SNPs drawn from a 1.314 Mb 1-LOD critical region on 20q13.2. A set of SNPs in the critical region were suggestive of association, consistent with incomplete tagging of low-frequency variants. These results demonstrate that genome-wide methods are applicable to discovery of loci that modify quantitative measures of disease severity in a monogenic trait. Genes of substantial biologic relevance to CF occur at both loci.

2353/T

Frequency of point mutations and intragenic rearrangements in REEP1 responsible for SPG31. *C. Goizet[1,2], C. Depienne[1,3], A. Boukhris[1,3,4], E. Mundwiller[1], E. Fedirko[3], E. Denis[3], D. Hannequin[5], P. Charles[3,6], I. Feki[4], J.F. Pinel[7], J. Yaouanq[7], B. Fontaine[6], A. Durr[1,3,6], A. Brice[1,3,6], G. Stevanin[1,3,8].* 1) CR-icm, INSERM / UPMC UMR_S975, NEB, Paris, France; 2) Université Victor Segalen Bordeaux 2, Laboratoire de Génétique Humaine, Bordeaux, France; 3) AP-HP, Pitié-Salpêtrière Hospital, Département de Génétique et Cytogénétique, Fédération de Génétique, Paris, France; 4) Service de Neurologie, Hôpital Habib Bourguiba, Sfax, Tunisia; 5) Service de Neurologie, CHU Rouen, France; 6) GHU Pitié-Salpêtrière, Fédération des Maladies du Système Nerveux, Paris, France; 7) Hôpital Pontchaillou, Clinique Neurologique, CHU Rennes, France; 8) EPHE, Paris, France.

Hereditary spastic paraplegias (HSP) constitute a clinically and genetically heterogeneous group of neurodegenerative disorders characterized by slowly progressive spasticity of the lower extremities. Mutations in the SPG31 gene, REEP1, were initially associated with a pure dominant form of the disease. Our objective was to estimate the frequency of SPG31 in Western European HSP families, to define the spectrum of mutations in REEP1 and to describe the associated phenotypes. To this end, we sequenced all exons of REEP1 in a large panel of 177 unrelated European HSP index patients from kindreds with dominant inheritance, with either pure (n=92) or complicated (n=85) forms of the disease. They mostly originated from France. We also looked for rearrangements using a dedicated MLPA (MRC_Holland) kit. We identified 12 different heterozygous mutations in 12 different families of French origin, with either a pure or a complex phenotype; 3 affected the splicing of the gene, 2 were exon deletions, 3 were missense mutations and 3 were small indel or point mutations introducing premature stop codons. In addition, we also found a variation in the 3'UTR that might alter the binding of microRNA. The overall mutation rate in our clinically heterogeneous sample was 7.3%, and 3.5% by taking into account HSP cases with other known entities.

2354/T

Genotype-phenotype correlations in subjects with X-linked hypohidrotic ectodermal dysplasia. *H. Schneider[1], J. Hammersen[1], K. Huttner[2], A. Bohring[3].* 1) Department of Pediatrics, University of Erlangen-Nuremberg, Erlangen, Bavaria, Germany; 2) Edimer Pharmaceuticals Inc., Cambridge, USA; 3) Institute of Human Genetics, University of Muenster, Germany.

Background: X-linked hypohidrotic ectodermal dysplasia (XLHED), the most common ectodermal dysplasia, is caused by EDA gene mutations. Reduced sweating is a major cause of XLHED-associated morbidity and mortality. To characterize the genotype-phenotype relationship, sweat gland function was assessed non-invasively in genotyped XLHED patients. **Subjects and Methods:** 27 male XLHED subjects aged 2 to 57 years and 17 healthy male controls aged 5 to 56 years were studied at a family conference in Germany (clinicaltrials.gov NCT01109290). Informed consent was obtained from all participants. Parameters determined included palmar sweat pore density, sweat volume produced after stimulation with pilocarpine using the Wescor 3700 device, and palmar skin conductance measured with the Med-Storm 1001 device. **Results:** Control subjects had 423 sweat pores/cm2 on average (range 294-669) and a mean sweat volume of 77 µl following stimulation (38-93 µl). In 5 of 14 XLHED children and 1 of 13 XLHED adults some sweat was detectable by the Wescor assay (1.0-11.0 µl). However, there was a clear demarcation between XLHED males and controls. Among XLHED subjects, 12/27 had neither detectable sweat pores nor inducible sweating, 13/27 showed a few sweat pores (6-113/cm2) correlating with absence of sweat or low sweat volumes (up to 11.0 µl), and 2/27 had normal sweat pore counts but reduced sweat volumes. Basal skin conductance, an indirect measure of skin hydration, was reduced (<20.0 µS) in 20 of 21 non-sweating and 1 of 6 low-sweating XLHED patients, but none of the 17 controls. Similarly, stimulated skin conductance following blood sampling was reduced (<10.0 µS) in all 21 non-sweating XLHED subjects, 3 of 6 with decreased sweating, and 4 of 17 controls. In this XLHED cohort representing 22 different EDA genotypes, exon deletions, nonsense and splice-site mutations and mutations affecting the furin cleavage site of ectodysplasin-A were consistently associated with compromised sweat gland function across all measurements. **Conclusions:** In contrast to prior reports on non-genotyped HED populations with qualitative sweat assessment, this study on XLHED males confirmed a consistent, quantifiable defect in sweat gland function as a disease biomarker, even in the setting of normal sweat pore counts. Skin conductance measurements may be useful as a screening tool, but have reduced sensitivity in detecting XLHED subjects with residual sweat gland function.

2355/T

High level deletion mosaicism in Cornelia de Lange with severe phenotype. *C. Gervasini[1], J. Azzollini[1], D. Rusconi[2], P. Castronovo[1], A. Cereda[3], M. Masciadri[4], S. Russo[4], A. Selicorni[3], P. Finelli[2], L. Larizza[1].* 1) Div Medical Genetics, Univ Milano San Paolo Sch, Milan, Italy; 2) Cytogenetics Laboratory Istituto Auxologico Italiano, Milano, Italy; 3) Ambulatorio Genetica Clinica Pediatrica Università Milano Bicocca Fondazione MBBM A.O S Gerardo Monza, Italy; 4) Molecular Laboratory Istituto Auxologico Italiano, Milano, Italy.

Cornelia de Lange (CdLS, MIM #122470, #300590, #610759) is a rare, congenital neurodevelopmental syndrome characterized by growth/mental retardation, dysmorphic face and limb reduction defects. Wide phenotypic variability is common in the CdLS patients. Mutations in NIPBL, SMC1A and SMC3 genes, encoding for a regulator and two subunits of the cohesin complex respectively, are found in 60-65% of CdLS patients. A CdLS patient with severe typical phenotype was tested by DHPLC and direct sequencing for NIPBL and SMC1A genes and found negative. The ERCC8 gene, responsible for Cockayne syndrome, was then screened as plausible candidate based on the partial clinical overlapping between the two syndromes, but no sequence alteration was detected. In order to exclude rare chromosomal rearrangements affecting the major CdLS genes, BAC-FISH was performed targeting the NIPBL gene. An asymmetric signal was apparent in a fraction of cells, suggesting a genomic deletion in mosaic condition. The finding was confirmed by aCGH analysis which showed a large deletion encompassing exons 3-23 in about 80% of the cells. This case provides the second evidence of mosaicism in CdLS and raises the issue of the variable associated clinical phenotypes. We have recently described a patient with low-level mosaicism for a point mutation who displayed a phenotype milder than predicted by a truncating mutation. Conversely the high deletion mosaicism here reported is associated with a severe presentation as attested by death of the proband at age one year. Further evidence of mosaicism could explain the clinical heterogeneity of CdLS, highlighting the likely underestimated mutation rate of known genes.

2356/T

Molecular & clinical analysis of the *retinoic acid induced 1* gene (*RAI1*) in patients with suspected Smith-Magenis Syndrome without the 17p11.2 deletion. *A.C.M. Smith[1], T. Vilboux[2], C. Ciccone-Stevens[2], J. Blancato[3], G. Cox[4,5], W. Introne[1], W.A. Gahl[1,2], M. Huizing[2].* 1) Office Clinical Dir, NHGRI/NIH, Bethesda, MD; 2) Medical Genetics Branch, NHGRI/NIH, Bethesda, MD; 3) Dept. of Oncology, Georgetown University Medical Center, Washington, DC; 4) Div. of Genetics, Children's Hospital Boston and Dept Pediatrics, Harvard Medical School, Boston, MA; 5) Genzyme Corporation, Cambridge, MA.

Smith-Magenis syndrome (SMS) is a complex developmental disorder characterized by multiple congenital anomalies. The syndrome is primarily ascribed to a ~3.7 Mb de novo deletion on chromosome 17p11.2. Haploinsufficiency of multiple genes likely underlies the complex clinical phenotype. *RAI1 (Retinoic Acid Induced 1)* is recognized as a major gene involved in the SMS phenotype. Extensive genetic and clinical analyses of 36 patients with SMS-like features, but without the 17p11.2 microdeletion, yielded 5 patients with *de novo* and 5 patients with novel *familial RAI1* variants. Haplotype analysis showed two major *RAI1* haplotypes in our primarily Caucasian cohort; the novel *RAI1* variants did not occur in a preferred haplotype. RNA analysis revealed for the first time that *RAI1* mRNA expression was significantly decreased in cells of patients with the common 17p11.2 deletion, as well as in those with *de novo RAI1* variants. Expression levels varied in patients with familial *RAI1* variants and in non-17p11.2 deleted patients without identified *RAI1* defects. No correlation between SNP haplotype and *RAI1* expression was found. Two clinical features, ocular abnormalities and polyembolokoilomania (object insertion), were significantly correlated with decreased *RAI1* expression. While not significantly correlated, the presence of hearing loss, seizures, hoarse voice, childhood onset of obesity and specific behavioral aspects and the absence of immunologic abnormalities and cardiovascular or renal structural anomalies, appeared to be specific for the *de novo RAI1* subgroup. Recognition of the combination of these features may assist in referral for *RAI1* analysis of patients with SMS-like features without detectable microdeletion of 17p11.2.

2357/T

The genetic perspective from a large ethnically mixed cohort of patients with BBS. *C. Deveault[1], G. Billingsley[1], J. Bin[1], R. Theal[1], A. Vincent[1], K.J. Fieggen[3], J.L. Duncan[4], C. Gerth[2], K. Ogata[5], S.S. Wodak[6, 7, 8], E.I. Traboulsi[9], G.A. Fishman[10], D. Chitayat[11, 12], T. Knueppel[13], J.M. Millan[14], F. Munier[15], D. Kennedy[16], S.G. Jacobson[17], M. Innes[18], G.A. Mitchell[19], E. Heon[1, 2].* 1) Department of Genetics and Genome Biology, The Hospital for Sick Children, Toronto, Ontario, Canada; 2) Department of Ophthalmology and Vision Sciences, The Hospital for Sick Children, Toronto, Ontario, Canada; 3) Division of Human Genetics, University of Cape Town, Cape Town, South Africa; 4) Dept of Ophthalmology, UCSF, San Francisco, CA, USA; 5) Centre for Computational Biology, The Hospital for Sick Children, Toronto, Ontario, Canada; 6) Molecular Structure and Function Program, The Hospital for Sick Children, Toronto, Ontario, Canada; 7) Department of Biochemistry, University of Toronto, Toronto, Ontario, Canada; 8) Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada; 9) Cole Eye Institute, Cleveland Clinic Foundation, Cleveland, OH, USA; 10) Dept of Ophthalmology & Visual Sciences, University of Illinois at Chicago, Chicago, IL, USA; 11) Prenatal Diagnosis and Medical Genetics Program, Mt. Sinai Hospital, Toronto, Ontario, Canada; 12) Dept of Clinical and Metabolic Genetics, The Hospital for Sick Children, Toronto, Ontario, Canada; 13) Department of Pediatric Nephrology, University Children's Hospital Heidelberg, Heidelberg, Germany; 14) Unidad de Genetica, Hospital Universitario La Fe, Valencia, Spain; 15) Hôpital Ophtalmique Jules Gonin, Lausanne, Switzerland; 16) MotherSafe Royal Hospital for Women, Randwick, New South Wales, Australia; 17) Scheie Eye Institute, University of Pennsylvania, Philadelphia, PA, USA; 18) Department of Medical Genetics, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada; 19) Division of Medical Genetics, CHU Sainte-Justine, Montréal, Quebec, Canada.

Bardet-Biedl syndrome (BBS) is a pleiotropic ciliopathy disorder characterized by retinal degeneration, obesity, polydactyly, renal and genital abnormalities and cognitive impairment. To date, mutations have been identified in fourteen genes (*BBS1-BBS14*) that account for ~70% of affected individuals. BBS was previously shown to genetically and clinically overlap with other ciliopathies such as McKusick-Kaufman syndrome (MKKS), Alström syndrome (ALMS), Meckel-Gruber syndrome (MKS) and Joubert syndrome (JBTS). We present the analysis of our full cohort consisting of 83 probands of varied ethnic origin (104 cases) and describe 27 unpublished novel changes and novel clinical observations.

Patients were recruited from The Hospital for Sick Children, Toronto and from collaborators abroad. Mutational analysis of *BBS1-12* used different approaches including direct sequencing, homozygosity mapping and commercially available DNA chip analysis of known mutations. Phenotype analysis was as comprehensive as possible.

Two putative disease-causing mutations (EPP=3) were identified in 62 probands (74.7%) of diverse ethnicity. Nineteen new mutations were found in 7 different BBS genes (1, 2, 4, 8, 9, 10 and 12). Eight variants of unknown significance (EPP=2) were identified. Sixteen cases were found to have more than 2 mutations (EPP=3).

In addition to having typical BBS features, most of our cases (65.4%) also showed features more characteristic of other ciliopathies. We found 53.8% that showed overlap (at least 2 or more features) with ALMS, 20.5% with MKKS and 9.0% with both. Features more typical of JBTS and MKS were also observed in a few patients. The overlap and the features were not specific to any BBS genes. These numbers for the overlapping features could be underestimated as not all clinical information was available on all features for all patients. The overlap between MKKS and BBS was previously reported to be seen in chaperonin-like genes (*BBS6*, *10* and *12*) but we also observed this in patients with *BBS2* mutations.

The analysis of larger cohorts and improved phenotyping will contribute to better defining genotype-phenotype correlations and the spectrum of disease features involved which is necessary to improved patient management.

2358/T

High frequency of triallelic inheritance in individuals with Leber Congenital Amaurosis. *W. Wiszniewski[1], R.A. Lewis[1,2,3,4], D. Stockton[10], J. Peng[5], G. Mardon[1,3,6,7,8], R. Chen[1,5,8], J.R. Lupski[1,2,9].* 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Dept Pediatrics, Baylor Col Medicine, Houston, TX; 3) Dept Ophthalmology, Baylor Col Medicine, Houston, TX; 4) Dept Medicine, Baylor Col Medicine, Houston, TX; 5) Human Genome Center, Baylor Col Medicine, Houston, TX; 6) Dept Neuroscience, Baylor Col Medicine, Houston, TX; 7) Dept Pathology, Baylor Col Medicine, Houston, TX; 8) Program in Developmental Biology, Baylor Col Medicine, Houston, TX; 9) Texas Children's Hospital, Houston, Texas; 10) Dept Pediatrics, Wayne State University, Detroit, Michigan.

Leber congenital amaurosis (LCA) is a clinically and genetically heterogeneous retinal dystrophy. The causes of LCA have been unraveled partially at the molecular level. At least 14 genes have been reported that, when mutated, result in LCA. To understand the roles of the known genes in LCA a group of outbred subjects from 60 apparently either recessive families, with one or more affected individuals, or isolated affected patients were evaluated. One affected individual from each family underwent comprehensive mutational analysis by direct DNA sequencing of all coding regions and splice junctions of 13 LCA genes. Mutations were indentified in 70% of individuals. CEP290 made the largest contribution to the identified mutations, providing 43% of those mutant alleles. We identified seven families in which affected individuals with two mutant alleles, sufficient to cause disease, had an additional mutation at a second LCA locus. The families with mutations in more than one gene show no evidence for a more severe ocular phenotype. Our findings suggest mutational load can be important to penetrance of the LCA phenotype.

2359/T

Resistance to Thyroid Hormone: description of a new mutation in a Colombian family. *J. Campos[1], P. Duran[2], F. Salguero[3], G. Laverde[4], P. Paez[5], H. Groot[1], MC. Lattig[1].* 1) Laboratorio de Genética Humana Departamento de Ciencias Biologicas Universidad de los Andes; 2) Hospital Infantil Fundacion Cardioinfantil- Instituto de Cardiología Bogota - Colombia; 3) Facultad de Medicina Universidad de los Andes Bogota - Colombia; 4) Laboratorio de Investigacion Hormonal Bogota - Colombia; 5) Fundación Clínica el Bosque, Facultad de Medicina el Bosque Bogota - Colombia.

Resistance to Thyroid Hormone (RTH) is a group of genetic entities with autosomic dominant inheritance. This syndrome is characterized by a decreased effect of the thyroid hormones with respect to their circulating levels; thyroid function tests demonstrate a persistent elevation of the thyroxine (T4) and triyodotironine (T3) and the free fractions, with an inappropriately normal or elevated TSH. Its incidence is 1 in every 40000 births, but could be higher given the greater sensitivity of the current molecular studies. RTH is caused in the majority of cases by mutations in the thyroid hormone beta receptor (TRβ), specifically in its T3 binding site. Currently, mutations in the TRβ gene have been identified in more than 343 families suffering RTH worldwide, and of these approximately 124 have been characterized as unique in each family; i.e. these mutations have not been identified in another family. We present here the clinical history of a Colombian family with RTH and report a new mutation in exon 8 of the TRβ gene, which we have named V264L. The family also presents individuals with hypothyroidism who do not manifest the V264L mutation, therefore we believe that two separate entities are segregating in the same family, one is RTH due to the V264L mutation and the other is hypothyroidism with an unknown etiology.

2360/T

Pathogenic-Or-Not -Pipeline for prediction of disease relevance of variations and their mechanism. *M. Vihinen[1, 2], J. Härkönen[1], A. Olatubosun[1], T. Tiirikka[1], J. Väliaho[1], J. Thusberg[1].* 1) Institute of Medical Techology, Univ Tampere, Tampere, Finland; 2) Science Center, Tampere University Hospital, Tampere, Finland.

Sequencing laboratories are producing ever increasing number of sequence information, which include numerous genetic variations. Analysis of the effects of these variations has become the bottleneck in many fields of genetics, especially in clinical laboratories. As normal non-harmful variations appear at very high frequency, every individual has millions of variations. Only small portion of these are disease-related or associated. Pathogenic-Or-Not -Pipeline (PON-P) is an integrated computer tool that allows - analysis whether a variation is pathogenic or benign - and for those identified as benign the analysis of putative mechanism PON-P integrates a large number of existing tools developed by our group and others and produces a summary of the predictions. The tool allows also submissions of relative large numbers of cases. The program provides a detailed summary of the predictions and the combined end result based on machine learning approach. Currently the methods included are for annotations, identification of known SNPs, tolerance of mutations, effects to stability, aggregation and disorder tendency, as well as to protein localization. PON-P is freely available at http://bioinf.uta.fi/PON-P/.

2361/T

The inheritance of missense c.487A>G mutation in GJB2 gene in two Iranian families. *M. Falah[1], M. Houshmand[2], S. Akbaroghli[3], S. Mahmodian[1], H. Emamdjomeh[1], M. Farhadi[1].* 1) Department and Research Centre of ENT & Head and neck Surgery of Iran Medical University, Tehran, Iran; 2) National Institute for Genetic Engineering and Biotechnology , Tehran, Iran; 3) Tehran welfare Organization, Tehran, Iran.

Abstract: Background: Mutations in GJB2 gene are the most common cause of hereditary hearing loss. The majority of GJB2 mutations are recessive, but a few dominant mutations have been associated with hearing loss. This study introduces some new fact about M163V mutation in GJB2 gene in two Iranian families. Material and Methods: Genomic DNA of Two unrelated Iranian families with hearing loss were obtained from six family members and screened for GJB2 mutations with direct sequencing. Results: Fathers of both families showed late onset hearing impairment in third or fourth decade of the life, but hearing loss in children was early onset in both families with more severity rather than fathers. Also, one grandfather of every family showed late onset hearing loss in seven decade. The analysis of familial pedigree revealed anticipation in phenotype and autosomal dominant inheritance. There was a substitution of A to G in exon 2 at nucleotide 487(M163V). This mutation was heterozygous in fathers and children while mothers were normal. Discussion: Previously, M163V always introduced as unknown heterozygous not even as compound heterozygous. Researcher showed the produced protein of M163V failed in the formation of homotypic junctional channel. Due to other mutation in this nucleotide was reported as M163L in autosomal dominant inheritance that defects trafficking to the plasma membrane and increase cell death. Our finding can confirm the autosomal dominant inheritance of this mutation. This hypothesis was further supported by conservation of the methionine residue at position 163 across the 23 mammalian species.

2362/T

Tyrosinase-related Oculocutaneous Albinism (OCA1A/B): report of the G47D mutation in Colombia. *D. Sanabria[1], H. Groot[1], J. Guzman[2], MC. Lattig[1].* 1) Laboratorio de Genetica Humana Departamento de Ciencias Basicas Universidad de los Andes Bogota - Colombia; 2) Fundación Universitaria del Área Andina Bogota - Colombia.

Oculocutaneous Albinism (OCA) is an autosomic recessive inherited disorder characterized by lack of the pigment melanin in melanocyte-containing tissues such as hair, skin, and eyes. The enzyme tyrosinase, codified by the gene TYR, has been shown to be responsible for Oculocutaneous Albinism Type 1 (OCA1A/B). To date approximately 230 mutations have been described as responsible for OCA1A/B in different populations. The aim of this study is to further understand the history of albinism in Colombia by following the G47D mutation found in a family that lives in its capital, Bogota, and its relationship to individuals with OCA1 in the small town of Cienega - Boyaca located on the eastern Andes Mountains. The family with OCA1 in Bogota has two affected individuals, one with OCA1A and the other is diagnosed with OCA1B where both members are heterozygous for the G47D and the second mutation has not been found. Two individuals that were born and raised in the town of Cienega, diagnosed with OCA1 are homozygous recessive for the mutation G47D. After examining official town and church records of births, deaths, and marriages and by personal interviews with people from the town it could be concluded that the individuals from Cienega and the family in Bogota have a common ancestral parent who lived approximately during 1850. The G47D mutation has been previously reported on Hispanic populations such as Canary Islands, Puerto Rico, Cuba and Mexico and it is believed to have originated in the Moroccan Jew and Sephardic (Spanish) populations. Haplotype analysis is on the way to verify if the G47D mutation found in Colombia has the same founder origin as the Puerto Rican/Moroccan Jew populations.

2363/T

Co-inheritance of Beta-Thalassemia and Fragile-X Syndrome in a Turkish Family. *I. Keser[1], T. Bilgen[1], Y. Arikan[1], E. Mihci[2], O. Duman[3], A. Yesilipek[4], S. Haspolat[3].* 1) Med Biol & Gen, Med Faculty,Akdeniz Univ, Antalya, Antalya, Turkey; 2) Pediatric Genetics, Med Faculty,Akdeniz Univ, Antalya, Antalya, Turkey; 3) Pediatric Neurology, Med Faculty,Akdeniz Univ, Antalya, Antalya, Turkey; 4) Thalassemia Unit, Pediatric Hematology, Med Faculty,Akdeniz Univ, Antalya, Antalya, Turkey.

Background: Hemoglobinopathy and mental retardation are the most severe health problems in the populations. Beta-thalassemia and fragile X syndrome (FXS) are common diseases among hemoglobinopathies and inherited cause of mental retardation, respectively. In this study, we aimed to investigate and to report the co-inheritance and effect of beta-thalassemia and FXS in a Turkish family. Methods: Following the pedigree analysis, DNA extraction from peripheral blood, amplification and DNA sequencing of the beta-globin gene and fragment analysis for CGG repeats of the Fragile X Mental Retardation 1 (FMR1) gene were done for each member at risk in the family. Results: We found the expansion from premutation to full mutation of the CGG repeats of FMR1 gene and the co-inheritance of this CGG instability and codon 39 (C-T) mutation of beta-globin gene in the family with the first cousin consanguinity. While proband, 4 year old male, had full mutation and carrier for codon 39, his brother had premutation and beta-thalassemia major phenotype due to codon 39 homozygosity. Proband's father had carrier for codon 39 mutation and mother's had carrier for both codon 39 and premutation for FMR1 gene. Conclusion: Although the association of beta-thalassemia and other diseases such as Down syndrome has been reported, the co-inheritation of beta-thalassemia and fragile X syndrome had not been reported in literature. Our findings suggest that co-inheritance of both diseases which were transmitted by two different modes is important to discuss the clinical findings due to both diseases and to give genetic counseling to the families at risk.

2364/T

Identification of novel mutations in myosin VIIA gene in two Chinese families with either high- or low-frequency non-syndromic deafness. *H. Yuan[1], Y. Sun[1], J. Chen[1,2], H. Sun[1,3], J. Cheng[1], J. Li[1], Y. Lu[4], Y. Lu[1], Z. Jin[1], Y. Zhu[1], P. Dai[1], X. Liu[1,5], R. Wang[1].* 1) Institute Of Otolaryngology, Chinese PLA General Hospital, Beijing 100853, China; 2) Wellcome Trust Sanger Institute, Hinxton, United Kingdom; 3) Department of Otolaryngology, General Hospital of Chinese People's Armed Police Force, Beijing, 100039, China; 4) Department of Obstetrics and Gynecology, Chinese PLA General Hospital, Beijing 100853, China; 5) Department of Otolaryngology, University of Miami, Miami, FL, USA.

The Myosin VIIA (MYO7A) gene encodes a protein classified as an unconventional myosin. Mutations within MYO7A can lead to both syndromic and non-syndromic hearing impairment in humans. Among 340 different mutations reported in MYO7A, only five led to non-syndromic sensorineural deafness autosomal dominant type 11 (DFNA11). Here, we present the clinical, genetic, and molecular characteristics of two large Chinese DFNA11 families with either high- or low-frequency hearing loss. Affected subjects of family DX-J033 have a sloping audiogram at young ages at high frequency, and then affected all test frequencies with increased age. Affected members of family HB-S037 present with an ascending audiogram affecting low frequencies at young ages, and then all frequencies were involved, with increasing age. Genome-wide linkage analysis mapped the disease loci within the DFNA11 interval for both families. DNA sequencing of MYO7A revealed two novel nucleotide variations, c.652G>A (p.D218N) and c.2011G>A (p.G671S), in the two families. Neither of these two mutations was detected in DNA from 100 unrelated Chinese controls. Sequence alignments of MYO7A revealed that the two novel mutations are highly conserved across species and among different myosin classes. The identified mutations in MYO7A in the present study are the first reported cases to be implicated in DFNA11 in Chinese population. For the first time, we tested the Electrocochleography (ECochG) in DFNA11 family with low-frequency hearing loss and speculated that the low-frequency sensorineural hearing loss in DFNA11 family was not related to endolymphatic hydrops.

2365/T

Candidate gene approach in identification of disease-associated genes in Myofibrillar myopathy. *H. Lee[1], P. Serdaroglu-Oflazer[2], M. Olivé[3], Z. Odgerel[1], P. van der Ven[4], D. Fürst[4], L. Goldfarb[1].* 1) 1National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MarylandNINDS, NIH, Bethesda, MD; 2) Medical Faculty, Istanbul University, Istanbul, Turkey; 3) IDIBELL-Hospital Universitari de Bellvitge and CIBERNED Hospitalet de Llobregat, Barcelona, Spain; 4) Department of Molecular Cell Biology, Institute of Cell Biology, University of Bonn, Bonn, Germany.

Myfibrillar myopathy (MFM; OMIM # 601419) is a group of neuromuscular disorders caused by mutations in genes coding for sarcomeric Z-disk proteins. Dominant germline and de novo mutations in seven genes (DES, MYOT, LDB3, CRYAB, BAG3, FLNC, and FHL1) have been established as the cause of MFM, but a significant number of MFM patients failed to show disease causing mutations in routinely tested genes. Fourteen additional Z-disk associated proteins closely interacting with MFM-causing proteins are likely to be involved in MFM, although such association has not been reported yet. In order to maximize the efficiency of etiological search, we used 10K-genotyping based approach for molecular testing in large families. The family under study was a recently identified large kindred of Macedonian ancestry affected with an MFM-type myopathy. According to the family history, MFM has been transmitted in at least four consecutive generations. Segregation analysis demonstrated autosomal dominant inheritance. Genome-wide 10K single nucleotide polymorphism (SNP) genotyping was performed in 21 family members including 10 MFM patients. Linkage analysis excluded six known MFM-causing genes and 14 potential candidates (1.6 Mb in total) with LOD scores of less than -2. Suggestive linkage was found in the FLNC region which showed a seven point LOD score of 2.9. Subsequent DNA sequence analysis of the FNLC coding region was complicated by inconsistencies caused by the interference with an unknown by that time highly homologous FLNC pseudogene located on chromosome 7q32-q35, about 53.6 kilobases downstream of the functional FLNC gene in inverted orientation. Using optimized procedures, the disease-causing mutation was eventually uncovered in exon 48 of FLNC. This mutation cosegregated with the disease. In conclusion, genome wide linkage analysis in sufficiently large MFM-affected families can effectively exclude candidate genes and focus sequencing effort on a gene showing evidence of involvement.

2366/T

Identification of novel ENaC gene mutations in African patients with cystic fibrosis-like disease. *L. Mutesa Sr[1, 2], C. Verhaeghe[1], J.F. Vanbellinghen[1], V. Dhennin[3], A. Uwineza[2], H. Cuppens[4], C. Korbmacher[5], V. Bours[1].* 1) Dept Medical Genetics, Univ Liege, Belgium, Liege, Belgium; 2) Center for Medical Genetics, National University of Rwanda, Rwanda; 3) GIGA-Genomics Facility, University of Liège, Belgium; 4) Department of Human Genetics, Katholieke Universiteit Leuven, Belgium; 5) Institut für Zelluläre und Molekulare Physiologie, Universität Erlangen-Nürnberg, Germany.

The defect in chloride and sodium transport in cystic fibrosis (CF) patients is a consequence of CFTR loss of function and/or an abnormal interaction between CFTR and ENaC. A few patients were described with CF-like symptoms with a single CFTR mutation and/or an ENaC mutation. In our previous study (Mutesa L. et al., Genetic analysis of Rwandan patients with cystic fibrosis-like symptoms: identification of novel CFTR and ENaC gene variants. Chest 2009, 135:1233-1242), we have identified in small group of CF African patients several novel ENaC gene mutations in association with CFTR mutations. In the present study, we extensively performed ENaC genes sequencing of the remaining CF patients without any CFTR mutation. Several novel ENaC gene mutations were identified and some of them were located in highly conserved domains and consistent with a pathophysiological role. Our data suggest that CF-like syndrome in Africa could be associated with ENaC mutations.

2367/T

A novel NF1 gene mutation in an Italian family with neurofibromatosis type 1. *A. Gabriele[1], M. Ruggieri[2], M. Muglia[1], A. Patitucci[1], A. Magariello[1], R. Mazzei[1], F.L. Conforti[1], C. Ungaro[1], G. Di Palma[1], L. Citrigno[3], W. Sproviero[4], A. Gambardella[1,4], A. Quattrone[5].* 1) Institute of Neurological Science (ISN), National Research Council (CNR), Cosenza, Italy; 2) Department of Paediatrics, University of Catania, Catania, Italy; 3) Department of Neurosciences, Psychiatric and Anesthesiological Sciences, University of Messina, Messina,Italy; 4) Institute of Neurology, University Magna Graecia, Catanzaro, Italy; 5) Neuroimaging Research Unit, National Research Council, Catanzaro, Italy.

Neurofibromatosis type 1 (NF1) is a common autosomal dominant disorder with an estimated incidence of one in 3,500 births. Clinically, NF1 is characterized by café-au-lait (CAL) spots, neurofibromas, freckling of the axillary or inguinal region, Lisch nodules, optic nerve glioma, and bone dysplasias. NF1 is caused by inactivating mutations of the 17q11.2-located NF1 gene. We present a clinical and molecular study of an Italian family with NF1. The proband, a 10-year-old boy, showed large CAL spots and freckling on the axillary region and plexiform neurofibromas on the right side only. His father (47 years old) showed, in addition to the similar signs, numerous neurofibromas of various sizes on his thorax, abdomen, back, and shoulder. The brother and the sister of the proband presented only small CAL spots. In the examined family with NF1, a novel frameshift insertion mutation of one adenine at the nucleotide 654 (c.654 insA) in exon 4c was identified. This novel mutation creates a shift on the reading frame starting at codon 218 and leads to the introduction of a premature stop at codon 227 (PTC). The mutation was also detected in all affected members of the family but it was not found in 200 normal chromosomes. In a previous study, Toliat et al. reported a high incidence of mutations in exon 4b of the NF1 gene. Later, Griffiths et al. also reported mutations in exon 4b in addition to exons 10a, 13, 16, 31, and 37. Our data suggest that exon 4 can be considered a mutation hotspot of the NF1 gene even if it is not located in the two functional domains: Cys-Ser rich domain encoded by exons 11-17 and GAP-related domain encoded by exons 21-27a. The mutation reported in this study determines the production of an abnormal mRNA that contains a premature termination codon at codon 227. This abnormal mRNA could result in a shorter non-functional protein, or it could be disrupted by a cellular surveillance mechanism, Nonsense-mediated decay (NMD), preventing the translation of such mRNA into truncated, and potentially harmful, protein.

2368/T

CHD7 mutations in CHARGE syndrome: implication for the genotype and phenotype correlation. *Y. Ke[1,2], D. Lee[1], G. Ma[1], M. Chen[1,3,4].* 1) Department of Genomic Medicine, Changhua Christian Hospital, Changhua, Taiwan; 2) Department of Pediatrics, Changhua Christian Hospital, Changhua, Taiwan; 3) Department of Obstetrics and Gynecology, and Department of Medical Genetics, College of Medicine and Hospital, National Taiwan University, Taipei, Taiwan; 4) Department of Obstetrics and Gynecology, Changhua Christian Hospital, Changhua, Taiwan.

CHARGE syndrome is a rare autosomal dominant disorder with characteristic features including Colooma, Heart malformation, choanal Atresia, Retardation of growth and/or development, Genital abnormalities and Ear anomalies with or without hearing loss, which give the name (an acronym) to this condition. The prevalence of CHARGE syndrome is in one of 10,000 live born infants. The diagnosis of CHARGE syndrome is mainly based on the clinical findings and temporal bone imaging although the diagnostic criterion for CHARGE syndrome was recently up dated and was stressed the importance of the 3C triad (Coloboma-Choanal atresia-abnormal semicircular Canals). At present, mutations in the chromodomain helicase DNA binding protein coding gene, CHD7, are known to cause CHARGE syndrome. There are approximately 60-70% of patients with CHARGE syndrome have mutation in CHD7 gene. Here, we reported on the clinical and molecular findings in five unrelated families with CHARGE syndrome. Since all of the mutations identified were different and novel, we also discussed the possible genotype and phenotype correlation for this condition.

2369/T

Discordant monochorionic diamniotic twins carrying a mosaic NF1 mutation. *J.G. Pappas[1], E. Ward[1], J.C. Allen[2], J. Xie[3], A. Poplawski[3], L.M. Messiaen[3].* 1) Dept Pediatrics,Human Gen, New York Univ Sch Med, New York, NY; 2) Dept Pediatrics, Div Ped Hematology/Oncology, New York Univ Langone Med Ctr, New York, NY; 3) Dept Genetics, Univ Alabama at Birmingham, Birmingham, AL.

We present 12 year old monochorionic diamniotic twin girls, twin A with multiple café-au-lait macules, axillary freckling and optic glioma and twin B without signs of neurofibromatosis type 1 (NF1). They were delivered by cesarean at the 35th week of gestation due to twin to twin transfusion. Both are growing and developing normally, have identical facial features and similar anthropometrics. Twin A is under treatment for optic glioma diagnosed by brain MRI. Twin B had normal brain MRI as well as multiple negative clinical examinations for signs of NF1 including skin examinations using the Woods lamp. Mutation analysis for NF1 in the blood lymphocytes revealed a truncating mutation, c.3047-3048delGT, in mosaic form in both girls. Mosaicism for this mutation was also present in both twins in a different sample, i.e. cells from saliva. The mutation was found in ~25 % of the blood cells in both twins and in ~15% of the saliva cells in twin A, versus 8-10% of the saliva cells in twin B. To the best of our knowledge this is the first description of monochorionic diamniotic twins mosaic for an NF1 mutation, with one twin clinically unaffected at age 12. Possible explanations for the discordance include occurrence of the mutation before the formation of the germ layers followed by unequal ratios of mutant cells in the resulting twins or mosaicism in the apparently unaffected twin solely in the hematopoietic cells due to twin - twin transfusion. The former possibility predicates occurrence of a mutation very early during development, before the 9th day of gestation when monochorionic diamniotic twins split. Hence, generalized mosaic NF1 would have been expected as a phenotype in both twins. Biallelic mutations in the NF1 have been proven to be associated with the different phenotypic features (Maertens O et al, 2007) and further study of other tissues from different embryonic cell layers for the distribution of the NF1 mutation may give us insights for explaining the phenotypic discordance.

2370/T

Nonsense mutations of *COL3A1* gene causing nonsense-mediated mRNA decay in two Japanese patients with Vascular type of Ehlers-Danlos Syndrome. *B. Than Naing[1], A. WATANABE[1,2], A. HATAMOCHI[3], H. MORISAKI[4], T. SHIMADA[1,2].* 1) Department of Molecular Genetics, Nippon Medical School, Tokyo, JAPAN; 2) Division of Clinical Genetics, Nippon Medical School, Tokyo, JAPAN; 3) Department of Dermatology, Dokkyo Medical University, JAPAN; 4) Department of Bioscience, National Cerebral and Cardiovascular Center, JAPAN.

Vascular type of Ehlers-Danlos syndrome (vEDS), also known as EDS type IV (MIM#130050), is a life-threatening autosomal dominant inherited disorder of connective tissue mainly caused by mutations in type III collagen, COL3A1 gene (total 52 exons). The majority of identified mutations are point mutations that result in single amino acid substitutions for glycine in the GLY-X-Y repeat of the triple helical region. About one-third of the known mutations occur at splice sites, and most result in exon skipping. The mutation of COL3A1 gene in vEDS patients have been detected by reverse transcriptase PCR (RT-PCR) direct sequencing using total RNA from patient's fibroblasts. No mutation in the COL3A1 gene using total RNA method was detected in two Japanese patients with typical clinical features of vEDS; patient 1 is a 21 year-old male with family history of sudden death of a brother at 25 years of age and patient 2 is a 43 year-old male with no family history. We analyzed known single nucleotide polymorphisms in coding region (cSNPs) using genomic DNA. Heterozygosities were found in the genomic DNA at two cSNPs (c.2244T>C and c.3613A>G) in patient 1 and one cSNP (c.2092G>A) in patient 2. These sequence discrepancies between mRNA and genomic DNA strongly suggest nonsense-mediated mRNA decay (NMD) which degradates mRNA carrying premature translation termination codons. Therefore, we analyzed the whole coding region of COL3A1 gene by PCR direct sequencing using genomic DNA. A nonsense mutation was found in each patient: c.2992C>T; p.Gln998Ter at exon 42 in patient 1 and c.4141C>T; p.Gln1381Ter at exon 51 (outside of triple helical region of COL3A1 gene) in patient 2. The level of protein expression was different in each patient. In patient 2 family, the severity differences were present within the same family because no clinical findings were detected in the mother having the same mutation. This is the first report of nonsense mutations in the COL3A1 gene which cause nonsense-mediated mRNA decay. Many nonsense mutations have been reported in other collagen genes causing diseases. Our findings indicate that cSNP analysis of genomic DNA is highly informative when mutations could not be found by standard RT-PCR of mRNA from patients having typical vEDS symptoms. Sequence discrepancies at the cSNPs between mRNA and genomic DNA suggest NMD and therefore, sequencing of the whole coding regions should be performed using genomic DNA.

2371/T

PRF1 and STX11 Mutations in Familial Hemophagocytic Lymphohistiocytosis Patients. *A. Alpman Durmaz[1], F. Ozkinay[2], B. Durmaz[1], O. Kirbiyik[1], D. Yilmaz[3], N. Cetingul[3], C. Vergin[4], H. Akin[1], H. Onay[1], C. Ozkinay[1].* 1) Department of Medical Genetics, Ege University Medical Faculty, Izmir, Turkey; 2) Department of Pediatric Genetics, Ege University Medical Faculty, Izmir, Turkey; 3) Department of Pediatric Hematology, Ege University Medical Faculty, Izmir, Turkey; 4) Department of Pediatric Hematology, Dr. Behcet Uz Pediatric Hospital, Izmir, Turkey.

Familial hemophagocytic lymphohistiocytosis (FHL) is a rare autosomal resesif hyperinflammatory syndrome characterized by fever, hepatosplenomegaly, pancytopenia, hypertriglyceridaemia, hypofibrinogenaemia, and neurological abnormalities. Mutations in Perforin, Syntaxin and Munc13-4 genes are found to be associated with FHL-2, FHL-4 and FHL-3, respectively. In this study, we analyzed the PRF1 and STX11 genes in patients with FHL. Thirty-two patients were analyzed for PRF1 mutations by DNA sequencing. Patients who were found to be normal for PRF1 mutations were analyzed for STX11 mutations. Among 51 patients sequenced for PRF1 mutations, 3 patients were found to be heterozygous for A91V and 3 patients homozygous for W374X mutations. Twenty-three of the perforin mutation negative patients were available for STX11 gene mutation analysis and 2 patients were found to be heterozygous for E115D which was not previously reported. Two patients were found to be compound heterozygous for V197M and E182E. In one of the 2 patients after parental DNA sequencing, mutations were considered to be on the same allele. These 2 mutations were not described as a mutation or polymorphism before. STX11 and PRF1 mutations play an important role in FHL patients and molecular spectrum in these genes may be different in different populations.

2372/T

ABCG2/BCRP as a major causative gene for gout. *H. Matsuo[1], T. Takada[2], K. Ichida[3,4], T. Nakamura[5], A. Nakayama[1,6], Y. Takada[7], H. Inoue[1], Y. Kawamura[1], Y. Sakurai[8], T. Hosoya[4], H. Suzuki[2], N. Shinomiya[1].* 1) Dept. Integrative Physiol., National Defense Med. College, Tokorozawa, Japan; 2) Dept. Pharm., Univ. Tokyo, Tokyo, Japan; 3) Dept. Pathophysiol., Tokyo Univ. Pharm. Life Sci., Tokyo Japan; 4) Dept. Intern. Med., Jikei Univ. School Med., Tokyo, Japan; 5) Lab. Math., National Defense Med. College, Tokorozawa, Japan; 6) Dept. Defense Med., National Defense Med. College, Tokorozawa, Japan; 7) Dept. Forensic Med., National Defense Med. College, Tokorozawa, Japan; 8) Dept. Prev. Med. Publ. Health, National Defense Med. College, Saitama, Japan.

Gout based on hyperuricemia is a common disease with a genetic predisposition. Recent genome-wide association study also showed that serum uric acid (SUA) levels and gout relates to ABCG2 gene, which is reported to locate in a gout-susceptibility locus (MIM 138900) on chromosome 4q revealed by a genome-wide linkage study. We previously reported that ABCG2 is an exporter that has polymorphic reduced functionality variants. As ABCG2 exports nucleotide analogs structurally similar to urate, these findings suggest that ABCG2 could be a urate secretion transporter and a cause of gout. Mutation analysis of 90 Japanese hyperuricemia patients in ABCG2 revealed six nonsynonymous mutations: V12M, Q126X, Q141K, G268R, S441N and F506SfsX4. ATP-dependent transport of urate was reduced by approximately half (46.7%) in Q141K, and was nearly eliminated in Q126X, G268R, S441N and F506SfsX4. Among these variants, relatively frequent two dysfunctional SNPs, Q141K (31.9%) and Q126X (2.8%), were then analyzed. Haplotype frequency analysis revealed that there is no simultaneous presence of Q126X and Q141K in one haplotype. As Q126X and Q141K are assigned to nonfunctional and half-functional haplotype, respectively, their six genotype combinations are divided into five functional groups. Gout risk of 75% function was increased with an OR of 3.02 (95% CI, 1.96-4.65; P=2.29x10-7) and that of 50% function was with an OR of 4.34 (95% CI, 2.61-7.24; P=2.23x10-9). Gout risk of £25% function was remarkably increased with an OR of 25.8 (95% CI, 10.3-64.6; P=3.39x10-21). 10.1% of gout patients had these genotypes of £25% function, while only 0.9% of control males have the same genotype combinations. In addition, genotype combinations of full function are detected in 50.8% of the control subjects but only in 21.4% of gout patients. Our function-based genetic analysis showed that combinations of dysfunctional variants are major causes for gout, thereby providing evidence for "a common disease common variant" hypothesis. We will show the latest progress on our study in this meeting.

2373/T

Mutation screening in a cohort of Chinese families with hereditary nuclear cataract. *KJ. Wang[1, 2], SQ. Zhu[2], CP. Pang[1].* 1) Opthalmology & Visual Sciences, Chinese University of Hong Kong, Hong Kong, Hong Kong; 2) Beijing Tongren Eye Center, Beijing Tongren Hospital, Capital Medical University, Beijing Ophthalmology & Visual Sciences Key Lab, Beijing, China.

PURPOSE: To investigate the molecular genetic background in Chinese families with autosomal dominant nuclear cataract. METHODS: Members of thirty unrelated Chinese families with congenital nuclear cataracts and 100 unrelated individual adults without congenital cataracts were recruited for this study. All study subjects were given full ophthalmological examinations. The genomic DNA was extracted from peripheral blood leukocytes. We conducted two-point linkage analysis and sequencing of 18 cataract candidate genes to find the causative mutations. RESULTS: There were variations in the clinical phenotypes among families but all involved nuclear opacifications. Among the thirty families, six showed nuclear cataract and microcornea. Mutation analysis revealed seven causative mutations, five novel and two reported. Five families (16.7%) have affected crystalline genes, CRYAA-R116C, R116H, CRYBB2-V146M, I21N, CRYBB1- R233H. Two families (6.7%) had mutations in the GJA8 gene, I31T and S258F. These mutations co-segregated with all affected individuals in the respective families and were not observed in unaffected family members or the 100 controls. CONCLUSIONS: Mutations were identified in 7 out of the 30 families (23.3%). All these mutations involve in crystallins and connexins. The CRYBB2 mutation (V146M) was first identified in this study to be associated with congenital cataract and microcornea. Our results, with 67.7% of families not identified for mutations in reported genes, strongly indicates the genetic heterogeneity of congenital cataracts, although the crystalline genes appear to be more commonly associated with hereditary nuclear cataracts than other genes in the Chinese population.

2374/T

Inheritance patterns in myofibrillar myopathy associated with a BAG3 mutation. *Z. Odgerel[1], A. Sarkozy[2], HS. Lee[1], C. McKenna[2], J. Rankin[3], V. Straub[2], H. Lochmüller[2], F. Paola[4], A. D'Amico[4], E. Bertini[4], K. Bushby[2], LG. Goldfarb[1].* 1) National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD; 2) Institute of Human Genetics, Newcastle upon Tyne, U.K; 3) Department of Clinical Genetics, Royal Devon and Exeter NHS Foundation Trust, Exeter, U.K; 4) Department of Neurosciences, Ospedale Bambino Gesu', Rome, Italy.

Myofibrillar myopathies (MFMs) are a group of heterogeneous disorders defined by disintegration of myofibrils. These disorders are known to be associated with mutations in DES, CRYAB, MYOT, ZASP, FLNC, FHL1, or BAG3. We conducted a study of the underlying mechanisms of disease transmission in three families with early onset MFM caused by BAG3 p.Pro209Leu mutation. Haplotype analysis, informative in Family 1, indicated that two affected siblings inherited the pathogenic allele from their asymptomatic father, suggesting that the father was germline mosaic. Transmission from asymptomatic parents also occurred in Families 2 and 3, although the transmitting parent could not be identified. The father of the two affected boys in Family 1 displays somatic mosaicism for the BAG3 p.Pro209Leu mutation. A recurrent transmission from an unaffected parent indicates that the mutation is present in a significant proportion of his germ cells. Somatic mosaicism for pathogenic mutations has important ramifications for counseling offered to families regarding the risk to younger children. Since the disease associated with the BAG3 p.Pro209Leu mutation starts in the childhood and results in incapacity and death before reaching reproductive age, transmission from affected individuals is unlikely.

2375/T

Molecular analysis in Belgian patients with late-onset glycogen storage disease type II. *W. Lissens, S. Seneca, S. Van Dooren, I. Liebaers.* Ctr Med Gen, UZ Brussel, Brussels, Belgium.

Glycogen storage disease type II (GSDII or Pompe disease) is an autosomal recessive disorder caused by a deficiency of the lysosomal alfa-glucosidase (GAA). Clinically, the disease represents a continuous spectrum of phenotypes from rapid progressive early-onset subtypes to late-onset milder subtypes. We present here the results of molecular analysis of all coding exons and parts of the flanking introns of the GAA gene in 16 unrelated patients, aged between 25 and 59 years, with late-onset GSDII on clinical and biochemical bases. All 16 patients were carriers of the leaky splice site mutation c.-32-13T>G (previously described as IVS1-13T>G) that is known in literature to occur at a high frequency in adult-onset patients of Caucasian origin. The mutations on the other alleles comprised 9 small deletions/insertions/duplications with c.258dupC present in 3 patients, 4 missense (3 not previously described) and one nonsense mutation, one donor splice site mutation and one deletion from I17 to I18. The independent segregation of the mutations was proven in all families. Our results confirm the high incidence of the c.-32-13T>G mutation in Caucasian patients with late onset GSDII. Therefore, mutation analysis in late-onset GSDII patients could be started by sequencing for the presence of this mutation in our population. The other mutations are scattered all-over the GAA gene; many of these mutations are insertions or deletions of a few bases. We would like to thank the Neurologists who have sent samples from their patients for molecular analysis.

2376/T

Mutation Analysis of the FRAS1 and FREM2 gene in Fraser Syndrome using Roche 454 GS-FLX System. *M.J. Vogel[1], P.H.A. van Zon[1], A.M.H. Langemeijer-Ketelaar[1], I.J. Nijman[2], E.E. Voest[3], E.P.J.G. Cuppen[1,2], P.J. Scambler[4], M.M. van Haelst[1], J.K. Ploos van Amstel[1], Fraser Syndrome Collaboration Group.* 1) Department of Medical Genetics, University Medical Center Utrecht, Utrecht, The Netherlands; 2) Hubrecht Institute-KNAW and UMC Utrecht, The Netherlands; 3) Department of Medical Oncology, UMC Utrecht, The Netherlands; 4) Molecular Medicine Unit, Institute of Child Health, London, United Kingdom.

Fraser syndrome (FS, MIM 219000) is an autosomal recessive malformation disorder characterized by cryptophthalmos, syndactyly, and abnormalities of the respiratory and urogenital tract. FS is considered to be the human equivalent of the murine blebbing mutants. In these mice mutations at five loci cause a phenotype that is comparable to FS in humans. Thus far mutations in two homologous human genes, FRAS1 and FREM2, have been identified to cause FS. FRAS1 contains 74 exons and encodes an extracellular matrix protein of 4,011 amino acids. FREM2 (FRAS1-related extracellular matrix protein 2) has 24 exons and encodes a protein of 3,169 amino acids. Recently, mutations in FREM1 were identified in patients with a congenital malformation syndrome consisting of bifid nose, renal agenesis and anorectal malformations. This condition may represent a previously unrecognized variant of FS. FREM1 has 37 exons encoding a protein of 2,197 amino acids. To date, a causative mutation has been detected in approximately 45% of the FS cases, of which the majority comprised FRAS1. Only 2 different FREM2 mutations have thus far been reported. The size of these genes makes the analysis of the genes laborious. A proper DNA-diagnostics for this syndrome is much needed as is a better insight in the genotype-phenotype relationship of Fraser Syndrome and related phenotypes. We therefore, developed a test on the 454 GS-FLX massive parallel sequencing (MPS) platform from Roche that allows simultaneously testing of large genes. Such a test could easily be expanded in the future with additional candidate genes. We have initially set up a test for FRAS1 and FREM2. This test involves 395 amplicons that cover all exons and their flanking sequences. The amplicons are pooled and subjected to MPS. We have analyzed six FS cases and identified mutations in the FRAS1 gene in three probands and FREM2 mutations in two probands. Seven out of eight of these mutations have not been reported before. Although the number of patients investigated in this study is small, the percentage of FREM2 families identified is remarkable (33%). All mutations that were identified could be confirmed by Sanger sequencing. In only one family we could not identify a pathogenic mutation in either of these two genes, although further improvement of coverage will be needed to strengthen this conclusion. In two cases the identification of the disease causing mutations led to prenatal testing.

2377/T

A COMMON PATTERN OF BRAIN MRI IMAGING IN MITOCHONDRIAL DISEASES WITH COMPLEX I DEFICIENCY. *A. Lebre[1], M. Rio[1], L. Faivre d'Arcier[1], D. Vernerey[1], P. Landrieu[2], A. Slama[2], C. Jardel[3], P. Laforêt[3], D. Rodriguez[4], N. Dorison[4], D. Galanaud[3], B. Chabrol[5], V. Paquis-Flucklinger[6], D. Grévent[1], S. Edvardson[7], J. Steffann[1], B. Funalot[8], N. Villeneuve[5], V. Valayannopoulos[1], P. de Lonlay[1], I. Desguerre[1], F. Brunelle[1], J.P. Bonnefont[1], A. Rötig[1], A. Munnich[1], N. Boddaert[1].* 1) Université Paris Descartes, AP-HP Hôpital Necker-Enfants Malades et Inserm U781 et U797, Départements de Génétique, de Radiologie pédiatrique et des Maladies du développement, Paris F-75015 France; 2) Université Paris XI, AP-HP Hôpital Bicêtre, Départements de Neurologie pédiatrique et de Biochimie, Kremlin-Bicêtre F-94270 France; 3) AP-HP Hôpital Pitié-Salpêtrière, Départements de Biochimie et de Radiologie et Institut de myologie, Paris F-75013 France; 4) UPMC Univ Paris 06, AP-HP Hôpital Armand Trousseau-La Roche-Guyon et Inserm UMR 975, Département de Neuropédiatrie, Paris F-75012, France; 5) Université de Marseille, AP-HM Hôpital de la Timone-Enfants, Départements de Neurologie et de pédopsychiatrie pédiatrique, Marseille F-13000, France; 6) Université de Nice Sophia Antipolis, Hôpital Archet 2, Département de Génétique Médicale, Nice F-06000, France; 7) Pediatric Neurology, Hadassah Hebrew University Medical Center, Jerusalem, Israel; 8) Université de Limoges, Hôpital Universitaire Dupuytren, Département de Neurologie, Limoges F-87000, France.

Objective: To identify a consistent pattern of brain MRI imaging in primary complex I deficiency. Complex I deficiency, major cause of respiratory chain dysfunction, accounts for various clinical presentations, including Leigh syndrome. Human complex I comprises seven core subunits encoded by mitochondrial DNA (mtDNA) and 38 nuclearly-encoded core subunits (nDNA). Moreover, its assembly requires six known and many unknown assembly factors. To date, no correlation between genotypes and brain MRI phenotypes has been found in complex I deficiencies. Design and Subjects: We have retrospectively collected the brain MRI of 30 patients carrying known mutation(s) in genes involved in complex I and compared them with the brain MRI of 11 patients carrying known mutation in genes involved in pyruvate dehydrogenase (PDH) complex and 10 patients with MT-TL1 mutations. Results: All complex I deficient patients showed bilateral brainstem lesions (30/30) and the 77% (23/30) showed putaminal anomalies. Supratentorial stroke-like lesions were only observed in complex I deficient patients carrying mtDNA mutations (8/19) and necrotizing leukoencephalopathy in patients with nDNA mutations (4/5). Conversely, the isolated stroke-like images observed in patients with MT-TL1 mutations, or corpus callosum dysmorphism observed in PDH deficient patients were never observed in complex I deficient patients. Conclusion: We identified a common pattern of brain MRI imaging with signal abnormalities in brainstem and subtentorial nuclei with lactate-peak as a clue of complex I deficiency. We suggest that combining clinico-biochemical data with brain imaging can help orient genetic studies in complex I deficiency.

2378/T

Mutations in quinone biosynthesis pathway results in up-regulation of CABC1 gene. *C. MEILLER, A. MUNNICH, A. ROTIG.* INSERM U781, Université René Descartes Paris V.

Quinone (CoQ10) plays a pivotal role in the mitochondrial respiratory chain (RC) as it distributes electrons between the various dehydrogenases and the cytochrome segments of the RC. CoQ10 deficiencies represent a rare cause of mitochondrial disorders. We have found CoQ10 deficiency in eight unrelated families and identified in the different patients mutations in various genes encoding enzyme of the ubiquinone biosynthesis pathway: PDSS1, PDSS2, COQ2 and CABC1. All these genes are ubiquitously expressed but their mutations are related to different clinical presentations: deafness, mental retardation and obesity for PDSS1 mutations, deafness, nephrotic syndrome, mental retardation, myopathy, ataxia for PDSS2 mutations, neonatal fatal neurological distress and liver failure for COQ2 mutations and ataxia for CABC1 mutations. The mechanisms leading to this tissue specific expression of the disease are yet unknown but it could be hypothesized that different expression level of these genes in different tissues could partially account for this feature or that specific regulations result from mutations of a specific gene. As a first step in investigating tissue-specific expression of quinone deficiency we analyzed the expression levels of all genes known or supposed to be involved in quinone biosynthesis in cultured skin fibroblasts of patients with PDSS1, PDSS2, COQ2 and CABC1 mutations. Total RNA extracted from fibroblasts of the eight patients and a control were reverse transcribed and the expression level of PDSS1, PDSS2, COQ2, COQ3, COQ4, COQ5, COQ6, COQ7, COQ8, COQ9, COQ10A, COQ10B, CABC1, ADCK1, ADCK2, ADCK4, ADCK5 genes was established by quantitative PCR. In all patients we observed a strong increase of CABC1 expression level and to a lesser extend of COQ3. CABC1 protein has a putative kinase activity based on the identification of four kinase conserved motifs in its aminoacid sequence but its biochemical function is currently unknown. It has been shown in yeast that COQ8, the counterpart of CABC1 has a regulatory function of quinone biosynthesis pathway. The up-regulation of CABC1 gene in fibroblasts of patients with quinone biosynthesis deficiency confirms the central role of CABC1 in this biosynthesis pathway. Finally, CABC1 has been shown to be a direct target of p53 and it should be hypothesized that this gene also acts as a sensor of RC deficiency.

2379/T

Mitochondrial genetics and sepsis. *A. Pyle[1], S.V. Baudouin[2, 3], P.F. Chinnery[1].* 1) Mitochondrial Research Group, Newcastle University, Newcastle upon Tyne, NE2 4HH, United Kingdom; 2) Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, NE2 4HH, United Kingdom; 3) Department of Anaesthesia, Royal Victoria Infirmary, Newcastle upon Tyne, NE1 4LP, United Kingdom.

Infectious disease has had a dramatic impact on human evolution. Our genetic makeup partially determines our ability to resist infection. There is emerging evidence that mtDNA plays an integral role in the evolution of the human species. Several studies have reported changes in mitochondrial function during severe infection. Mitochondrial DNA is highly polymorphic; as a result there is considerable variation in the mtDNA genome both within and between human populations. Consequently, mtDNA is divided into a discrete number of mtDNA haplogroups. A comprehensive analysis of mtDNA haplogroups and sepsis found that patients who were haplogroup H are more likely to survive after admission to an intensive care unit. The functional cause of these associations remains uncertain but there is increasing evidence that these inherited genetic variations influence respiratory chain efficiency. Mitochondrial copy number alterations have also been found in a number of diseases, with a reduction in mtDNA copy number observed in sepsis patients. This reduction has been attributed to the reciprocal increase in blood neutrophils after infection. The mtDNA content of circulating monocytes and lymphocytes is decreased in the acute phase of sepsis. It has been suggested that mtDNA depletion could serve as a prognostic marker in acute sepsis, or directly relevant to the mechanisms of survival. The mitochondrial genome has been much less studied in patients with infection and sepsis. Here we describe a mtDNA genetic study into the polymorphic changes in sepsis patients.

2380/T

Poikiloderma with Neutropenia: delineation of novel mutations and geographic clusters. *E.A. Colombo[1], G. Roversi[2], N. Elcioglu[3], G. Fremont[4], L. Volpi[5], L. Larizza[1]*. 1) Dipartimento Medicina, Chirurgia e Odontoiatria, Università di Milano, Milano, Italy; 2) Unit of Medical Genetics, Fondazione IRCCS, Istituto Nazionale Tumori, Milano, Italy; 3) Department of Pediatric Genetics, Marmara University Hospital, Istanbul, Turkey; 4) Departement of Dermatology, Hôpital Saint-Louis, Paris, France; 5) Dipartimento di Biologia e Genetica per le Scienze Mediche, Università degli Studi di Milano, Milano, Italy.

Patients affected with Poikiloderma with Neutropenia (OMIM #604173) show persistent neutro penia, early onset poikiloderma, palmoplantar keratosis and a peculiar pachyonychia on big toes.

Recently genome-wide scan of an inbred Italian pedigree identified genetic linkage to the16q13 and targeted sequencing appointed the *C16orf57* disease gene. Three mutations of the gene were described: c.502A>G, c.504-2A>C and c.666_676+1del12: surprisingly all affect the splicing process leading to complete skipping of exon 4, exon 5 and exon 6, respectively**1**.

We herein describe three new patients with non-splicing mutations in the *C16orf57* gene: i) a French woman of NorthAfrican origin has a homozygous mutation in exon 2 c.179delC, predicting a truncated product 113 aminoacids long (p.Pro60LeufsX55). The same mutation has been just described by Tanaka in three Moroccan siblings**2**, suggesting either geographic consanguinity or founder effect. ii) Two Turkish PN boys carry a homozygous c.531delA deletion in exon 5, predicting a truncated product (p.His179-MetfsX86). They are both born from inbred families but no common ancestor and not even a common origin from a geographic isolate could be traced back.

It's not yet clear if this autosomal recessive genodermatosis results from complete haplo insufficiency or a residual protein activity is sustained by hypomorphic mutations. Delineating the mutational repertoire of the gene is one of the tools suitable to unravel its function. Including the present report only 10 out of 29 PN patients have the mutation identified**1,2**; we don't know the genetic alteration/s specific to the Navajo population**3,4** neither to the other reported Turkish patients**4,5** but it could be interesting to establish whether different ethnic groups have private founder mutations and whether the clinical phenotype is modulated by the type of mutation.

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1 Volpi L. et al. (2010) AJHG 86: 72-6; **2** Tanaka A. et al. (2010) Am J Med Genet 152A: 1347-8; **3** Clericuzio C. et al. (1991) AJHG 49: A661; **4** Wang LL. et al. (2003) Am J Med Genet 118A: 299-301; **5** Van Hove JL. et al. (2005) Am J Med Genet 132A: 152-8.

2381/T

Mutation Analysis of FBN1 Gene in Marfan sydrome Probands and Patients with Marfanoid Habitus or Fibrillinopathy. *A. Kuskucu[1], K. Oztarhan[2], K. Yararbas[3], S. Gursoy[3], A. Tukun[3]*. 1) Medical Genetics, Bakirkoy Women and Children's Hospital , Bakirkoy, Istanbul, Turkey; 2) Pediatric Cardiology, Bakirkoy Women and Children's Hospital , Bakirkoy, Istanbul, Turkey; 3) Duzen Laboratory Groups,Turkey.

Marfan syndrome (MFS) is an autosomal dominant connective tissue disorder with a high degree of clinical variability, usually caused by a mutation in the fibrillin 1 (FBN1) gene. Cardinal manifestations involve the ocular, skeletal, and cardiovascular systems. Using sequencing analysis methodology, we studied the mutation spectrum in 20 probands with Marfan syndrome and related phenotypes. It is well known that mutations in the FBN1 gene cause MFS and have been associated with a wide range of milder overlapping phenotypes. A proportion of patients carrying a FBN1 mutation does not meet enough diagnostic criteria for MFS, and are diagnosed with fibrillinopathy or Marfanoid habitus. We would like to report our findings and related genotype phenotype correlations.

2382/T

Genetics of Primary Dystonia. *T. Fuchs[1], K.M. Lamar[1], D. Raymond[2], R. Saunders Pullman[2], N. Sharma[3], I. Peter[1], S. Bressman[2], L.J. Ozelius[1]*. 1) Gen & Genomic Sci, Mount Sinai Sch Med, New York, NY; 2) Department of Neurology, Beth Israel Medical Center, New York, NY; 3) Department of Neurology, Massachusetts General Hospital, Boston, MA.

Primary torsion dystonia (PTD) is a movement disorder characterized by involuntary twisting, repetitive movements and abnormal postures where dystonia is the only neurological feature. Seven genes have been mapped for PTD but only two have been identified: TOR1A which is responsible for DYT1 - early onset generalized dystonia and THAP1, which is responsible for DYT6 - dystonia of mixed type. Deciphering the genetic basis of primary dystonias is a critical step in understanding the molecular pathophysiology of the disease and finding a better treatment or cure for the disease. Our program for deciphering the genetic basis of PTD comprises multiple gene discovery approaches including traditional linkage analysis and positional cloning, candidate gene association studies, genome wide association (GWAS) and next generation sequencing. Recently, using the positional cloning approach, we discovered THAP1 as the gene underlying DYT6 dystonia. An insertion/deletion mutation in this gene was shown to be causative founder mutation in Amish-Mennonite families. Additionally, THAP1 missense, nonsense and truncating mutations were found in families and sporadic individuals of diverse ethnic origins. We performed a whole genome scan and linkage analysis in a cervical dystonia family with 8 affected individuals which identified four putative linkage regions. The enriched exome libraries of these regions will be constructed and sequenced using next generation sequencing. Polymorphisms in the TOR1A/TOR1B region have been implicated as being associated with primary focal and segmental dystonia. In a cohort of subjects with either focal or segmental dystonia we found a strong association between rs3842225 SNP in TOR1A and protection from focal dystonia. In order to find additional genes involved in etiology of focal dystonias, we genotyped approximately 100 Ashkenazi Jews focal dystonia samples using Affymetirix 6.0 platform. The GWAS is being performed on these data.

2383/T

Uniparental disomy and progressive clonal selection a common mechanism causing late onset β-thalassemia major? *C.L. Harteveld[1], C. Rifaldi[2], A. Giambona[3], C. Ruivenkamp[1], M. Hoffer[1], C. Borgnia-Pignatti[4], A. Maggio[3], M. Cappellini[2], P.C. Giordano[1]*. 1) Human and Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands; 2) Fondazione Ospedale Maggiore Policlinico Istituto di Ricovero e Cura a Carattere Scientifico Universita di Milano, Milano, Italia; 3) U.O.C. Ematologia II con Talassemia, Azienda Ospedaliera Vincenzo Cervello Via Trabucco 180, 90146 Palermo, Italia; 4) Department of Clinical and Experimental Medicine-Pediatrics University of Ferrara, Ferrara, Italy.

We present three cases of "severe late onset β-thalassemia" in three independent subjects, all presenting with the mild phenotype of beta thalassemia minor up to adult age and developing a severe transfusion dependent phenotype in the third and fourth decade of life when a presumed homozygosity for the cd39 (C®T) mutation is observed. We demonstrate that uniparental isodisomy of part of chromosome 11p15 accounts for the observed mosaicism for the cd 39 (C®T) mutation. Clonal selection for hematopoietic stem cells containing the uniparental isodisomy for the mutant beta-globin gene during life may account for the progressive development of the disease. We base our conclusion on the fact that we have observed in all cases molecular rearrangements on the short arm of chromosome 11, together with sequences showing T/T homozygosity in the presence of a small but consistent C signal in DNA extracted from peripheral blood, from buccal mucose and from erythroid cultures. Loss of heterozygosity due to a deletion of one allele was excluded by MLPA analysis. In addition, two subjects had a father who was a carrier and a non-carrier mother and one male subject was married and had 3 children of which only one was a carrier. A similar observation for a single case was made by Chang et al., who found this in a single patient with late-onset β-thalassemia Major. Our study demonstrates that uniparental isodisomy of chromosome 11p15 is apparently more frequently associated with late-onset transfusion dependent β-thalassemia in presumed carriers at birth, representing a novel mechanism leading to this special form of beta-thalassemia and possibly in other late-onset genetic diseases. Chang et al. Haematologica 2008, 93(6):913-916.

2384/T

RGR and USH2A Digenic Recessive Retinitis Pigmentosa Causes an Early Onset Stable Retinal Phenotype. *P. Bitoun[1], E. Pipiras[2], B. Benzacken[2], A. Delahaye[2].* 1) Gen Med, CHU Paris-Nord, Hopital Jean Verdier, Bondy, France; 2) Embryo-Cytogenetics and Assisted Reproductive Biology, CHU Paris-Nord, Hopital Jean Verdier, Bondy, France.

Purpose: Identification of causal genes by Molecular analysis of Retinitis Pigmentosa patients DNA allows for characterisation of disease , prognostic factors, prenatal diagnostic possibilities if indicated and possible treatment by gene replacement therapy or pharmacologic substances. A cohort of 25 RP patients was screened for recessive, dominant or X-linked RP genes mutation depending on the family history/clinical presentation using automated chip analysis. Materials and Methods: Patients were examined clinically and had ERG VEP OCT and color vision testing and gave informed consent for genotyping. For patients with a clinical or pedigree phenotype compatible with a recessive disorder The following genes were tested for a total of 584 known mutations and SNP's : CERKL, CNGA1, CNGB1, MERTK, PDE6A,PDE6B, PNR,RDH12, RGR,RLBP1, SAG,TULP1, CRB, RPE65, USH2A, USH3A, LRAT using automated genechip analysis. Results:A 19 year old young woman with early onset but slowly progressive RP and ERG photopic and scotopic anomalies was identified with a digenic mutation in both RGR and USH2A genes as follows RGR, exon 4:454 C>A which predicts H152N HET Mut. USH2A exon 12: 2137ÝG>C which predicts G713R HET Mut.She has normal hearing. RGR is an RPE retinal G-protein coupled receptor also found in Muller cells. It is a Rhodospin homolog which unlike rhodopsin binds all trans retinal converted to 11-cis retinal by light . it is implicated in recessive RP and dominant choroidal sclerosis. USH2A/ usherin is a basement membrane protein, with laminin EGF and fibronectin type III domains, found in many tissues including capillary and structural basement membranes in retina and inner ear; USH2A causes 30-40% of Usher syndrome type 2 and 10-15% of recessive RP. The putative interaction of these 2 proteins to cause the RP phenotype warrants further investigation Discussion: Digneic RP has been rarely reported in a handful of patients with RDS/Peripherin and ROM1 since 1994 as well as Peripherin 2 and ROM1 ; RHO and RP1 ans also SP4 and GNB1 rod Transducin . Digenic invovlment has also been described in albinism. Triallelism has been rarely identified in Bardet-Biedl syndrome and in digenic RP with USH2A with PDZD7. The availability of multiplex genechip analysis and next gen sequencing allows testing of multiple genes to characterise patients with digneic disorders and better understand gene interaction in the retina and their role in phenotype and pathogenesis of RP.

2385/T

Molecular Characterization of Beta-Globin Gene Mutations in Filipino Beta-Thalassemia Patients. *C.L.T. Silao[1,2], M.C.C. Manuel[3], M.L.T. Naranjo[4], R.P. Laude[3], P.D. Fajardo[2], E.M.A. Melendres[2], C.D. Padilla[1,2], E.dJ. Yuson[4].* 1) Institute of Human Genetics, National Institutes of Health, Manila, Philippines; 2) Department of Pediatrics, University of the Philippines-Philippine General Hospital, Manila, Philippines; 3) Institute of Biological Sciences, College of Arts and Sciences, University of the Philippines Los Baños, Laguna, Philippines; 4) Thalassemia Center, Fe del Mundo Medical Center, Quezon City, Philippines.

BACKGROUND: Beta-thalassemia is an autosomal recessive disorder marked by deficiency or absence of synthesis of the beta-globin chain of the hemoglobin protein. It is caused by the inheritance of one or two diseased alleles of the human beta-globin gene on chromosome 11. Clinical manifestations include anemia and in extreme cases, splenomegaly, bone deformities, impaired growth, and endocrinopathies among others. This study characterizes the spectrum of beta-globin gene mutations in a Filipino population. METHODS: This involves 35 Filipino beta-thalassemia patients detected through complete blood count, red blood cell indices, and hemoglobin electrophoresis or HPLC. Genomic DNA was extracted either from dried blood spots or from whole blood using the Qiagen QIAamp DNA Blood Mini/Midi Kit. Polymerase chain reaction (PCR) analysis was used to detect the 45 kb beta thalassemia deletion and direct sequencing was done to determine other beta globin gene mutations. RESULTS: The 45 kb deletion was confirmed to be the most common mutation found. It was detected in 22/70 (31 percent) alleles analysed. This was followed by codon 67 (-TG) in 8 alleles (11 percent). CONCLUSIONS: Filipino beta-thalassemia patients show considerable heterogeneity, both phenotypically and genotypically. Obtained data were useful for genetic counseling.

2386/T

Microcytic anemia in three siblings associated with a nonsense codon of STEAP3 and low expression of the normal allele. *B. Grandchamp[1,5], C. Balser[2], C. Kannengiesser[1,5], G. Hetet[1], C. Oudin[1], E. Kohne[3], C. Beaumont[4], H. Heimpel[5].* 1) Department of Biochemistry and Genetics, CHU Bichat, Paris, France; 2) Department of Hematology and Oncology, Erlenring Center, Marburg, Germany; 3) Department of Pediatrics, University Hospital Ulm, Ulm, Germany; 4) INSERM UMR 773, University Paris Diderot, Paris, France; 5) Department Internal Medicine III, University Hospital Ulm, Ulm, Germany.

Three siblings, two boys and a girl born to healthy parents from Pakistan origin had a transfusion-dependent poorly regenerative anemia and iron overload. A moderate microcytosis with distinct anisopoikilocytosis was present. All siblings had repeated viral and bacterial infections. Thalassemia, Congenital Dyserythropoietic Anemia , sideroblastic anemia and other known genetic causes were excluded and several candidate genes were sequenced from DNA of the proband. STEAP3, an iron reductase involved in the iron-transferrin endocytic cycle was found to be mutated with a premature STOP codon (c.C110X) at the heterozygous state. The same mutation was found in the 2 other affected siblings and their unaffected father. We did not detect any abnormality in the maternal alleles. Interestingly, a genome-wide expression study showed that STEAP3 displays significant allele-specific expression that is associated with SNPs in the 3' flanking region of the gene (Emilsson V et al, Nature, 2008: vol 452: 423431, supplementary 5c). We studied the relative expression of STEAP3 alleles in immortalized lymphocytes from family members after treatment the cells with emetin to prevent the degradation of the mutated allele resulting from nonsense mediated mRNA decay. We found that the relative expression of the normal allele is about twice higher in the father than in his three affected offspring. Quantitative PCR from blood RNA confirmed that STEAP3 mRNA is significantly more decreased in the affected siblings than in their father. We confirmed that STEAP3 expression in blood from healthy unrelated volunteers is highly variable, allele specific and associated to 3'UTR SNPs. Recent reports demonstrated that allele specific expression is a rather common finding that may concern 5-20% of the genes. The coinheritance of a mutated and a low-expressed normal allele has been previously reported in erythropoietic protoporphyria and in pyropoïkylocytosis and may mimic either a dominant or a recessive transmission depending on the frequency of the low expressed allele in the population. We postulate that STEAP3 deficiency leads to severe anemia in human and may result from the genotypic combination of a mutated allele and a low expressed allele.

2387/T

Pathogenetic mechanism of Diamond-Blackfan Anemia (DBA). *I. Dianzani[1], A. Aspesi[1], E. Pavesi[1], I. Boria[1], F. Avondo[1], P. Roncaglia[2], M. Caterino[3], D. Lazarevic[2], M. Ruoppolo[3], S. Gustincich[2], C. Santoro[1].* 1) Dept Med Sci, Univ Piemonte Orientale, Novara, Italy; 2) International School for Advanced Studies (SISSA/ISAS), Trieste, Italy; 3) Department of Biochemistry and Medical Biotechnologies, Università degli Studi di Napoli Federico II, Napoli, Italy.

DBA (MIM 105650) is a congenital, autosomal dominant, hypoplasia of erythroid progenitors often associated with physical malformations. Though rare this disease is interesting for hematologists because it is the prototype of pure erythroid aplasia. Half of the patients respond to steroid therapy. Non-responders require lifelong blood transfusions. DBA is the first ribosomapathy ever reported. In approximately 50% of cases it is due to heterozygous mutations in a ribosomal protein (RP) gene of the small (RPS19, RPS24, RPS17, RPS26, RPS10) or the large (RPL5, RPL11, RPL35A) subunit. The link between mutated RPs and defective erythropoiesis is unknown. To shed light on the molecular alterations responsible for the disease we compared gene and protein expression profiles in control and RP haploinsufficient cells. Differentially expressed genes were expected to reveal which pathways are impaired in DBA. We used human erythroleukemic cell line TF1 expressing inducible siRNA against RPS19, the gene most frequently mutated in DBA. This model is widely employed in DBA studies since proper amounts of bone marrow patient cells are not available and mouse models do not show a hematological phenotype. A proteomic analysis by DIGE on RPS19 downregulated TF1 cells was also performed. Results show an abnormal expression of proteins involved in cytoskeleton organization, protein synthesis and apoptosis. These variations may be due to multiple cellular mechanisms that we decided to investigate. Transcriptome analysis was performed using Affimetrix microarrays to assess whether abnormal transcription was present. Validation of a set of DIGE and microarray results were carried out by Western blot and qRT-PCR, respectively, on RPS19 downregulated cells (both TF1 and CD34+ cord blood derived cells). A previous study from our group showed that fibroblasts from patients with mutations in RPS19 presented expression anomalies as compared with control fibroblasts (Avondo et al 2009). This study showed that expression anomalies are not limited to hemopoietic cells in DBA. We have compared the expression profile from these cells and that from TF1 cells downregulated for RPS19. Notwithstanding the different cell type and model we found a common set of genes that were dysregulated: they represent a molecular signature of RPS19 deficiency, that may be used to develop a diagnostic assay.

2388/T

Identification of a Novel *C16orf57* Mutation in Athabaskan Patients with Poikiloderma with Neutropenia, Clericuzio-Type. *L. Wang[1], K. Harutyunyan[1], S. Plon[1], R. Erickson[2], A. Irvine[3], R. Bagatell[4], T. Griffin[5], T. Shwayder[6], C. Clericuzio[7].* 1) Dept Ped, 1102 Bates St., Suite 1200, Baylor College of Medicine, Houston, TX. 77030; 2) Departments of Pediatrics and Molecular and Cell Biology, University of Arizona Health Science Center, 1501 N. Campbell Ave, Tucson, AZ 85724; 3) Departments of Clinical Medicine, Trinity College Dublin and Paediatric Dermatology Our Lady's Children's Hospital Crumlin, Dublin 12 Ireland; 4) Division of Oncology, The Children's Hospital of Philadelphia, 3501 N. Civic Center Blvd., Philadelphia PA 19104; 5) Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, Department of Pediatrics, 3333 Burnet Avenue, Cincinnati, Ohio 45229; 6) Department of Dermatology, Henry Ford Hospital, 3031 W Grand Blvd, Suite 800, Detroit, MI 48202; 7) Department of Pediatrics, University of New Mexico School of Medicine, MSC10 5590, Albuquerque, NM 87131.

Background: Poikiloderma with Neutropenia (PN), Clericuzio-Type (OMIM #604173) is characterized by poikiloderma, nail dystrophy, neutropenia, recurrent sinopulmonary infections, and chronic bronchiectasis. First described by Clericuzio in 1991 in 14 patients of Navajo descent, it was initially referred to as "Navajo Poikiloderma." Subsequently, it was described in non-Navajo patients and was renamed "Poikiloderma with Neutropenia." Recently, *C16orf57* was identified as the causative gene mutated in three separate PN kindreds. The purpose of the current study was to confirm the presence of *C16orf57* mutations in a small cohort of PN subjects including several subjects of Athabaskan (Navajo and Apache) ancestry. **Patients and Methods:** Nine subjects from seven kindreds were enrolled in an IRB-approved study at Baylor College of Medicine. One subject from each family was studied. Three subjects were of Athabaskan ancestry (100% Navajo, 100% Apache, and 25% Navajo, respectively). PCR amplification and sequencing of the *C16orf57* gene was performed on genomic DNA using previously published primers and conditions (Volpi et al, Am J Hum Genet 2010; 86:1-5) and included the entire coding region and conserved splice site junctions of the gene. **Results:** We identified deleterious mutations in *C16orf57* in all PN subjects analyzed in our cohort. The seven mutations consisted of deletion (2), nonsense (3), and splice site (2) mutations. Four subjects were homozygous and three subjects were compound heterozygous for mutations. The three subjects of Athabaskan ancestry all had a deletion mutation (c.496delA) which was not found in the four other non-Athabaskan subjects. The full Athabaskan subjects were homozygous for this mutation while the quarter Navajo subject was compound heterozygous for this mutation and another deletion mutation. **Conclusions:** Mutations in the *C16orf57* gene have been identified thus far in all described subjects carrying the clinical diagnosis of PN. We have identified a new mutation (c.496delA) present in all three subjects of Athabaskan descent, suggesting that this mutation represents the underlying cause of PN in this sub-population of patients.

2389/T

Characterizing expression of Proteus marker proteins in skin using immunohistochemistry. *M.J. Lindhurst[1], J.C. Sapp[1], C.R. Lee[2], L.G. Biesecker[1].* 1) GDRB, NHGRI/NIH, Bethesda, MD; 2) NCI/NIH, Bethesda, MD.

Proteus syndrome (PS) is a rare disorder that is hypothesized to be caused by a mosaic gene alteration lethal in the non-mosaic state. The phenotype of PS is highly variable. Objective diagnostic criteria have been published that has allowed for a more precise definition of the disorder. These criteria require: a mosaic distribution of lesions, a sporadic occurrence, and a progressive course of the disease plus a combination of characteristic manifestations. Characteristic manifestations include cerebriform connective tissue nevi (CCTN), linear epidermal nevi (LEN), vascular malformations, disproportionate overgrowth, and others. While it is straightforward to recognize a PS lesion in an affected patient or to recognize a histological section from a patient with PS as abnormal, a cellular phenotype had not been established. Recently, we reported the results of a micorarray experiment comparing cultured skin fibroblasts from affected PS lesions to skin fibroblasts cultured from non-PS skin. We found seven genes, *CD9*, *COL14A1*, *COL15A1*, *COL21A1*, *COL6A3*, *EML1*, and *FBN2*, that were up-regulated in the PS-affected cultures. When qRTPCR was used with a larger sample set to validate the micorarray results, *CD9*, *COL14A1*, *COL21A1*, and *EML1* showed significant differential expression. Immunohistochemistry using antibodies to CD9, COL15A1, and FBN2 showed increased staining of dermal fibroblasts and the surrounding extracellular matrix in five plantar CCTN sections from four PS patients when compared to plantar skin controls from five non-PS individuals. This staining was non-uniform within a section and varied among the PS and control sections. Presently, we are characterizing the staining in more detail to see if additional structures within the skin show any differential expression and to look for correlations between the areas with increased staining and other characteristics such as morphology or staining of other types of skin markers. In addition, we are testing antibodies to COL21A1 and COL6A3 to see if they are up-regulated in vivo. We are also examining skin biopsies taken from other areas, both affected and unaffected, of PS patients and are planning to compare the staining to other types of collagenomas and lesions found in other overgrowth syndromes. This survey will help not only in understanding the biology of skin lesions in PS, but may also lead to the development of markers that can be used to distinguish different overgrowth syndromes.

2390/T

Homozygosity Mapping is an efficient strategy for mutation identification in Consanguineous Families with a Genetically Heterogenous Disease. *R. Segel[1,2], S. Zeligson[1], T. Falik-Zaccai[3,4], S. Perlberg[1], M. Neufeld[5], P. Cohen[5], P. Renbaum[1], S. Zuckerman[1], A. Nir[2,6], E. Levi-Lahad[1,2].* 1) Medical Genetics Institute, Shaare Zedek Medical Center, Jerusalem, Israel; 2) Hebrew University, Jerusalem, Israel; 3) Institute of Human Genetics, Western Galilee Hospital-Nahariya, Nahariya, Israel; 4) Rappaport Faculty of Medicine, Technion, Israel Institute of Technology, Haifa, Israel; 5) Department of Ophthalmology, Shaare Zedek Medical Center, Jerusalem, Israel; 6) Department of Pediatric Cardiology, Hadassah University Medical Center, Jerusalem, Israel.

Molecular diagnosis in patients with genetically heterogeneous diseases is challenging and costly. We report the use of genome-wide Homozygosity Mapping (HM) using Affymetrix 250K Nsp arrays in two families, each with a genetically heterogeneous autosomal recessive disease. The strategy was to determine homozygous regions associated with the disease, and sequence only the known disease gene(s) within such regions. We identified and sequenced only one candidate gene in each family, and identified the disease-causing mutations, which were both novel. Family 1: A consanguineous Arab family in which two siblings were diagnosed with Xeroderma Pigmentosum (XP). The diagnosis was confirmed using a functional DNA repair assay with the affected patients' cells. XP is caused by mutations in at least nine genes. HM of two affected children and their healthy brother detected eight regions of homozygosity. Only one known XP gene, ERCC5, was present in one of these homozygous regions. ERCC5 sequencing identified a novel frameshift mutation, generating a stop codon located three amino acids downstream. Family 2: A consanguineous Arab family with isolated anophthalmia. Mutations in four genes are known to cause isolated anophthalmia, but these account for only a small proportion of all cases, indicating further genetic heterogeneity. HM of three affected patients indicated one homozygous area (chr14 68,922,826 - 94,033,531), which encompasses CHX10, a known isolated anophthalmia gene. CHX10 seqeuncing revealed a novel missense mutation in a highly conserved residue. Computational analysis indicated that this mutation is likely to cause a conformational change of functional significance in the tertiary structure of the protein. Discussion: The cases described illustrate that HM is an efficient first step in pursuing molecular diagnosis for genetically heterogenous diseases in consanguineous families. In highly consanguineous populations, HM often reveals multiple regions of homozygosity, but as these cases demonstrate, this approach can still pinpoint a single gene to be sequenced from among the gene set known to be associated with a specific disease, significantly reducing the time and cost of genetic analysis in such families. Since HM is also useful in outbred populations (Hildebrandt F et al, PLoS Genet., 2009), this strategy may have general utility as well.

2391/T

The spectrum of PORCN mutations in Focal Dermal Hypoplasia in a cohort of 31 consecutive diagnostic cases. *G. Richard, I. Olivos-Glander, D. Ho, S. Bale.* GeneDx, Gaithersburg, MD.

Focal Dermal Hypoplasia (FDH) is an X-linked disorder with highly variable features due to faulty focal development of ectodermal and mesodermal tissues resulting from a block in Wnt signaling. Greater than 90% of affected individuals are females, who harbor a de novo mutation in the PORCN gene that facilitates of the secretion of Wnt signaling proteins. All affected males, and some females, are reported to be mosaic for a post-zygotic PORCN mutation. Such mosaic mutations can be challenging to detect. To date, 59 distinct PORCN mutations and 6 patients with microdeletions involving several exons or the entire PORCN gene have been published. Our clinical diagnostic laboratory has performed molecular testing for FDH in 31 individuals by sequence analysis of the PORCN gene combined with deletion testing with exon-level resolution using a custom-design microarray ('ExonArrayDx'). 10 of 26 females referred to GeneDx with a diagnosis of FDH were found to be heterozygous for a novel PORCN mutation identified by DNA sequencing. In addition, one female from a 3-generation family of affected females had a genomic deletion of the entire PORCN gene. Follow-up genome-wide aCGH was recommended to determine the exact boundaries of the genomic deletion and its gene content. The mutation spectrum in our cohort included 2 single-base pair deletions, 3 nonsense, 1 splice site, and 3 missense mutations. One of these, Gly342Asp was observed in two unrelated patients. Each of the missense variants altered highly conserved residues in the membrane-bound O-acyltransferease domain, where all previously reported missense mutations are located. No mutation or gene deletion was identified in the remaining 15 females nor any of the 5 males submitted for PORCN testing. Nevertheless, low-level mosaicism for a PORCN mutation or deletion could be missed, and analysis of DNA derived from tissues other than blood (e.g. cultured fibroblasts) was recommended. The mutation distribution we observed was comparable to earlier reports, with the majority of mutations being either gene deletions (9%) or mutations leading to premature stop of translation (55%), and the remainder (36%) being missense mutations presumably resulting in loss of protein function. This experience shows that DNA sequencing combined with gene copy number analysis is an efficient protocol for mutation identification in FDH in a clinical diagnostic laboratory, and improves the sensitivity of diagnostic testing.

2392/T

Terminal Osseous Dysplasia is caused by a single recurrent mutation in the FLNA gene. *J.T. Den Dunnen[1], Y. Sun[1], R. Almomani[1], E. Aten[1], J. Celli[1], J.W.F. van der Heijden[1], H. Venselaar[2], S.P. Robertson[3], A. Baroncini[4], B. Franco[5], L. Basel-Vanagaite[6], E. Horii[7], R. Drut[8], Y. Ariyurek[1], M.H. Breuning[1].* 1) Human & Clinical Genetics, Leiden University Medical Center, Rotterdam, Netherlands; 2) NCMLS, UMC Nijmegen, the Netherlands; 3) Department of Paediatrics and Child Health, Dunedin School of Medicine, Dunedin, University of Otago, New Zealand; 4) Unit of Medical Genetics, ASL Department of Maternal and Child Health, Imola, Italy; 5) Medical Genetics, Department of Pediatrics, Federico II University, Naples, Italy; 6) Schneider Children's Medical Center of Israel and Raphael Recanati Genetics Institute, Rabin Medical Center, Tel Aviv University, Tel Aviv, Israel; 7) Nagoya 1st Red Cross Hospital, Nagoya, Japan; Yokkaichi Municipal Hospital, Yokkaichi, Japan; 8) Servicio de Pathologia, Hospital de Niños, 1900 La Plata, Argentina.

Terminal Osseous Dysplasia (TOD) is a rare X-linked male-lethal disease characterized by skeletal dysplasia of the limbs, pigmentary skin defects and recurrent digital fibroma with onset in infancy. Females have variable severity and 100% skewed X-inactivation. Mainly because only a few cases are known world-wide, attempts to identify the disease-causing gene were hitherto not successful. We applied the new possibilities of next-generation sequencing combined with exome capture to sequence all X-chromosome genes. In the female patient analysed we identified in total 351 exonic and 25 splice site variants. Combining all available information, incl. limited published linkage data, segregation, X-inactivation and computational analysis of the variants identified, a novel variant in the FLNA gene emerged as the most likely cause of the disease. The variant was on the inactivated X-chromosome and was found in all TOD cases we could analyse; 6 unrelated cases (three families and three sporadic cases). In all cases the variant segregated with the disease and it was transmitted 4 times from a mildly affected mother to a more seriously affected daughter. The mutation identified, the last nucleotide of an exon, was suspected to interfere with normal splicing. Since the variant allele was inactivated in all patient samples analysed, we were not able to analyse the effect on RNA level. However, expression was detectable when we cultured cells obtained from an archived sample; fibroma tissue surgically removed 15-year ago. In this sample, the mutation of the normal splice donor site turned out to activate an exonic cryptic site, removing the last 48 nucleotides from the exon. At the protein level this results in a loss of 16 amino acids, predicted to remove a sequence at the surface of filamin repeat 15. Mutations in FLNA are known to cause a range of diseases with partially overlapping phenotypes (see the FLNA sequence variant database we established at www.LOVD.nl/FLNA). The unique consequences of the TOD mutation we identified might explain why it results in a clearly distinct phenotype. Overall our data strongly support the conclusion that TOD is caused by a single recurrent mutation in the FLNA gene.

2393/W

A comprehensive evaluation of whole genome sequence data in five HapMap individuals, inclusive of one parents-child trio. *L.T. Guey[1], M.E. Schaffer[1], J.M. Laramie[1], K. Pant[2], D. Ballinger[2], S. Lincoln[2], D.R. Cox[1], A.B. Seymour[1].* 1) Applied Quantitative Genotherapeutics, Pfizer Inc., Cambridge, MA; 2) Complete Genomics Inc., Mountain View, CA.

Whole genome sequence data was evaluated in five individuals used in the International HapMap Project (four of European ancestry [NA12878, NA12891, NA12892, NA06990] and one of African ancestry [NA18507]), three of which comprise a father (NA21891) - mother (NA12892) - child (NA12878) trio. DNA from these HapMap individuals was sequenced by Complete Genomics Inc. with a nanoarray-based short-read sequencing-by-ligation technology. The median depth of coverage by mapped reads was 45- to 52-fold across all samples and variants were called using local de novo assembly on approximately 96% of the genome for each sample. We estimated a Mendelian inheritance error (MIE) rate of 7.4x10^-6 from 20,396 trio inconsistencies, which is well above the estimated mutation rate of 1.1x10^-8. A similar MIE rate was observed when the analysis was limited to the exome, or the coding regions of the genome. MIEs were more likely to have significantly lower depth of coverage and lower “variation scores” from the assembly software. These measures could be used in future analyses to identify potential errors. Lastly, a comparison of the data with 3.7 million genotypes generated by the HapMap project showed an overall concordance rate of 99.5%. Specifically, variants called reference homozygote and non-reference homozygote had overall concordance rates of 99.9% and 99.5% respectively while variants called heterozygote had a concordance rate of approximately 98%.

2394/W

Metabolic SNPs and Nicotine and Cotinine Metabolism. *A.W. Bergen[1], H.S. Javitz[1], M. Michel[1], R. Krasnow[1], D. Nishita[1], C.N. Lessov-Schlaggar[2], C.L. Sanders[3], N.J. Markward[3], E.J. Stanek[3], F.W. Freuh[3], R.D. Hockett[4], J. Kaufman[4], N. Benowitz[5], G.E. Swan[1].* 1) Center for Health Sciences, SRI International, Menlo Park, CA; 2) Department of Psychiatry, Washington University School of Medicine, St Louis, MO; 3) Personalized Medicine Research and Development, Medco Health Solutions, Bethesda, MD; 4) Affymetrix, Inc., Sunnyvale, CA; 5) Departments of Medicine, Bioengineering and Therapeutic Sciences, University of California, San Francisco, CA.

One billion individuals currently smoke tobacco worldwide and millions of individuals die each year from tobacco-related illness. All available interventions will be needed to meet the demand for effective, sustainable tobacco control, including pharmacogenetically-informed treatments for smoking cessation. Investigation of both the time course of nicotine concentration in the body (pharmacokinetics) and the response of the brain and body to nicotine (pharmacodynamics) will be part of developing such treatments. The nicotine metabolic ratio (NMR), the ratio of the nicotine metabolites trans-3'-hydroxycotinine to cotinine, is modestly associated with measures of smoking intensity and smoking cessation. We performed a pharmacogenetic study of the fixed-dose NMR utilizing the Affymetrix DMET™ Plus Assay, which interrogates over 1900 markers at 225 drug metabolizing enzyme and transporter genes, in a sample of 336 individuals (twins and siblings) from 159 sibships who have received a standardized dose of deuterium-labeled nicotine and cotinine and from whose blood samples nicotine metabolite levels were measured using liquid chromatography-mass spectrometry. The plasma NMR mean (SD) and range was 0.26 (0.13) and 0.04-0.79, respectively. We performed hierarchical linear modeling of NMR estimates adjusted for age, gender, smoking status, menopausal status, reproductive hormone use and BMI, and of twenty-two common (minor allele frequency ≥ 0.05) biallelic non-insertion-deletion polymorphisms at CYP2A6, CYP2B6, FMO3, UTG1A4 and UGT1A9, genes which are known to code for proteins involved in nicotine metabolism. We observed associations of P£0.0001 at rs28399433 (CYP2A6 -47G>T) and rs4803418 and rs4803419 (CYP2B6 IVS4+1735C>G and +2332C>T), uncorrected for multiple testing of polymorphisms or models. Retrospective power calculations from this modest study support known gene associations with the NMR, but suggest that the power to detect novel associations with substantially less impact on NMR variance is more limited.

2395/W

Cellular genetic approaches to defining drug toxicity pathways. *N. Butz[1], O. Suzuki[1], B. Steffy[1], D. Scoville[1], B. Parks[2], J. Trask[2], R. Thomas[2], T. Wiltshire[1].* 1) Division of Pharmacotherapy and Experimental Therapeutics, University of North Carolina at Chapel Hill, Chapel Hill, NC; 2) The Hamner Institute for Health Sciences, Research Triangle Park, NC.

Despite recent success in defining pharmacogenetically relevant gene variants, genomic determinants of drug toxicity are poorly understood. To identify quantitative trait genes critical to drug response phenotype we developed a novel high-throughput screening platform using cultured primary embryonic fibroblasts from the Mouse Diversity Panel. A library of 70 compounds (pharmaceutical drugs and environmental toxins) has been profiled for cytotoxicity utilizing high content imaging and simultaneous evaluation of cell health parameters at a single cell level across 32 mouse inbred strains. Genome-wide association analyses between observed cellular phenotypes and known genotypes were performed using haplotype association mapping to identify quantitative trait loci (QTLs) influencing cellular responses to tested compounds. This approach generated robust and reproducible results yielding significant inter-strain variation for all measured phenotypic endpoints. We have discovered a large number of QTL and successfully defined in silico high-confidence candidate genes. For instance, a locus underlying phenotypic variations in cell viability after rotenone exposure revealed a gene encoding member A3 of the ATP-binding cassette family (Abca3) involved in transport and drug response. In vitro validation of target genes using overexpression and siRNA knock down technology is currently underway. Gene enrichment analysis allowed association of these haplotypes to specific signaling pathways underlying toxicological responses. We have identified a network enriched for cell viability assay endpoints from combined set of candidate genes in 20 compounds screen that is centered on p53 as a key node, possibly indicating a common toxicity pathway.

2396/W

A genome wide association study (GWAS) to identify markers of weight gain and response to Pregabalin (Lyrica) in Fibromyalgia. *C. Hyde[1], S. John[2], P. Banerjee[3].* 1) Clinical Research Statistics, Pfizer, Groton, CT; 2) 2Human Genetics, Molecular Medicine, Pfizer Worldwide Research and Development, Groton, CT; 3) 3Human Genetics, Molecular Medicine, Pfizer Worldwide Research and Development, New York, NY.

FM is a symptomatic condition that is characterized by musculoskeletal pain, multiple tender points, disordered sleep, fatigue and represents an area of significant unmet medical need. Lyrica is an alpha 2 delta ($\alpha 2\delta$) ligand that has analgesic, anxiolytic and anticonvulsant activity and has been developed primarily for the treatment of epilepsy, relief of neuropathic pain and FM. Upon administration of Lyrica patients experience weight gain, but variability has been observed. Similarly, variability in response to the drug has also been observed, and there are currently no predictors of response. We carried out a GWAS in 1120 individuals diagnosed with FM enrolled in three clinical trials of Lyrica, genotyping 501,550 Single Nucleotide Polymorphisms (SNPs) using the Perlegen 600K chip. The aim was to determine if there are common DNA variants that would have value in predictive response to Lyrica. We analyzed four statistical models to determine whether SNPs genotyped were associated with weight gain or efficacy. No markers in any of the four models reached statistical significance on a genome-wide basis, the most significant of the p-values being in the range of p<1.0e-06. Therefore, either there are no SNPs that have an effect on weight gain or efficacy, or the largest true effects are below what we were powered to detect in this cohort of 1160 individuals. To increase confidence in our results from the first dataset, we also ran a meta-analysis with a fourth clinical trial (N=686) on the efficacy data and did not observe any markers significant at a genome-wide level (one marker - rs12143420 had a p = 4.15e-06). We also analyzed a set of candidate genes based on prior evidence of involvement in obesity (for weight gain) as well genes in the mechanistic pathway of Lyrica including the alpha 2 delta ($\alpha 2\delta$) genes. This was done to facilitate hierarchical multiple testing corrections. The set of candidate gene SNPs comprised a total of 499 SNPs for the weight gain analysis and 239 SNPs for the efficacy analysis. For both the weight gain and efficacy analysis, no SNPs were statistically significant. Thus, the CACNA2D1 and CACNA2D2 genes ($\alpha 2\delta$ genes - targets for Lyrica) are not involved in weight gain or efficacy. Based on these results, we can conclude there are unlikely to be any common DNA variants that influence weight gain or efficacy with large effect sizes that can be utilized for therapeutic intervention in the administration of Lyrica.

2397/W

Association between *GIRK3* gene polymorphisms and postoperative analgesic requirements after major abdominal surgery. *D. Nishizawa[1], M. Nagashima[2], R. Kato[2], Y. Sato[3], M. Tagami[3], S. Kasai[1], Y. Ogai[1], J. Hasegawa[1], M. Hayashida[4], K. Ikeda[1].* 1) Molecular Psychiatry Reseach, Tokyo Institute of Psychiatry, Tokyo, Japan; 2) Department of Surgery, Toho University Sakura Medical Center, Sakura, Japan; 3) Department of Anesthesiology, Toho University Sakura Medical Center, Sakura, Japan; 4) Department of Anesthesiology, Saitama Medical University International Medical Center, Hidaka, Japan.

Objectives: Opioids are commonly used as effective analgesics for the treatment of acute and chronic pain. However, considerable individual differences have been widely observed in sensitivity to opioids. To explore polymorphisms responsible for the inter-subject difference in the opiate sensitivity, we performed an association analysis focusing on the SNPs in *GIRK3*, which is the gene encoding a subtype of G protein-activated inwardly rectifying potassium (GIRK) channel, known to play a key role in transmitting the analgesic effects of opioids. **Methods**: In our initial polymorphism search, a total of ten single-nucleotide polymorphisms (SNPs) were identified in the whole exon, 5'-flanking, and exon-intron boundary regions of the *GIRK3* gene. The subjects in the association study were a total of 112 patients with written informed consent who underwent major open abdominal surgery in hospitals and were treated with analgesics including opioids after surgery. The study protocol was approved by the Institutional Review Board at each related Institute. Total genomic DNA was extracted from peripheral blood or oral mucosa samples by standard procedures and used for genotyping. **Results and Discussion**: In an association study, carrying T alleles in the C1781T SNP and in the C1817T SNP were significantly associated with decreased postoperative 24-h analgesic requirements. The total dose (mean±SEM) of rescue analgesics converted to equivalent fentanyl doses was 1.10±0.23 and 0.61±0.11 μg/kg for the C/C and combined C/T and T/T genotypes in the C1781T SNP, respectively (p=0.035), and 1.00±0.16 and 0.40±0.10 μg/kg for the C/C and combined C/T and T/T genotypes in the C1817T SNP, respectively (p=0.002), suggesting that the subjects carrying T allele in these SNPs have higher analgesic sensitivity. The results indicate that these SNPs could serve as markers that predict decreased analgesic requirements. Our findings will provide valuable information for achieving satisfactory pain control and open new avenues for personalized pain treatment.

2398/W

Polymorphisms in BCRP, MRP2 and OATP1B1 Drug Transporters Influence Cerivastatin Transport. *B. Tamraz[1,2], H. Fukushima[3], D. Kroetz[3], PY. Kwok[1,2].* 1) Cardiovascular Research Institute, UCSF, San Francisco, CA; 2) Institute for Human Genetics, UCSF, San Francisco, CA; 3) Department of Bioengineering and Therapeutic Sciences, UCSF, San Francisco, CA.

Genetic variation in drug metabolizing enzymes and membrane transporters can modulate the beneficial, as well as the deleterious, effects of drugs. In a study of patients who developed rhabdomyolysis while taking the HMG-CoA reductase inhibitor, cerivastatin, we sought to identify genetic variants that might explain the high incidence of rhabdomyolysis associated with this medication. We re-sequenced three drug transporter genes, ABCC2 (coding for MRP2), ABCG2 (coding for BCRP) and SLCO1B1 (coding for OATP1B1) involved in efflux and uptake of cerivastatin and identified 10, 4 and 7 non-synonymous coding variants in ABCC2, ABCG2 and SLCO1B1 genes respectively. These variants were suspected to have functional consequences for cerivastatin transport. To test the hypothesis that the variants in these transporters affect the transport of cerivastatin, we stably transfected HEK293 cells with plasmids containing the variants, created for all three transporters by site-directed mutagenesis, and measured the function of the transporter in in vitro cellular assays. Our data on the OATP1B1 uptake shows that 6 polymorphism and three haplotypes (2 novel) cause a significant reduction in cellular uptake of Cerivastatin compared to wild type. This cellular uptake functional data along with data on variants of MRP2 and BCRP and their influence on cellular efflux of cerivastatin in stably transfected HEK293 cell lines will be presented.

2399/W

Pharmacogenetic determinants of 6-mercaptopurine metabolites concentrations variability during maintenance therapy for children with acute lymphoblastic leukemia. *T. Adam de Beaumais[1], M. Fakhoury[1], Y. Medard[1], S. Azougagh[1], D. Zhang[1], K. Yacouben[2], E. Jacqz-Aigrain[1].* 1) Pharm Dept, Robert Debre Hosp, Paris, France; 2) Haematology Dept, Robert Debre Hospl, Paris, France.

6-mercaptopurine (6-MP) is an inhibitor of de novo purine synthesis widely used in acute lymphoblastic leukemia (ALL) maintenance treatment. Its metabolites concentrations in red blood cells (RBC) were related to the risk of toxicities and relapses. We investigated the impact of pharmacogenetic polymorphisms affecting thiopurine methyltransferase (TPMT) and inosine triphosphate pyrophosphatase (ITPA) on 6-MP metabolites concentrations variability. Their relation with hepatotoxicities occurrence during maintenance therapy was analyzed. Sixty-six children treated according to EORTC 58951 protocol were included. Genotypes for TPMT*2, *3B, *3C, ITPA 94C>A and IVS2+21A>C were performed. RBC 6-thioguanine nucleotides (6-TGN) and 6-methylated mercaptopurine nucleotides (6-MMPN) concentrations were measured by HPLC technique and their variability evaluated by mixed-models. Six patients were TPMT heterozygous (4 TPMT*1/*2, 1 TPMT*1/*3A and 1 TPMT*1/*3C). Mutated allelic frequencies were 0.07 for ITPA 94C>A and 0.13 for ITPA IVS2+21A>C. TPMT heterozygous patients had 6-TGN levels 2.25 fold higher than wild-type patients (estimation and standard error: 757±69 versus 336±34 pmol/8x108RBC; p<0.0001). Inversely, TPMT wild-type patients had 2.25 fold higher 6-MMPN concentrations than heterozygous patients (11290±1219 versus 5010±2459 pmol/8x108RBC; p=0.006). A combined effect of TPMT and ITPA 94C>A on 6-MMPN concentrations was identified: lowest 6-MMPN concentrations were observed in patients with a multilocus TPMT variant/wild-type ITPA genotype (mean ± standard deviation: 1862±1850 pmol/8x108RBC), wild-type patients for both genes had concentrations 4.5 fold higher and the highest concentrations reached for patients wild-type TPMT/variant ITPA genotype (16468±9467 pmol/8x108RBC). A 6-MMPN concentrations threshold was defined by ROC curve at 5000 pmol/8x108RBC above which there is a great risk of hepatotoxicities. Our findings support the importance of TPMT polymorphism in 6-MP concentrations variability and suggest a potentially clinically relevant combined impact of TPMT and ITPA 94C>A on 6-MMPN concentrations related to hepatotoxic risk.

2400/W

Functional impact of a common copy number variant in dexamethasone induced apoptosis. *J. Aigner[1], S. Villatoro[1], R. Rabionet[1], L. Armengol[2], E. Marti[1], X. Estivill[1].* 1) Center for Genomic Regulation, Barcelona, Spain; 2) Quantitative Genomics, Barcelona, Spain.

Structural variations including copy number variants (CNVs) have been recognized as an important source of human genetic diversity. Phenotypic effects of CNVs are commonly observed, but the functional consequences of structural variations in general, and more specifically, on sensitivity to drug treatment have been poorly assessed so far. In the present study we describe the functional analysis of a 45-kb deletion CNV, and the genotype-based different consequences after treatment with the glucocorticoid dexamethasone. Glucocorticoids are steroidal anti-inflammatory agents that are widely used to treat several clinical diseases, including inflammatory and autoimmune diseases, organ transplantation and cancer. The 45-kb CNV deletion affects two genes of the same family, BTNL8 and BTNL3 and results in a new, in-frame, fusion mRNA (BTNL8*3). The presence of the CNV affects the expression levels of several genes, e.g. MMP9, FPR1, PDGFRB and PML, including down-regulation of both, BTNL3 and BTNL8, and the neighboring gene BTNL9. These three affected genes belong to the family of butyrophilin-like proteins, which are expressed in haematopoetic-linage cells. Expression of all three mRNAs was significantly induced after dexamethasone treatment in lymphoid cell-lines. Moreover, cells homozygous for the wild-type allele showed a significant increase in dexamethasone-induced apoptosis rate and over-expression of GFP-BTNL9 fusion-protein in (-/-) cells was sufficient to rescue this phenotype. BTNL9 has been shown to be upregulated in acute lymphoblastic leukemia and in germinal center B-cell like-type of diffuse large B cell lymphoma. For both cancers, glucocorticoids are an important component of the standard treatment protocols. Therefore, BTNL8*3 and BTNL9 protein expression status may provide useful clinical information in terms of clinical outcome of lymphoid malignancies and numerous other diseases following treatment with dexamethasone.

2401/W

PharmGKB: A decade of catalyzing pharmacogenomics research. *K. Sangkuhl[1], M.W. Carrillo[1], L. Gong[1], J.M. Herbert[1], C.F. Thorn[1], M. Gong[1], F. Liu[1], R. Tang[1], R. Whaley[1], M. Woon[1], T.C. Truong[1], T. Zhou[1], R.B. Altman[1,2], T.E. Klein[1].* 1) Department of Genetics, Stanford University, Palo Alto, CA; 2) Department of Bioengineering, Stanford University, Stanford, CA.

The Pharmacogenomics Knowledge Base (http://www.pharmgkb.org) marked its 10th anniversary as the NIH supported public online resource devoted to disseminating pharmacogenomics knowledge and data to catalyze research and support hypothesis generation. PharmGKB curates knowledge to capture more complex relationships between genes, variants, drugs, diseases and pathways. Popular features are the very important pharmacogene (VIP) summaries and drug-centered pathways, many of which have been published. PharmGKB's variant-mining project focuses on functional annotation of polymorphisms with pharmacogenomic relevance. PharmGKB is committed to mapping genetic variants to a common framework, providing uniform access to all polymorphisms and support search and sorting operations. These variant annotations are the foundation for our efforts recently published in clinically annotating an entire human genome. PharmGKB's user interface is tailored to provide easy direct access to knowledge and information for genes, drugs, variants, pathways, and diseases. The website also provides excellent educational material including a summary of well known pharmacogenetics pairs of gene-drug relationships and a web-based tool for teaching high school students about pharmacogenomics. To highlight the translation of pharmacogenomics findings into clinical practice the Clinical Pharmacogenomics page catalogues drugs with pharmacogenomic information in the context of FDA-approved drug labels.

Lastly, the PharmGKB has continued its role in consortia as an independent broker to accept, integrate and curate disparate data into a form that facilitates analyses and subsequent dissemination for use by the global research community as well as providing guidance to clinicians for pharmacogenetic tests to be used in the clinic. Current consortia involve (1) determining the role of CYP2D6 genetic variation as a predictor of breast cancer recurrence and survival in postmenopausal women (ITPC), (2) discovering new genetic variants that are important for warfarin dosing using genome wide association studies (IWPC-GWAS), and, (3) creating, curating, reviewing, and updating written summaries and recommendations for implementing specific pharmacogenomic tests and practices (CPIC).

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2402/W

Genetic and environmental influences on cognitive response of healthy volunteers to topiramate. *E.T. Cirulli[1], D.K. Attix[2,3], K.N. Linney[1], V. Dixon[2,3], J.M. McEvoy[1], R.A. Radtke[2], D.B. Goldstein[1].* 1) Center for Human Genome Variation, Duke Univeristy School of Medicine, Durham, NC; 2) Neurology, Department of Medicine, Duke University Medical Center, Durham, NC; 3) Psychiatry and Behavioral Sciences, Duke University Medical Center, Durham, NC.

Topiramate is an antiepileptic drug with marked, specific and well-characterized effects on cognition. However, only certain individuals appear to experience these side-effects; furthermore, only certain aspects of cognition, such as verbal fluency and working memory, seem to be affected. Taken together, these observations indicate both that there may be a genetic component influencing response to topiramate and that the identification of genetic variants contributing to this response may provide information about the biology behind the specific aspects of cognition that are affected. Topiramate has been shown to affect cognition within two hours of taking the first dose. Here, to characterize individual responses to an acute dose of topiramate, we have administered a 100 mg dose of topiramate to 158 healthy volunteers; two hours later they were administered a battery of eleven standardized cognitive tests. All subjects had taken this same battery without topiramate an average of one year prior, and a control group of 206 subjects took the battery twice without topiramate. Using the control data to build regression models predicting the amount of change between tests expected for each individual who took topiramate, we calculated the difference between the observed change and the expected change for each individual for each test and then performed principal component analysis. The first principal component was taken as a measure of the overall response of the individual to topiramate. We were clearly able to show the strong effect of topiramate on cognition in this controlled setting. However, individual response to topiramate varied widely. We found that weight and ethnicity had an impact on this response, but that gender, age, education and baseline intelligence did not. Furthermore, the majority of the variability in response could not be explained by covariates, implying that genetic variants play a role. Nevertheless, a genome-wide association study of response in this cohort did not find a significant association with any common variants, although this result is not unusual given the relatively small sample size. Further work will involve both an expansion of the sample size for the genome-wide association study, with the hope to find common variants affecting this phenotype, and whole-genome or whole-exome sequencing of some study participants to find rarer variants influencing this adverse drug reaction.

2403/W

Identifying Subtypes of Patients with Neovascular Age-Related Macular Degeneration by Genotypic and Cardiovascular Risk Characteristics. *M. Feehan[1], R. Durante[1], J. Hartman[1], M. DeAngelis[2].* 1) Observant LLC, 1601 Trapelo Rd., Ste 255, Waltham MA 02451; 2) Ocular Molecular Genetics Institute, Department of Ophthalmology, Harvard Medical School, Massachusetts Eye and Ear Infirmary, 243 Charles Street, Boston, MA 02114.

One of the challenges in the interpretation of research showing associations between environmental and genotypic data with disease outcomes such as neovascular age-related macular degeneration (AMD) is understanding the phenotypic heterogeneity within a patient population vis-à-vis any risk factor associated with the condition. This is critical when considering the potential therapeutic response of patients to any probative drug developed to treat the condition. We evaluated a sample of patients with neovascular AMD, that in previous published studies had been shown to be at elevated risk for the condition through environmental factors such as tobacco consumption and strongly associated genetic variants including the complement factor H gene (CFH) on chromosome 1q25 and variants in the in ARMS2/HtrA serine peptidase 1 (HTRA1 and) gene(s) on chromosome 10q. We conducted a segmentation analysis of these patients, and in a multivariate model factors such as tobacco consumption failed to differentiate subtypes of patients. Rather, 4 meaningfully distinct clusters of patients were identified that were most strongly differentiated on their cardiovascular (CV) health status (histories of dyslipidemia and hypertension) and the alleles of the marker rs1049331 for ARMS2/HTRA1. These results have significant personalized medicine implications for drug developers attempting to determine the effective size of the treatable AMD population. Patient suptypes or clusters may represent different targets for development (based on genetic pathways in AMD and cardiovascular pathology), and also treatments developed that may elevate cardiovascular risk, may be ill advised for particular the clusters identified.

2404/W

Maternal Smoking, Drug-Metabolizing Gene Variants, and Risk for Gastroschisis. *M.M. Jenkins[1], M.L. Gallagher[1], J. Reefhuis[1], J.S. Witte[2], S.A. Rasmussen[1], T. Hoffmann[2], D.A. Koontz[1], C. Sturchio[3], A. Smith[3], P. Richter[1], E.J. Lammer[4], M.A. Honein[1], the National Birth Defects Prevention Study.* 1) Centers for Disease Control and Prevention (CDC), Atlanta, GA; 2) University of California, San Francisco, CA; 3) Battelle contractor to CDC, Columbus, OH; 4) Children's Hospital Oakland Research Institute, Oakland, CA.

Maternal smoking during pregnancy is one proposed risk factor for gastroschisis, but reported associations have been modest, suggesting that differences in genetic susceptibility might play a role. We analyzed 6 infant and maternal polymorphisms in 4 genes (*CYP1A1*, *CYP1A2*, *CYP2A6*, and *NAT2*) that code for drug-metabolizing enzymes to determine the potential effect they have on gastroschisis risk. Maternal interview data and cytobrush-derived DNA from a multi-site, population-based case-control study of major birth defects were used. Control infants were a sample of live-born infants with no major birth defects. Infants had estimated dates of delivery between Oct 1, 1997 and Dec 31, 2003. This analysis included 192 case families and 1687 control families with available DNA specimens from the infant and/or mother. Included specimens had DNA concentrations ≥0.1ng/µl. Families with any genotypes that were inconsistent with Mendelian inheritance were excluded. Genotyping of 6 variants was completed on genomic DNA using Pyrosequencing® technology. Genotyping call rates were 99-100%. Logistic regression models stratified by periconceptional smoking status (any or none) and adjusting for maternal age at birth (continuous), maternal race-ethnicity (White, Hispanic, other), and periconceptional alcohol use (any or none) were used to estimate adjusted odds ratios (ORs). Preliminary results provide no evidence of modifying effects of maternal or infant drug-metabolizing gene variants on risk of gastroschisis among mothers who reported smoking; some gene effects were observed among nonsmoking mothers. Decreased ORs were observed among nonsmoking mothers who had or whose infant had the *CYP1A2*1F* CC or AC genotype compared with nonsmoking mothers who had or whose infant had the AA genotype (OR_{mother} 0.6, 95% confidence interval [CI] 0.4-0.9; OR_{infant} 0.5, 95% CI 0.3-0.8). Increased ORs were observed among nonsmoking mothers who had the *NAT2*6* AA or GA genotype compared with nonsmoking mothers who had the GG genotype (OR 1.5, 95% CI 1.0-2.4), and among nonsmoking mothers whose infants had the *CYP1A2*1C* AA or GA genotype compared with nonsmoking mothers whose infant had the GG genotype (OR 1.9, 95% CI 1.0-3.6). Preliminary results suggest some direct gene effects among nonsmoking mothers. Although no interaction between smoking and these genetic risk factors was observed, effect of smoking frequency was not assessed. Further analyses will include family based methods.

2405/W

Development and Implementation of a Broad-Based ADME Genotyping Assay. *A.M.K. Brown[1,2], Y. Renaud[1,2], C. Ross[3], M. Hansen[4], U. Zanger[5], J-C. Tardif[1,2], M.S. Phillips[1,2].* 1) Montreal Heart Institute Pharmacogenomics Centre, Montreal, QC, Canada; 2) Université de Montréal, Montreal, QC, Canada; 3) University of British Columbia, Vancouver, BC, Canada; 4) Illumina, Inc. San Diego, CA; 5) Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, Germany.

The testing of evidence based functional markers in drug metabolism genes, such as those identified by the pharmaADME initiative (www.PharmaADME.org) or those included on many commercially available products, can serve as a valuable tools in drug development. However, a more thorough list of variants in genes involved in Absorption, Distribution, Metabolism and Excretion (ADME) is required if one wishes to investigate the inter-individual variability observed in the response to medications. In order to address this need, we have created a custom genotyping panel that uses unique assay designs to query key ADME genes. These well know genes and pathways are associated with pharmacokinetic mechanisms; however there has been little effort to interrogate a large number of these genes simultaneously with a single investigative tool. The assay consists of two types of variation, functional markers with known consequences and haplotype tags that account for blocks of linkage disequilibrium. This panel design can be used in two different ways, functional markers can be used to screen populations to make evidence based decision (i.e. stratification in clinical trials) and tag markers can be used to uncover novel genotype-phenotype correlations. The panel was developed using a rigorous development process which involved multiple phases of testing. The final assay designs incorporate novel assay conversion strategies to overcome areas of genomic interference, such as regions of homology and underlying polymorphisms. The present assay consists of 3000 markers with a conversion rate of 97.4%. To validate the assay, we recently screened 150 liver samples that have been extensively genotyped both using a genome-wide approach, and for many functional markers in ADME genes. Furthermore these livers have been phenotypically characterized for ADME enzyme activity, gene expression levels and many clinically relevant lab values. We will present novel genotype-phenotype correlations observed in these samples. The assay has also been used in studies involving chemotherapeutic agents and lipid lowering drugs and will continue to have broad applicability in any study or clinical trial that would benefit from the evaluation of an extensive list of ADME genes.

2406/W

The Application of Pharmacogenetics in Clinical Medicine and Drug Development. *L. Zhang.* CDER, Food and Drug Administration, Silver Spring, MD.

Pharmacogenetics (PGx) enables clinicians to prospectively identify patients most likely to derive benefit from a drug, with minimal likelihood of adverse events. Polymorphisms in drug metabolizing enzymes, drug targets, and disease pathogenesis genes provide substantial knowledge about the mechanisms of inter-individual differences in therapeutic response and toxicity. Moreover, PGx transforms the way clinical trials are conducted by allowing for the selection of a more homogeneous study population, thereby reducing the size and cost of drug design. Over 50 PGx biomarkers have now been included on FDA-approved drug labeling. In addition, FDA encourages opportunities through Voluntary Exploratory Data Submission (VXDS) and Biomarker Qualification program to identify new PGx evidences that may expedite the drug development process. PGx applications consist of next-generation sequencing, candidate approach, genome wide association study (GWAS), expression analysis and biological validation etc. The session will present some PGx exploratory case studies which have impact on clinical practice and drug discovery strategy.

2407/W

Genome wide association analysis of rheumatoid arthritis patients treated with anti-TNF medication. Results of the DREAM registry. *MJH. Coenen[1], M. Umicevic-Mirkov[1], SH. Vermeulen[1,2], EJM. Toonen[1], A. Lee[3], F. Batliwalla[3], W. Kievit[4,8], H. Scheffer[1], T. Jansen[5,8], EJ. Knijff-Dutmer[6,8], TRDJ. Radstake[4], MAFJ. van de Laar[7,8], P. Barrera[4], PLCM. van Riel[4,8], PK. Gregersen[3], B. Franke[1].* 1) Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 2) Department of Epidemiology, Biostatistics, and HTA, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 3) The Feinstein Institute for Medical Research, Manhasset, New York; 4) Department of Rheumatology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 5) Department of Rheumatology, Medical Centre Leeuwarden, Leeuwarden, The Netherlands; 6) Department of Rheumatology, Gelderse Vallei Hospital, Ede, The Netherlands; 7) Department of Rheumatology, University Twente & Medisch Spectrum Twente, Enschede, The Netherlands; 8) on behalf of the Dutch Rheumatoid Arthritis Monitoring registry.

Background: Treatment strategies blocking tumour necrosis factor (TNF) have proven very successful in patients with rheumatoid arthritis (RA). However, a relevant subset of patients does not respond for reasons that are unknown. There are currently no means of identifying these patients prior to treatment start.

Objective: We aimed at identifying genetic factors predicting anti-TNF treatment outcome in patient with RA using a genome-wide association approach.

Methods: We selected patients with RA treated with antibodies directed against TNF (anti-TNF) from the Dutch Rheumatoid Arthritis Monitoring (DREAM) registry. Disease activity scores (DAS) 28 at baseline and after 14 weeks were available of 449 patients. Single nucleotide polymorphisms (SNPs) were genotyped using the Illumina HumanHap550-Duo BeadChip or the Human660W-Quad. Association analysis using the DAS28 change as outcome was performed using the whole genome association analysis toolset in PLINK.

Results: 511.499 SNPs passed quality control. No findings passed the threshold for genome-wide significance (p-value £$1x10^{-8}$). 53 SNPs showed suggestive association (uncorrected p-value <10^{-4}) with DAS28 change. Eight of the top ten associated SNPs were located in the following genes; *SCARA5, QKI, EP400, LPHN3, ATXN7, MYO1E* and *HDAC9*. Candidate genes that can be linked to anti-TNF or RA are *EP400* and *HDAC9*. EP400 plays a role in cell death, one of the working mechanisms of anti-TNF therapy; HDAC9 has a proposed function in hematopoiesis. In addition, endogenous HDAC activity is thought to regulate Th1 and 2 responses.

Conclusions: The genome wide association approach is a potent tool for the identification of new candidate biomarkers predicting anti-TNF response. However, our suggestive findings need to be replicated in larger, independent patient cohorts before any conclusions about the association of these SNPs with treatment outcome can be drawn.

2408/W

A Genome-Wide Association Study of Bronchodilator Response in Four Asthma Drug Trial Cohorts. *B.E. Himes[1,2,3,4], A.C. Wu[5], J. Lasky-Su[2], B. Klanderman[2], J. Ziniti[2], J. Senter-Sylvia[2], R. Lazarus[2], J.J. Lima[6], C.G. Irvin[7], S.P. Peters[8], K. Tantisira[2], A.A. Litonjua[2], S.T. Weiss[2,4].* 1) Div Hlth Sci & Tech, Harvard Med Sch, Boston, MA; 2) Channing Laboratory, Brigham and Women's Hospital and Harvard Medical School, Boston, MA; 3) Children's Hospital Informatics Program, Boston, MA; 4) Partners Center for Personalized Genetic Medicine, Boston, MA; 5) Department of Population Medicine, Harvard Pilgrim Health Care and Harvard Medical School, Boston, MA; 6) Nemours Children's Clinic, Center for Pharmacogenomics and Translational Research, Jacksonville, Florida; 7) Vermont Lung Center, Department of Medicine and Physiology, University of Vermont, Burlington, Vermont; 8) Center for Human Genomics, Wake Forest University School of Medicine, Winston-Salem, NC.

Asthma is a chronic inflammatory airway disease with well-established heritability that affects over 300 million people around the world. The most common medications used for the treatment of asthma are β_2-agonists (e.g. albuterol). The effect of β_2-agonists is commonly assessed using the bronchodilator response (BDR) test, which measures reversible airway obstruction and aids in the diagnosis of asthma. The physiological response to a bronchodilator is a complex trait that is heritable and partly under genetic control. We performed a genome-wide association (GWA) study of BDR using measures at randomization of 1,196 non-Hispanic white subjects with mild to severe asthma from four drug trial cohorts: the Childhood Asthma Management Program (CAMP), the Leukotriene Modifier or Corticosteroid Salmeterol study (LOCCS), the Effectiveness of Low Dose Theophylline as an Add-on Treatment in Asthma trial (LODO), and a medication trial conducted by Sepracor, Inc. After stringent quality-control filters were applied, data for 469,884 single nucleotide polymorphisms (SNPs) were used to measure the association of SNPs with BDR using a linear model as implemented in PLINK. Covariates included for adjustment were age, gender, height, study cohort, and the top four eigenvectors calculated in EIGENSOFT to account for residual population stratification among subjects. Of the top 15 SNPs with the lowest p-values (ranging from 2.9×10^{-5} to 2.7×10^{-6}), one is within an intron of *CRISPLD2*, a lung development gene that promotes matrix assembly, one is within an intron of *SPAT2SL*, a gene that is highly expressed in bronchial epithelial cells, and one is near *ANXA1*, a gene that is highly expressed in bronchial epithelial cells, lung, and fetal lung and is part of a family of Ca^{2+}-dependent phospholipid binding proteins that is thought to have anti-inflammatory activity. Replication of these association results is ongoing in two additional cohorts of asthmatics. To date, this is the largest genetic study of BDR that has been performed, and its results suggest that there is promise in gaining a better understanding of the biological mechanisms of differential response to β_2-agonists through GWA studies.

2409/W

DRD2 Single Nucleotide Polymorphisms are associated with nicotine dependence in an urban population of African American smokers. *B. Arrey[1,4], P. Karla[1], C. Williams[2], S. Wilson[3], L. Ricks-Santi[2,4], T. Mason[4], M. Abbas[4], V. Apprey[4,5], G. Bonney[4,5], W. Anthony[1], G. Dunston[4,6].* 1) Pharmaceutical Sciences, Howard University, Washington, DC; 2) Cancer Center, College of Medicine, Howard University, Washington, DC; 3) Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD; 4) The National Human Genome Center, Howard University, Washington, DC; 5) Community and Family Medicine, Howard University, Washington, DC; 6) Microbiology, Howard University, Washington, DC.

Statement of Purpose: This study addresses tobacco-related health disparities by exploring the genetics of nicotine dependence. The dopamine receptor D2 (DRD2), which binds dopamine neurotransmitters and influences the pleasure intensity signals transmitted to the brain, is involved in nicotine dependence. DRD2 single nucleotide polymorphisms (SNPs) have been associated with dopamine receptor expression. Recent studies have shown the association of SNPs in the 3' untranslated region of this gene with nicotine dependence. The TaqA1 allele in DRD2/ANKK1, occurs more frequently in populations of African descent. Our laboratory utilizes SNPs in candidate genes to investigate the functional significance of common variants in health disparities. Therefore, we investigated the association of DRD2 SNPs with nicotine dependence in African Americans. Methods: We used Restriction Fragment Length Polymorphism (RFLP) and Pyrosequencing to examine the association between several DRD2 SNPs (rs6274; rs6276; rs6279 and rs1800497/Taq1A with nicotine dependence in a cohort of 519 African American current smokers in the Baltimore, MD-Washington, DC metropolitan area. Clinical symptoms of nicotine dependence were defined in accordance with the Diagnostic and Statistical Manual of Mental Disorders. Results: We report a significant association of rs1800497 with heavy smoking (OR=0.48 95% CI: 0.25-0.93; p=0.03) and the frequency of smoking (OR=0.16, 95% CI: 0.16-1.11; p=0.05). Additionally, rs6276 was associated with frequency of smoking (OR=0.24, 95% CI: 0.06-1.05; p=0.02) while rs6279 was associated with overall clinical nicotine dependence(OR=0.46, 95% CI: 0.11-0.74); p= 0.01). Conclusions: These data provide evidence for an association between DRD2 SNPs and nicotine dependence in African Americans. Identification of SNPs associated with nicotine dependence may be instructive in the design of genotype-based clinical diagnosis and targeted intervention in the emergence of personalized genomic medicine for diverse population.

2410/W

Variants of the *NR3C1* gene associated with glucocorticoid response to patients with nephrotic syndrome. *T. Ozeki[1], T. Mushiroda[1], N. Kamatani[2], Y. Nakamura[1,3].* 1) Laboratory of Pharmacogenetics, RIKEN Center for Genomic Medicine, Yokohama, Kanagawa, Japan; 2) Statistical Analysis, RIKEN Center for Genomic Medicine, Yokohama, Kanagawa, Japan; 3) Laboratory of Molecular Medicine, Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

Nephrotic syndrome (NS) is characterized by proteinuria, hypoalbuminemia, edema, and hyperlipidemia. There is a considerable variability in the sensitivity to glucocorticoids (GCs) for NS among individuals. About 10% of NS patients show GC-resistant and refractory NS. GCs exert their effect by binding to GC receptor (GR), forming a complex that translocates to the nucleus, then regulate the expression of target genes by interaction with a cis-element, GC-responsive element (GRE). The genetic variations of *NR3C1* encoding the GR have been reported to affect GC sensitivity. Thus, we investigated associations of SNPs of the *NR3C1* gene with GC response to patients with NS.

From the registered samples in the BioBank Japan Project, we obtained 172 NS patients diagnosed as a minimal-change NS or a membrane nephropathy. The subjects included 43 patients with GC-resistant or refractory NS, and 129 patients who achieved remission. We genotyped 40 tag-SNPs of the *NR3C1* gene by means of multiplex PCR-based Invader assays.

Among the SNPS screened, 4 SNPs (rs41423247, rs174047, rs33388 and rs10482634) on the *NR3C1* gene showed significant association with GC-resistant and refractory NS after the correction of multiple testing, and rs12709096 revealed the lowest *P*-value for association ($P = 1.72 \times 10^{-6}$, odds ratio = 6.1 (95% CI = 2.9-12.9)). Our findings should facilitate development of a genetic test to identify individuals at risk for the GC-resistant and refractory NS.

2411/W

Suppressive effects of black seed oil on ovalbumin induced acute lung remodeling in E3 rats. *M. B. Raza Asim[1, 2], M. Shahzad[3], X. Yang[1, 2], Q. Sun[1, 2], F. Zhang[1, 2], Y. Han[1, 2], L. Shemin[1, 2].* 1) Genetics and Molecular Biology, SChool of Medicine, Xi'an Jiaotong university, Xi'an, shaanxi, China; 2) Key Laboratory of Environment and Genes Related to Diseases (Xi'an Jiaotong University) , Ministry of Education, P.R. China; 3) Department of Pharmacology, University of Health Sciences, Lahore 54600, Punjab, Pakistan, Pakistan.

Abstract Both the seed and oil of Nigella sativa (black seeds) have been used as traditional medicine for centuries to treat asthma, bronchitis, rheumatism and other related inflammatory diseases. Lung remodeling in allergic asthma is the structural alteration of lung tissues due to the deposition of different types of collagen and cell proliferation. In this current study, we examined whether pure black seed oil (BSO) has inhibitory effect on acute lung remodeling in ovalbumin induced asthma model in E3 inbred rats. Rats were randomly divided into three groups; Control, OVA and BSO. Two weeks after intraperitoneal sensitization, the rats were subjected to intranasal challenge to ovalbumin (OVA) and treated intraperitoneally with pure black seed oil for seven days after each challenge. The collagen deposition, goblet cell hyperplasia and infiltration of inflammatory cells were determined by Masson's Trichome, Periodic acid-Schiff, and hematoxylin and eosin staining respectively. The activities of total arginase, ODC and proline level were determined by photometry. HPLC was used to determine the level of polyamine in lung homogenate of experimental rats. The mRNA expression of arginase I, endothelin1, MMP3 and growth factors such as TGF- β, FGF2, VEGF was determined by real time RT-PCR. The administration of pure black seed oil suppressed inflammatory cells infiltration in the airways, collagen deposition and goblet cell hyperplasia. The activity of total arginase and ODC, proline and polyamines levels in the lung homogenate were suppressed after treatment in the lungs of asthmatic rats. Black seed oil treatment abrogated the endothelin1, MMP3, TGF-β, FGF2 and VEGF mRNA expression in the lung of OVA challenged rats. In conclusion, black seed oil suppressed the OVA induced acute lung remodeling by attenuating endothelin1, MMP3, factors related to remodeling, total arginase activity and its downstream products such as proline and polyamine. These results show that Black seed oil might be useful for the treatment and future research for allergen-induced lung remodeling.

2412/W

ACTG DACS 250: Mitochondrial Genetic Variation Is Associated with CD4 T Cell Recovery in HIV-infected Persons Initiating Antiretroviral Therapy. *B. Grady[1], D.C. Samuels[1], G.K. Robbins[3], D. Selph[1], J.A. Canter[1], R.B. Pollard[4], D.W. Haas[2], R. Schafer[5], S. Kalams[2], D. Murdock[1], M.D. Ritchie[1], T. Hulgan[2].* 1) CHGR, Vanderbilt Univ, Nashville, TN; 2) Division of Infectious Diseases, Department of Medicine, Vanderbilt Univ, Nashville, TN; 3) Division of Infectious Diseases, Massachusetts General Hospital, Harvard Medical School, Boston, MA; 4) Department of Internal Medicine, University of California Davis, Sacremento, CA; 5) Division of Infectious Diseases, Department of Medicine, Stanford University, Stanford, CA.

Mitochondrial genetic variation has been associated with many aspects of HIV infection, ranging from time to progression to AIDS to adverse effects from antiretroviral therapy (ART). In this study, we used the GeneChipR Human Mitochondrial Resequencing Array v2.0 (Affymetrix, Inc., Santa Clara, CA, USA) to obtain full mitochondrial DNA sequence data from U.S.-based adult participants in the AIDS Clinical Trials Group (ACTG) study 384 and examine links between mitochondrial DNA variants and the extent of CD4 T cell recovery with ART. Variation in the mitochondrial genome could influence apoptotic activity in cells with certain mitochondrial DNA variants which might lead to reduced CD4 T cell recovery during ART. In ACTG 384, data on CD4 T cell count was available at baseline, and 48 and 96 weeks after ART initiation on 423 participants, 30% of which self-identified as non-Hispanic black. The primary outcome was CD4 T cell count as an absolute change from baseline at 48 weeks dichotomized at a 100 cell increase. A race-stratified analysis of mitochondrial DNA variants with a minor allele frequency greater than 1% showed several variants associated ($P < 0.05$ before Bonferroni correction) with both increased and decreased magnitude of CD4 cell recovery. The most significantly associated mitochondrial DNA variant was found in the stratum of (self-identified) non-Hispanic blacks and indicated a primary association between the African L2 subhaplogroup and decreased magnitude of CD4 cell recovery ($P = 0.002$, Odds Ratio = 0.022). In addition to variants tagging the L2 subhaplogroup, there were other mitochondrial DNA polymorphisms not associated with haplogroup status which warrant follow up in a replication set. We plan to continue study of the effect of mitochondrial genetic variation on CD4 T cell recovery utilizing additional data from the ACTG cohort and other populations. Findings from these studies have the potential to improve ART outcomes in patients by enhancing prognostic information and improving treatment decisions.

2413/W

Gene expression studies of TRANK1, a candidate gene for bipolar disorder, demonstrate marked upregulation after chronic valproate treatment. *X. Jiang, J. Wendland, W. Coronal, S. Detera-Wadleigh, F.J. McMahon.* Genetic Basis of Mood and Anxiety Disorders, National Institute of Mental Health, National Institutes of Health, US Dept. of Health and Human Services, Bethesda, MD.

Previous studies have implicated TRANK1 in the etiology of bipolar disorder (Wellcome Trust Case Control Consortium 2007; Secolin et al. 2010), and in an accompanying abstract Wendland et al (WCPG 2010) report genome-wide significant association between common variants near TRANK1 and bipolar disorder. TRANK1 (also known as lupus brain antigen1, LBA1) encodes a tetratricopeptide and ankyrin repeat containing protein of unknown function that is highly expressed in brain and other tissues. To explore the biology of TRANK1 and its possible relationship to the pathophysiology of bipolar disorder, we carried out several in vitro gene expression studies. First, we investigated the effect of mood stabilizing drugs, lithium and valproate (VPA), on TRANK1 expression in cultured cell lines. We found that VPA increased LBA1 expression in a time and concentration dependent manner. This held true in SH-SY5Y, HEK 293, and HeLa cell lines, despite highly variable levels of expression at baseline. While 0.5 mM VPA increased TRANK1 expression only slightly at 24 h, 3 mM VPA increased TRANK1 mRNA expression more than 10- fold after 72 h of treatment. In contrast, 2 mM lithium had no effect on TRANK1 even after up to 72 h of treatment. In order to survey the biological impact of changes in TRANK1 expression, we conducted gene expression array studies with HeLa cell lines in which siRNA-mediated knockdown of TRANK1 achieved >70% loss of expression. We used the Illumina HumanHT-12_v3 BeadChips. Analysis revealed differential expression of many genes that are involved in diverse cellular processes. This preliminary work suggests that TRANK1 expression is markedly upregulated by chronic VPA and has an impact on downstream signaling pathways that may be involved in the pathogenesis of bipolar disorder.

2414/W

Scanning variations in genes related to anesthetic disorder by using custom resequencing array. *S. Levano[1], Ph. Demougin[2], M. Singer[1], A. Urwyler[1], Th. Girard[1].* 1) Departments of Anaesthesia and Biomedicine, University of Basel, Switzerland; 2) Life Sciences Training Facility, Pharmazentrum, Basel, Switzerland.

In anesthesia field there are two inherited pharmacogenetic disorders that are worthy of consideration; butyrylcholinesterase (BCHE; MIM# 177400) deficiency due to its high incidence and malignant hyperthermia (MH; MIM# 145600) due to the fatal consequences. While the hot spots of BCHE mutations are few, the presence of multiple mutations is frequent in this gene. Today the genetic basis of MH lies in the RYR1 and CACNA1S causative mutations. About 70% and 1% of MH cases are linked to RYR1 and CACNA1S mutations. Despite the early hypothesis of RYR1 hot spots, today it is known that the mutations are spread along the whole coding region, which comprises more than 15,000 nucleotides. Our and other genetic groups have exhaustively examined the two principal genes, RYR1 and CACNA1S, encompassing an extensive collection of detected mutations. Taking this information into account, we have realized the need for an intensive mutation-detection approach. Recently, we have established a resequencing DNA array that contains 153 exons of the three genes (RYR1, CACNA1S, and BCHE) and serves as a detection tool for the simultaneous and highly specific identification of variants. We compared the results of the array with the dideoxy sequencing results, which were previously obtained. Array data was analyzed using Affymetrix GeneChip Sequencing Analysis software v4.1 (GSEQ). For further analysis and statistics we used a SQL database (MySQL) and own perl-scripts. Five different quality scores (QS0 to QS4) were used to analyze the resequencing arrays. The QS value affects the stringency for calling the correct bases. As we require very low false negatives to avoid missing a causative mutation, the array data were tested with different QS values at the lower end. The genetic data, obtained from 130 DNA samples in about 66 DNA arrays, is currently being analyzed. From our preliminary results, the error rate is 0.003-0.028 at QS4 and 0.008-0.056 at QS0. The overall call rate ranges from 83 to 99% at quality scores QS4 and QS0, respectively. The averages of cross hybridization in unused places ranged from 1 to 2.66% at QS4 and QS1, respectively.

2415/W

Genetic Predictors of Relapse in Major Depressive Disorder. *G. Laje[1], S. Paddock[1], A.J. Rush[2], F.J. McMahon[1].* 1) Intramural Research Program, National Institute of Mental Health, Bethesda, MD; 2) Duke-National University of Singapore, Singapore.

Major depressive disorder (MDD) will become the second greatest cause of disability in the next decade. Only a fraction of patients with MDD respond to antidepressant treatment and even fewer achieve full symptomatic remission. Moreover, of those patients who respond to treatment about one third relapse. Some predictors of relapse have emerged such as incomplete remission and stressful life events, however, to date, genetic predictors have been elusive. The Sequenced Treatment Alternatives to Relieve Depression study (STAR*D) offered up to one year of follow up treatment for those patients who improved on a specific treatment strategy (n=862). We defined relapse as those patients who reported a QIDS-SR total score <11 at the first follow-up visit, had at least one additional follow-up visit, and reported a QIDS-SR score of 11 or more at any follow-up visit (n=255). Those whose QIDS-SR score remained <11 at all follow-up visits were used as the comparison group (n=430). A candidate gene study was conducted with 780 SNPs representing 68 genes in neurotransmitter, neurotrophin, and other pathways. Results were adjusted for population stratification and Bonferroni-corrected for multiple testing. The marker rs2251388 was associated with relapse (OR: 1.82, X2=4.12, unadjusted p-value: 3.8e-5, adjusted p-value: 0.03). This is an intronic marker on GRIK1 in chromosome 21. This gene encodes the gluR5 subunit of the glutamate-kainate receptor, known to regulated by stress and corticosteroids. We conclude that genetic variation in GRIK1 is associated with relapse of depressive symptoms.

2416/W

Genome-wide association study identifies genetic determinants of warfarin responsiveness for Japanese. *P.C. Cha[1], T. Mushiroda[2], A. Takahashi[3], M. Kubo[4], S. Minami[5], N. Kamatani[3], Y. Nakamura[1].* 1) Laboratory Molecular Medicine, Institute of Medical Science, University of Tokyo, Japan; 2) Research Group for Pharmacogenomics, RIKEN Center for Genomic Medicine, Japan; 3) Laboratory for Statistical Analysis, RIKEN Center for Genomic Medicine, Japan; 4) Laboratory for Genotyping Development, RIKEN Center for Genomic Medicine, Japan; 5) Department of Bioregulation, Nippon Medical School, Kawasaki, Japan.

Warfarin is a commonly-used anticoagulant, whose dose needs to be determined for each individual patient owing to large inter-individual variability in its therapeutic dose. Although several clinical and genetic variables influencing warfarin dose have been identified, uncovering additional factors is critically important for safer use of warfarin. Through a genome-wide association study, we identified SNP rs2108622 (*CYP4F2*) as a genetic determinant of warfarin responsiveness for Japanese. Stratifying subjects who have been pre-classified according to genotypes of SNP rs10509680 (*CYP2C9*) and SNP rs9923231 (*VKORC1*), based on their genotypes of rs2108622 allowed identification of subjects who require higher dose of warfarin. Besides, incorporating genotypes of rs2108622 into warfarin dosing algorithm that considers age, body surface area (BSA), status of amiodarone co-administration, and genotypes of SNPs in the *CYP2C9* and *VKORC1* genes improved model's predictability. In this study, association of *CYP4F2* with warfarin dose of Japanese has been established for the first time. Besides, a warfarin dosing algorithm that incorporates genotypes of rs2108622 and amiodarone co-administration status has been suggested for the first time for the Japanese. Finally, our study also implied that common SNPs other than those in the *CYP2C9*, *VKORC1*, and *CYP4F2* genes that show strong effect on therapeutic warfarin dose might not exist.

2417/W

Association study of Melanocortin-4 receptor gene polymorphisms with antipsychotic induced weight gain. *N.I. Chowdhury[1], A.K. Tiwari[1], R.P. Souza[1], S.A. Shaikh[1], H.Y. Meltzer[2], J.A. Lieberman[3], D.J. Mueller[1], J.L. Kennedy[1].* 1) Neurogenetics, Centre for Addiction & Mental Health, Toronto, Ontario, Canada; 2) Psychiatric Hospital at Vanderbilt University, Nashville, TN, USA; 3) New York State Psychiatric Institute, Columbia University Medical Center, New York City, NY, USA.

Introduction Antipsychotic induced weight gain results in the metabolic syndrome in schizophrenia patients. Single nucleotide polymorphisms (SNPs) located downstream, including rs17782313, of the Melanocortin- 4 receptor (MC4R) gene have been associated with body mass index and waist circumference in healthy human populations. These findings have been replicated in obese and paediatric populations. Thus, we hypothesized that candidate single-nucleotide polymorphisms near MC4R can influence the antipsychotic-induced metabolic syndrome in schizophrenia patients. **Methods** Four tagged SNPs (rs2229616, rs17782313, rs11872992, rs8087522) were analysed in 224 patients who underwent treatment for chronic schizophrenia and were evaluated for antipsychotic induced weight gain for up to 14 weeks. Our refined sample consisted of 67 African Americans and 87 European Americans on clozapine/olanzapine, prospectively. We compared weight change (%) across genotypic groups using analysis of variance and covariance for the three tagSNPs ($r2 \geq 0.8$) near the MC4R gene. Variants were genotyped using ABI TaqMan assays. **Results** The rs2229616 was monomorphic in our population. No significant genotypic or allelic associations were found between rs11872992 and rs17782313 polymorphisms and weight gain ($p > 0.05$). A trend towards association was found between rs8087522 and weight gain in patients of European Ancestry taking either olanzapine/clozapine ($p = 0.088$). The haplotype comprised of rs8087522-rs11872992-rs17782313 was nominally significant. **Conclusion** In this study we suggest that the polymorphisms near and within the MC4R gene may be associated with antipsychotic-induced weight gain in chronic schizophrenia patients. However these observations were made in a relatively small patient population. These results need to be replicated in larger sample sets. *Corresponding author: Dr. James Kennedy Neurogenetics Department 250 College Street Toronto, Ontario M5T 1R8 Phone: (416) 535-8501 ext. 4987 Fax: (416) 479-4666.

2418/W

A novel CYP2C9 mutation in a warfarin hyper- sensitive patient. *C. Ciccacci[1], N. Paolillo[1,2], M. Falconi[3], D. Di Fusco[1], F. Oteri[3], V. Forte[4], A. Desideri[3], G. Novelli[1], P. Borgiani[1].* 1) Department of Biopathology and Diagnostic Imaging, Section of Genetics, School of Medicine, Tor Vergata University, Rome, Italy; 2) IRCCS C. Mondino Institute of Neurology, Foundation, Pavia, Italy; 3) Department of Biology, Tor Vergata University, Rome, Italy; 4) Azienda Policlinico Tor Vergata, Center of Haemostasis and Thrombosis, Rome, Italy.

Warfarin (Coumadin) is a worldwide prescribed anticoagulant, largely utilized for the long term treatment and prevention of thromboembolic events. It is known that the interindividual variability in the warfarin dosing is a multifactorial character and both environmental and genetic factors influence the dose necessary for the therapeutic effect. The genes resulted to be more involved in warfarin dosing are CYP2C9 and VKORC1 and, more recently, CYP4F2. Anyway, this common variability can not explain the abnormal warfarin dose response in rare individuals with very high warfarin sensitivity or resistance. Here we report the case of an Italian patient with very high warfarin-hypersensitivity. The patient, an 81 years man that started anticoagulant therapy for atrial fibrillation, required only 2.25 mg/week to achieve therapeutic effect; this dose is definitely underneath the normal variability. In order to elucidate the molecular basis of his very high warfarin sentitivity, we conducted a pharmacogenetic analysis. In a first phase, we analyzed the common polymorphisms in VKORC1, CYP2C9 and CYP4F2 genes, already known to be associated with warfarin dosing. Anyway these common polymorphisms could not explain the very low dosing of our patient since he resulted a mix of low dosing and high dosing polymorphic variants. We hypothesized that this hyper-sensitive patient could present some other rare genetic variant. For this reason we searched for rare variations by direct sequencing of the entire coding regions of VKORC1, CYP2C9 and CYP4F2 genes. We found a novel mutation in the CYP2C9 gene, never described before, that, by means of ARMS Pcr, resulted to be in cis with CYP2C9*2 allele. This new mutation, c.374G>T, produces an aminoacid change in the enzyme, from arginine to leucine in position 125 of the protein. In order to clarify the structural/functional role of this mutation we have carried out electrostatic calculation studies on the basis of previous papers that have shown the relevance of the electrostatic interactions for the cytochrome function. Our results indicate the presence of an electrostatic potential alteration on the enzyme surface which can lead to a significant decrease of the recognition between the cytochrome and its redox partner NADPH P450 reductase. In conclusion this new rare variation causes an electrostatic alteration in the enzyme that may explain the warfarin hyper-sensitivity observed in this patient.

2419/W

Inclusion of CYP2C19*4 (c.1A>G) and *17 (g.-806C>T) for Clopidogrel Responsiveness Genotyping. *R.J. Desnick, S. Martis, Y. Kasai, R. Kornreich, S.A. Scott.* Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, NY.

Clopidogrel is a commonly prescribed antiplatelet prodrug that requires oxidation to its active metabolite by the cytochrome-P450 superfamily, most notably CYP2C19. Although the drug is standard of care in most patients undergoing percutaneous coronary intervention, the response to clopidogrel varies widely among individuals, in part due to inherited CYP2C19 alleles that impair enzymatic activity. The most commonly studied CYP2C19 variant is *2 (c.681G>A), which generates an aberrant splice site and results in a truncated and non-functional enzyme. CYP2C19*2 carriers have reduced responsiveness to clopidogrel with significantly increased risks of stent thrombosis, stroke, myocardial infarction, and death following treatment. In addition, the CYP2C19*17 (g.-806C>T) allele recently has been associated with increased CYP2C19 transcription, enhanced clopidogrel response and an increased risk of bleeding; however, the frequency of CYP2C19*17 has not been established in various ethnic groups. We previously reported the CYP2C19*2-*8 allele frequencies for the Ashkenazi Jewish (AJ) population which were similar to those in the general Caucasian population. To determine the frequency of additional variant CYP2C19 alleles in the AJ population and to assess the impact of including *17 in testing panels, we genotyped 180 AJ individuals, who previously had undergone CYP2C19*2-*8 testing, for CYP2C19*2-*10, *13, and *17 using the Osmetech® 11 allele panel. The frequencies of CYP2C19*1, *2, *4, and *17 were 0.622, 0.150, 0.022 and 0.206, respectively, and no other tested alleles were detected. Complete concordance between *2 and *4 was observed between the two genotyping panels. However, the frequency of extensive metabolizers (*1/*1) changed from 69% to 40% by inclusion of CYP2C19*17, which also resulted in 29% of AJ individuals being reclassified as ultrarapid metabolizers (*1/*17 or *17/*17). Notably, all CYP2C19*4 carriers were also *17 carriers, suggesting a *4/*17 genotype. However, all *2/*4 individuals also were *17 carriers, indicating that *4 (c.1A>G), a null allele, can occur on a *17 background (*4-*17). Thus, these findings identify a novel *4-*17 allele that would significantly alter the interpretation of CYP2C19 genotyping when testing for *17 without *4. These results underscore the importance of including both *4 and *17, in addition to the commonly tested *2 and *3, when assessing CYP2C19 for clopidogrel response among cardiovascular patients.

2420/W

Evaluating the benefits of using genetic information to design clinical trials. *Y. Hu[1], L. Li[2], N. Bing[2], K. Song[3], M. Nelson[2], M. Ehm[2], G. Abecasis[1], D. Waterworth[3], C. Spino[1], J. Whittaker[4], H. Kang[1].* 1) Department of Biostatistics, University of Michigan, Ann Arbor, MI, 48105; 2) GlaxoSmithKline, RTP, NC, USA; 3) GlaxoSmithKline, Upper Merion, PA, USA; 4) GlaxoSmithKline, Harlow, UK.

Designing a clinical trial for disease prevention is a complex and costly endeavor focused on identifying patients most likely to develop a disease in a short timeframe and then following these patients. Given that the GWAS era has produced many replicated genotype phenotype signals and that resequencing studies are poised to add to that bank of information, it is natural to ask whether these genetic findings be used, in conjunction with clinical information, in the design of prevention trials. We develop a general framework that explores the potential benefits of enriching the clinical trial sample by selecting individuals with greater genetic risk. Our framework takes into account screening and recruitment costs for each potential trial participant, follow-up costs for subjects who meet genetic or other screening criteria, and the current state of knowledge about trait-specific susceptibility variants, as exemplified by type 1 diabetes (T1D), type 2 diabetes (T2D), cardiovascular disease and age-related macular degeneration (AMD). These diseases differ in their genetic architecture: a number of markers robustly associated with T2D and cardiovascular disease only contribute small changes in disease risk; whilst loci with large effect size T1D and AMD have been shown to be effective in predicting disease risk. Using published data in conjunction with several proposed designs for prevention trials, we quantify the potential benefits -- in terms of trial cost and duration -- of incorporating genetic data in conjunction with clinical information in trials for a T1D, T2D, AMD and cardiovascular disease. Our results illustrate settings where incorporating genetic information will reduce the sample size by up to 20% to 37%; even with current sets of robustly associated loci, but considerable reduction of total trial cost may be achieved only in T1D and AMD. We also show these benefits may be further increased as the list of robustly associated markers for each trait grows. Our investigation illustrates that while current studies have the power to identify markers associated with disease risk, developing further resources such as combined clinical/genetic datasets to assess disease risk in the time dimension along with close collaboration between clinical trial scientists and geneticists will be required to translate this genetic information into the clinical trial context.

2421/W

Genome wide association analysis of elevated alanine aminotransferase levels in pazopanib treated patients. *L. Huang[1], B.H. Reck[1], V.L. Goodman[2], Z. Xue[1], C.F. Spraggs[1], V.E. Mooser[1], L. Pandite[2], C.F. Xu[1].* 1) GSK R&D: Genetics Division, Harlow, UK, North Carolina and Pennsylvania, USA; 2) GSK R&D: Oncology Research and Development, North Carolina and Pennsylvania, USA.

Pazopanib (Votrient™, GlaxoSmithKline) is an oral angiogenesis inhibitor that was approved by the US Food and Drug Administration for the treatment of patients with advanced renal cell carcinoma (RCC). However, elevations in alanine aminotransferase (ALT) have been observed in patients exposed to pazopanib. To evaluate the potential genetic effect for the observed increases of ALT, genome wide association analysis (GWAS) was performed using data from participants of two clinical studies for RCC. Genetic markers from the Illumina Human 1M Beadchip were analyzed. Maximum on-treatment ALT was analyzed for association with each SNP as a continuous variable in a quantitative trait analysis (QTA) and as a binary variable according to predefined thresholds in a case-control (CC) analysis. The effect of population stratification was minimized using genetic ancestry estimates obtained by principal component analysis. GWAS was performed on a genetically determined European ancestry cluster, which was composed of 240 patients, including 42 cases and 97 controls. None of the markers met the threshold of genome-wide significance ($P \leq 10^{-8}$). Two hundred and eight markers from 60 genes and intergenic regions had a P-value $\leq 10^{-4}$ in either QTA or CC analysis. Although some markers with modest effects on pazopanib related ALT elevation were identified among the GWAS markers investigated in this study, polymorphisms with a large effect for association with ALT elevation were not revealed. Results from this analysis suggest that multiple genetic factors, each with a modest or small effect, may contribute to the observed ALT elevation in pazopanib-treated patients.

2422/W

Cyclosporine Pharmacogenetics in Transplantation: Preliminary Results from a Multicenter Consortium. *P. Jacobson[1], A. Brearley[2], A. Israni[3], R. Leduc[4], D. Schladt[4], A. Matas[5], V. Lamba[6], W. Guan[4], R. Mannon[7], W. Oetting[8], DeKAF Investigators.* 1) Experimental and Clinical Pharmacology, University of Minnesota, Minneapolis, MN; 2) Clinical and Translational Science Institute, University of Minnesota, Minneapolis, MN; 3) Department of Medicine, Hennepin County Medical Center, Minneapolis, MN; 4) Division of Biostatistics, University of Minnesota, Minneapolis, MN; 5) Department of Surgery, University of Minnesota, Minneapolis, MN; 6) Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, MN; 7) Division of Nephrology, University of Alabama at Birmingham, Birmingham, AL; 8) Department of Experimental and Clinical Pharmacology and Institute of Human Genetics, University of Minnesota, Minneapolis, MN.

Background: Cyclosporine (CSA) is a common immune suppressant used in organ transplantation. Genetic variants within of the cytochrome (CYP) P450 family of genes and multidrug resistant (MDR1) genes have been extensively studied for their effect on CSA metabolism and pharmacokinetics. The majority of these studies have either not established an association or the associations have been weak. We have analyzed CSA pharmacogenomics in a large, well characterized cohort of kidney transplant recipients to identify genetic variation associated with CSA levels. Methods: CSA pharmacogenetics were studied through a multicenter consortium in the U.S. and Canada in 325 adult kidney transplantation recipients. CSA trough concentrations were measured in each patient as part of clinical care twice in weeks 1-8 posttx and then once in each of months 3, 4, 5 and 6 posttransplant. CSA trough concentrations (n=5101) were normalized for CSA dose and weight. CSA doses were adjusted to maintain whole blood concentrations in the therapeutic range as defined by the enrolling center. Recipient DNA was genotyped using a customized Affymetrix SNPChip containing 2724 single nucleotide polymorphisms (SNP) of which around 1000 were from drug absorption, distribution, metabolism and excretion (ADME) genes. Results: Mean±sd age and weight of the recipients were 50.36±13.79 yrs and 82.45±19.49 kg, respectively. 259 recipients were Caucasian and 66 were nonCaucasian. There were 202 male and 123 female recipients. Median (IQR) CSA troughs and daily dose were 173 ng/mL (136-220) and 350 mg (250-400), respectively. Time posttransplant, center and age were important factors towards log transformed CSA troughs. In repeated measures regression analysis, adjusted for the above factors, the top associated SNP towards troughs was within CYP3A7 (rs2687140, p=2.34x10-3) although it was not significant after controlling the false discovery rate at 20%. ATF6 (activating transcription factor 6, rs2271012) was the top SNP (p=1.33x10-3) after adjustment for rs2687140 and above factors. No MDR1 SNPs were found to be significant. Conclusion: We found the CYP3A7 SNP to be associated with a reduction in troughs whereas the ATF6 SNP was associated with an increase in troughs. These novel variants may further explain the substantial interpatient variability in CSA concentrations and will be validated in our accruing cohort of over 2000 patients.

2423/W

A Pharmacogenetic Approach to the Selection of Fabry Patients for Pharmacological Chaperone Therapy. *E. Katz[1], M.C. Della Valle[1], X. Wu[1], K. Mascioli[1], K. Chang[1], D. Greene[1], R. Schiffmann[2], D.J. Lockhart[1], K.J. Valenzano[1], E.R. Benjamin[1].* 1) Amicus Therapeutics, Cranbury, NJ; 2) Baylor Research Institute, Dallas, TX.

Fabry disease is an X-linked lysosomal storage disorder caused by mutations in the gene (*GLA*) that encodes α-galactosidase A (α-Gal A), and is characterized by pathological globotriaosylceramide (GL-3) accumulation. More than 600 pathogenic *GLA* mutations have been reported, of which more than 60% are missense. The iminosugar AT1001 (migalastat hydrochloride, 1-deoxygalactonojirimycin) is a pharmacological chaperone for α-Gal A that is currently in clinical development as a therapy for Fabry disease. AT1001 selectively binds α-Gal A, thereby increasing physical stability, lysosomal trafficking, and total cellular activity of some mutant forms (defined as "responsive"). As such, Fabry patients expressing responsive mutant forms of α-Gal A have the potential to benefit from AT1001 treatment. Thus, we have developed a single, systematic approach to select male and female patients that may be candidates for AT1001 therapy. First, each of 449 known Fabry disease-causing missense and small in-frame insertion and deletion mutations were individually engineered into *GLA*, expressed in HEK-293 cells, and tested for response to incubation with AT1001 after 4 to 5 days. Concentration-dependent increases in α-Gal A levels were seen for ~60% of the mutant forms. Next, to identify the subset most likely to respond to AT1001 *in vivo*, criteria were developed that consider the magnitude of the enzyme response to a clinically-relevant AT1001 concentration. Mutant forms that met the *in vitro* criteria were generally found to be responsive *in vivo* as measured in white blood cells (WBCs) from male patients orally administered AT1001 during Phase 2 clinical trials. Those that did not meet the *in vitro* criteria showed very limited or no *in vivo* α-Gal A response. A similar level of consistency for a larger number of mutant forms was observed between the HEK-293 cell results and responses measured in lymphoblasts derived from male patients. To date, 189 mutant forms of α-Gal A have been identified that meet the specified criteria. Taken together, these results suggest that a pharmacogenetic reference table comprised of AT1001-responsive mutant forms of α-Gal A and the corresponding *GLA* mutations can be used to select male and female Fabry patients for pharmacological chaperone therapy based on genotype.

2424/W

Replication of Association of the NTRK2 gene with Lithium Response in Bipolar Disorder in a Prospective Sample. *J. Kelsoe[1,2], S. Leckband[1,2], A. Demodena[1,2], R. McKinney[1,2], T. Shekhtman[1,2].* 1) Dept Psychiatry, MC 0603, Univ California, San Diego, La Jolla, CA; 2) Dept Psychiatry, VA San Diego Healthcare System, La Jolla, CA.

Lithium is the oldest mood stabilizer medication and the gold standard for treatment of bipolar disorder. Lithium responders comprise a clinically distinct subset of bipolar disorder patients many of whom have a excellent response to the drug. The identification of genes that predict response would be invaluable in guiding clinicians in treatment selection. 92 lithium responders and 92 non-responders were identified by retrospective review of research interviews and medical records. A SNP (rs1387923) 3' of the gene for the Trkb neurotrophin receptor (NTRK2) was associated to response (p=0.006). This association was observed only in patients who had predominantly euphoric rather than dysphoric mania. Though retrospective assessment of response is easier, a prospective trial is more definitive. We now report initial results from a prospective trial of lithium response. 77 subjects were entered into a clinical trial the goal of which was to stabilize the patients on lithium monotherapy over 3 months and then follow them for 2 years. In this initial analysis, total time in the study was examined using Cox proportional hazard survival analysis. After incorporating several clinical co-variates, the same allele of the same SNP in NTRK2 also showed association in the prospective sample (X2=14.1, p=0.028). These data provide further support for the role of NTRK2 in lithium response.

2425/W

Gene expression changes in peripheral blood as potential biomarkers of antidepressant exposure. *M.A. Kennedy[1], J.A. Harley[1,2], K. Doudney[1], M. Allington[1], L. McNoe[3], J. Pearson[4], M. Black[3], P.R. Joyce[2,4].* 1) Dept Pathology, University of Otago, Christchurch, Christchurch, New Zealand; 2) Department of Psychological Medicine, University of Otago, Christchurch, New Zealand; 3) Department of Biochemistry, University of Otago, Otago School of Medical Sciences, Dunedin, New Zealand; 4) Department of the Dean, University of Otago, Christchurch, New Zealand.

The actions of antidepressants depend on the promotion of neurogenesis and modulation of synaptic plasticity, which require modification of gene expression in the brain. Changes induced in the brain by these drugs may be reflected by expression differences in a more readily sampled tissue, the blood. To identify peripheral blood gene expression changes produced by antidepressants, we have used a rat model and delivered citalopram, paroxetine, haloperidol (a non-antidepressant control) or vehicle for 12 days via subcutaneously implanted osmotic minipumps. RNA was isolated using PAXgene blood RNA collection tubes, quantified, labelled and hybridised to rat whole genome expression arrays (Affymetrix Rat gene 1.0-ST). Analysis was carried out using the R package aroma.affymetrix (significance for differential expression was set at a false discovery rate p-value of <0.05) and tools from the Broad Institute Gene Pattern webserver (www.broadinstitute.org/cancer/software/genepattern/index.html). Significant transcript expression differences were detected between paroxetine, citalopram and haloperidol treated and vehicle-only treated samples which include genes involved in neuronal signalling and development. A total of 23 transcripts were altered by both SSRIs and not by haloperidol. These have become candidates for further investigation within our laboratory. We aim to use these preliminary data to guide analysis of peripheral blood mRNA samples from human patients initiating treatment with antidepressants, and to begin evaluating them as potential blood-based biomarkers for drug exposure and perhaps treatment.

2426/W

Association between Polymorphisms in CYP2D6 and ABCC2 and Clinical Outcomes of Adjuvant Tamoxifen Therapy for Breast Cancer Patients. *K. Kiyotani[1], T. Mushiroda[1], C.K. Imamura[2], N. Hosono[3], T. Tsunoda[4], M. Kubo[3], Y. Tanigawara[2], F. Aki[5], K. Hirata[6], Y. Takatsuka[7], M. Okazaki[8], S. Ohsumi[9], T. Yamakawa[10], M. Sasa[11], Y. Nakamura[1,12], H. Zembutsu[12].* 1) Laboratory for Pharmacogenetics, RIKEN Centor for Genomic Medicine, Yokohama, Japan; 2) Dept. of Clinical Pharmacokinetics and Pharmacodynamics, School of Medicine, Keio University, Tokyo, Japan; 3) Laboratory for Genotyping Development, RIKEN Centor for Genomic Medicine, Yokohama, Japan; 4) Laboratory for Medical Informatics, RIKEN Centor for Genomic Medicine, Yokohama, Japan; 5) Itoh Surgery and Breast Clinic, Kochi, Japan; 6) 1st Dep. of Surgery, Sapporo Medical University, Sapporo, Japan; 7) Dept. of Breast Surgery, Kansai Rosai Hospital, Hyogo, Japan; 8) Sapporo Breast Surgical Clinic, Sapporo, Japan; 9) Dept. of Breast Oncology, Shikoku Cancer Center, Ehime, Japan; 10) Yamakawa Breast Clinic, Kochi, Japan; 11) Tokushima Breast Care Clinic, Tokushima, Japan; 12) Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

The clinical efficacy of tamoxifen is suspected to be influenced by the activity of drug-metabolizing enzymes and transporters involved in the formation and elimination of its active forms, endoxifen and 4-hydroxytamoxifen. The purpose of this study was to investigate associations of polymorphisms in transporter genes as well as CYP2D6 to clinical outcome of patients with tamoxifen treatment. We studied 282 patients with hormone receptor-positive, invasive breast cancer receiving tamoxifen monotherapy. We investigated the effects of allelic variants of CYP2D6 and haplotype-tagging single nucleotide polymorphisms (tag-SNPs) of ABCB1, ABCC2 and ABCG2, which are possibly involved in the transport of tamoxifen or its metabolites, on recurrence-free survival using the Kaplan-Meier method and Cox regression analysis. We measured plasma concentrations of endoxifen and 4-hydroxytamoxifen in 98 patients receiving tamoxifen at a dose of 20 mg/day. CYP2D6 variants were significantly associated with shorter recurrence-free survival (P = 0.000036; hazard ratio 9.52 [95% CI 2.79-32.45] in patients with 2 variant alleles vs without variant allele). Among 51 tag-SNPs in transporter genes, a significant association was found at rs3740065 in ABCC2 (P = 0.00017; hazard ratio 10.64 [95% CI 1.44-78.88] in patients with AA vs GG genotypes). The number of risk alleles of CYP2D6 and ABCC2 showed cumulative effects on recurrence-free survival (P = 0.000000055). Patients carrying 4 risk alleles had 45.25-times higher risk compared to those with 0 or 1 risk allele. CYP2D6 variants were associated with lower plasma levels of endoxifen and 4-hydroxytamoxifen (P = 0.0000043 and 0.00052), whereas no significant difference was found among ABCC2 genotype groups. These results suggest that polymorphisms in CYP2D6 and ABCC2 may be important predictors for the clinical outcome of tamoxifen treatment for individual breast cancer patients.

2427/W

Disentangling baseline and on-treatment total bilirubin associations with UGT1A1*28. *L. Li[1], S.A. Bacanu[1], J.C. Whittaker[2], M.R. Nelson[1].* 1) GlaxoSmithKline, RTP, NC; 2) GlaxoSmithKline, Harlow, UK.

UGT1A1*28 is a common TA repeat polymorphism in the promoter region of the uridine diphosphoglucuronyl transferase. The TA7 allele causes an elevation in unconjugated and total bilirubin levels (Gilbert's syndrome). With the availability of UGT1A1*28 genotypes and total bilirubin (TBL) measurements from clinical trial participants for five compounds investigated in GSK, we could demonstrate that the *28 allele explains about 8% of the variation in pretreatment (baseline) TBL. Given its effect on baseline TBL, it would be important to differentiate the influence of the genotype on TBL levels in response to drug treatment from the drug independent effect. This is challenging, particularly when the comparator/placebo arm has not been genotyped or has limited sample size. We carried out a simulation study to assess whether commonly used analysis models can differentiate the drug-independent and dependent effects. Estimates of minor allele frequency and effect size on baseline TBL from the clinical trials are used to simulate drug-independent effects on TBL levels. We find that for analysis of maximum on-treatment TBL level as the endpoint, using baseline as a covariate (Model I) does not remove the background genotype effect and results in a very high false positive rate for drug-dependent effect. However, by analyzing change from baseline (i.e. subtracting baseline from the maximum on treatment TBL) as the endpoint without including baseline as a covariate (Model II), the analysis correctly removes the genetic association with baseline and correctly controls the type 1 error rate. In the analysis of the five GSK compounds, Model I results in significant genetic associations in four out of five compounds whilst Model II only identifies one compound, pazopanib, with statistically significant association. Pazopanib is the only one of the examined drugs known to be an inhibitor of UGT1A1. These results suggest that the *28 allele interacts with pazopanib to cause increases in TBL levels beyond those expected from the drug and genotype effects, separately. We recommend that where genotype is highly correlated with baseline, analyses should be carried out with both change from baseline and the commonly used approach in which baseline is included as a covariate and their results should be carefully compared. Genotyping markers of interest in the placebo/comparator arms can also help to facilitate the interpretation of results.

2428/W

Genome-wide association analysis of serum amyloid-A and statin response. *X. Li[1], Y.I. Chen[1], X. Su[1], K. Wojnoonski[2], J. Smith[3], M. Rieder[3], D. Nickerson[3], R.M. Krauss[2], J.I. Rotter[1].* 1) Cedars-Sinai Medical Center, Los Angeles, CA; 2) Children's Hospital Oakland Research Institute, Oakland, CA; 3) University of Washington, Seattle, WA.

Purpose: To identify genetic determinants of variation in plasma concentration of serum amyloid-A (SAA), a sensitive marker of inflammatory activity, and its response to statin treatment. Methods: Plasma SAA, lipids and C-reactive protein (CRP) levels were measured at baseline and after 6 wk treatment with simvastatin 40 mg/day in 566 Caucasian subjects in the Cholesterol/Atherosclerosis Pharmacogenetics Study. Genotyping was performed in about half the subjects using Illumina 317K beadchips and in the remaining subjects using Illumina 610K beadchips. Further imputation to ~2.5 million SNPs was peformed using BIMBAM v0.95. After excluding SNPs with minor allele frequency <0.05 and/or with poor imputation quality, ~2.1 million SNPs were available for the final analysis. Under the additive model, a linear model analysis (SNPTEST 1.15) was performed with log transformed SAA values and adjustment for age, gender and body mass index (BMI). Results: For baseline SAA, we identified two regions (11p15.1-p14 and 5q22.1) with 44/2 SNPs reaching genome-wide significance ($p<5\times10^{-8}$). Several candidate genes, such as SAA1, SAA2 and SAA4, are clustered at 11p15.1. The minor allele of the top SNP, rs4638289, located near 5' of the SAA1 gene, was associated with increased SAA with a p value of 2.1×10^{-20}. Two haplotype blocks were identified for these significant SNPs: one contained top 4 SNPs including rs4638289; the other one consisted of the remaining positive SNPs in this region. In addition, a candidate gene in 5q22.1, SLC25A46, has previous been reported to be associated with cardiac function (Vasan et al, 2009). After statin treatment, mean log transformed SAA decreased minimally overall (-0.02 ± 0.78 ng/mL in log-transform). This was significantly associated with reductions of CRP ($p<0.001$). No SNP associations with SAA response to simvastatin reached genome-wide significance, although 11 SNPs exhibited associations of $p<10^{-6}$ significance. Notably at 11p15.1, rs4638289 was associated with SAA response with a borderline p value of 0.06. Conclusions: Using GWAS, we have identified 2 regions associated with variation in SAA level, one of them containing a cluster of genes encoding SAA proteins. No loci were predictive of SAA change with statin treatment at the genome-wide level of significance. In addition, we have shown that statin-induced decreases in SAA are associated with decreases in CRP.

2429/W

CYP2D6 genetic polymorphism in fluoxetine and amitriptyline antidepressant response. *M. Lopez[1], E. Peñas-LLedo[2], H. Trejo[3], P. Dorado[2], J. Guerrero[3], M.E. Alonso[3], A. LLerena[2].* 1) Sistemas Biologicos, Univ Aut Metropolitana, Mexico City DF, Mexico; 2) 2Clinical Research Centre (CICAB), Extremadura University Hospital and Medical School, Badajoz, Spain; 3) National Institute of Neurology and Neurosurgery Manuel Velasco Suárez. Mexico City. Mexico.

CYP2D6 is involved in the metabolism of many antidepressants. It is characterized by a high individual variability in catalytic activity mainly due to more than 75 CYP2D6 alleles that determine metabolizer status. The role of CYP2D6 genetic polymorphism in the metabolism of amitriptyline and fluoxetine was previously demonstrated [LLerena et al, 2004]. Herein, we analyzed the relevance of CYP2D6 genetic polymorphism for the clinical response to the antidepressant drugs fluoxetine and amitriptyline. Sixty-five patients (DSM-IV) diagnosed with Major Depression and a score equal or greater than 17 on the Hamilton-Depression (HAM-D) were prospectively studied. They were treated either with fluoxetine or amitriptyline under antidepressant monotherapy. Informed written consent was obtained from all patients. Clinical Response was evaluated with HAM-D. Patients were evaluated every month. A two months period evaluation is reported here. Patients with a 50% decrease on HAM-D were considered as "responders". CYP2D6 genotyping was assayed by PCR-RFLP and RT-PCR. A first month evaluation showed that 49 out of the initial 65 remained (16 dropped-out) in the study and second month evaluation showed that 41 patients remained (8 more dropped-out). Among responders there were 56.6% and 60% to fluoxetine, and 50% and 70% to amitriptyline, at first and second follow up evaluations, respectively. Responders were characterized by presenting one or two CYP2D6 active genes. Furthermore, the number of active genes was related to better clinical response to both drugs. The percentage of responders was higher for those with two active genes than for patients carrying just one: (a) fluoxetine, 81 % vs.18 % at first month; 87% vs. 13% at second month; (b) amitriptyline, 60 % vs.40 % at first month; 83% vs. 17% at second month. All Ultrarapid metabolizers (n=3 UMs; those with more than two CYP2D6 active genes) were found to drop out during the first month. The only Poor Metabolizer patient in the study (PM; with none CYP2D6 active genes) was found among "non-responders" in both follow-up evaluations. The number of CYP2D6 active genes seems to be related to clinical response to the antidepressant drugs amitriptyline or fluoxetine. Among responders, the frequency of patients carrying two CYP2D6 active genes is higher than those with one copy. Moreover, UMs and PMs were not found in this group.

2430/W

Pharmacogenomics of Alzheimer's disease (AD): association of two novel genes (TAP1 and CYP7B1) in the ADME panel with efficacy of acetylcolinesterase inhibitors drugs. *F. Martinelli Boneschi[1], G. Giacalone[1], S. Lupoli[1,6], P. Brambilla[1], F. Esposito[1], G. Magnani[2], F. Caso[2], E. Coppi[2], C. Vismara[2], D. Galimberti[3], E. Scarpini[3], M. Franceschi[4], F. Macciardi[6], G. Forloni[5], G. Comi[1], D. Albani[5].* 1) Laboratory of genetics of complex disease, INSPE, Scientific Institute San Raffaele, Milan, Italy; 2) Department of Neurology, Memory disorder Unit, INSPE, Scientific Institute San Raffaele, Milan; 3) Department of Neurology, Ospedale Policlinico, Università degli studi di Milano, Milan, Italy;; 4) Department of neurology, Fondazione Multimedica, Castellanza, Italy; 5) Istituto di ricerche farmacologiche Mario Negri, Milan, Italy; 6) Dept of Science & Biomedical Technologies, School of Medicine, Università degli Studi di Milano.

Acetylcholinesterase inhibitors (AChI) are still today the mainstay of drug treatment for Alzheimer's disease (AD). They work by inhibiting the breakdown of acetylcholine, an important neurotransmitter associated with memory, by blocking the enzyme acetylcholinesterase. However, their effect is partial and limited to a subgroup of AD patients who are difficult to identify a priori. Our aim was to identify genetic markers predictive of response to AChI treatment in a cohort of AD-treated patients. A whole-genome case-control association study (WGAS) has been performed on 183 AChI-treated AD patients enrolled at several memory disorder clinics located in the North of Italy. Genotyping has been performed using the Illumina® Human660K BeadChip. Mean age at onset of the disease was 72 years, female:male ratio was 1.4:1, and baseline mini mental state examination (MMSE) was 20.4. AD patients have been classified into responders (R) and non-responders (NR) to treatment based on the change of MMSE during a 1-year treatment period. A parallel candidate-gene approach has been used to investigate the role of 8.037 SNPs present in the coding regions of 267 ADME (Absorption, Distribution, Metabolization, Excretion) genes (www.pharmaadme.org). SNPs have been tested for association after filtering for minimum allele frequency, Hardy-Weinberg equilibrium in controls, and genotyping failure with a logistic regression model of MMSE change during the treatment period as dependent variable. SNPs have been ranked based on their allelic association p value with the dependent variable. Among the top 10 SNPs, one is located in the coding region of the TAP1 gene and two in the CYP7B1 gene (p-values of 1.5*10-4, 7.7*10-4 and 8.1*10-4 respectively). The CYP7B1 gene catalyzes the synthesis of neurosteroids, such as dehydroepiandrosterone (DHEA) which are elevated in postmortem prefrontal cortex and temporal cortex of AD patients compared with cognitively intact control subjects. The TAP1 gene is a member of the MDR/TAP subfamily involved in multidrug resistance, and it is also implicated in the transport of antigens from the cytoplasm to the endoplasmic reticulum for association with MHC class I molecules, supporting the role of inflammation in the AD ethiopathogenesis and response to treatment. Replication of these three SNPs and additional ones identified using the WGAS approach is ongoing in an independent cohort of AD patients, using SEQUENOM® technology.

2431/W

Cytochrome-P450 Copy-Number Profiling among Caucasian, African-American, and Hispanic Individuals Identifies a Novel CYP2E1 Duplication Allele. *S. Martis[1], R. Vijzelaar[2], L. Edelmann[1], R. Kornreich[1], R.J. Desnick[1], S.A. Scott[1].* 1) Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, NY; 2) MRC Holland, Willem Schoutenstraat 6, Amsterdam, The Netherlands.

The polymorphic cytochrome-P450 (CYP450) system is a superfamily of over 50 hemoproteins which are the major enzymes involved in drug metabolism and bioactivation. Inherited genetic variation among the different CYP450 genes is common in all racial, demographic and ethnic groups and contributes to disease susceptibility and interindividual differences in drug response, including adverse drug reactions. In addition to single nucleotide polymorphisms, copy-number variation also influences CYP450 activity, particularly for CYP2D6; however, little is known about the role of copy-number variation among other CYP450 family members. To determine if other CYP450 genes have common deletion or duplication alleles, multiplex ligation-dependent probe amplification (MLPA) with probes (three to five per gene) that interrogated CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, and CYP3A5 was performed on over 90 DNA samples each from anonymous Caucasian, African-American, and Hispanic individuals. Among the CYP450 genes studied, copy-number variation was identified in CYP2A6 (19q13.2), CYP2B6 (19q13.2), CYP2D6 (22q13.2), and CYP2E1 (10q26.3). Specifically, among Caucasians, African-Americans and Hispanics, the frequencies of CYP2A6 deletion were 0.6%, 3.8% and 1.4%, and the frequencies of CYP2D6 deletion were 1.7%, 6.9% and 2.8%, respectively. CYP2B6 deletion was also identified among African-Americans (0.6%), possibly representing the recently described CYP2B6*29 partial deletion allele. Moreover, among Caucasians, African-Americans and Hispanics, the frequencies of CYP2A6 duplication were 0%, 0.8% and 0%, and the frequencies of CYP2D6 duplication were 3.5%, 6.9% and 2.1%, respectively. Interestingly, CYP2E1 duplication (MLPA probes at exons 5, 6, and 8) was found among 3.5%, 3.1% and 4.2% of Caucasian, African-American, and Hispanic individuals, respectively. To our knowledge, this is the first report of a CYP2E1 duplication allele, with a carrier frequency of ~1 in 14 among Caucasians, African-Americans, and Hispanics. Given CYP2E1 is involved in the metabolic activation of numerous hepatotoxic and procarcinogenic compounds, CYP2E1 duplication may have an important role in the development of certain chemically-mediated cancers and the risk for tumor development.

2432/W

Frequency of trinucleotide repeat variants in the promoter of the thiopurine S-methyltransferase gene. *M. Mikula, A. Buller Burckle, W. Sun, B. Crossley, C. Strom.* Dept Molecular Genetics, Quest Diagnostics Nichols Inst, San Juan Capistrano, CA.

Thiopurine S-methyltransferase (TPMT) catalyses the S-methylation of thiopurine immunosuppressants. At least 22 variants in the TPMT gene decrease methylation activity, which increases the risk for myelosuppression. However, 1-2% of Caucasians have ultra-high TPMT enzyme activity, which can lead to treatment resistance and hepatotoxicity. In vitro studies indicate that a trinucleotide repeat element in the promoter is responsible for the ultra-high TPMT activity in some individuals. Variants with 5 [(GCC)5] or 7 [(GCC)7] GCC repeats have been observed to result in ultra-high TPMT activity, while (GCC)6 is associated with normal TPMT activity. The goal of this study was to generate frequency data for the general U.S. population, since such data have not been reported. Fluorescent PCR was utilized to detect the described variants [(GCC)5-7]. Our genotypic analysis revealed 2 additional variants of unknown significance in the same region of the TPMT promoter: (GCC)8 and a 9-bp deletion (-6327_-6319delCGCCGCCGC). All variants were confirmed by DNA sequencing. A total of 1571 de-identified clinical specimens previously submitted to our laboratory for cystic fibrosis (CF) screening (n = 1393) or beta globin sequencing (n = 178) were analyzed for variation in the GCC repeat region of the TPMT promoter. Promoter variants were detected in 17 (1.08%). Of these, 5 were heterozygous for the (GCC)7 variant and 2 were heterozygous for (GCC)5 (7/1571 = 0.45%); 10 were heterozygous for variants of unknown significance, including (GCC)8 (n=1) and a 9-bp deletion (n=9). The rest (1554/1571=98.9%) were wild-type [(GCC)6]. (GCC)8 and the 9-bp deletion affect the number of GCC repeats in the TPMT promoter. Our data indicate that the carrier frequency for variants affecting the GCC repeats at -6329 in the TPMT promoter is approximately 1.0%, while the frequency of variants with known function ([GCC]5 and [GCC]7) is around 0.4%. Further studies are necessary to determine the effect of the novel variants on TPMT activity.

2433/W

CYP2D6 and CYP2C19 gene testing in psychiatric practice: A report from the Pharmacogenetics Research Clinic in Toronto. *D.J. Mueller, J.E. Sturgess, A.K. Tiwari, O. Likhodi, J.L. Kennedy.* Neurogenetics Section, Centre for Addcition and Mental Health, Toronto, ON, Canada.

Antipsychotic and antidepressant medication are widely used for psychiatric conditions such as schizophrenia or anxiety disorders including obsessive-compulsive disorder (OCD). Two polymorphic enzymes, CYP2D6 and CYP2C19, metabolize a large number of these medications. Functional polymorphisms in these enzymes can confer altered enzymatic activity, potentially leading to toxic or subtherapeutic drug levels. As part of our AmpliChip © Study, 39 individuals with OCD were genotyped for CYP2D6 and CYP2C19. Abnormal CYP2D6 activity (i.e. non-extensive metabolizer) was significantly associated with non-response to antidepressants. Two individuals who were ultrarapid metabolibzers (UM) failed to respond with 9 antidepressants and showed only repsonse with two trials, and one individual who was poor-responder did not respond to any SSRIs trial (p = .006). This data, including a thorough chart review, show that CYP2D6 non-extensive metabolizers are significantly more likely to have complicated medication histories and suggest that genotyping could have aided in selecting medications and dosages, potentially improving treatment outcome. As part of a new study at our Pharmacogenetics Research Clinic, the first set of patients with a diagnosis of schizophrenia and mood disorders with complicated medication histories have been enrolled prospectively and genotyped for CYP2D6 and CYP2C19. At study entry, patients are asked for consent for the genetic testing of their liver enzyme activities. Patients are assessed of current and previous treatment response and occurrence of side effects. After 6 weeks, the physicians are provided with an interpretation of the genotypic results and informed about the potential clinical implications which they will then discuss with their patients. The physicians are asked to complete a questionnaire evaluating the usefulness of the genotypic information provided by the study. After 12 weeks, the clients are assessed again to monitor potential adjustments of medications and their overall treatment outcome. Overall, physicians have returned excellent feedback that the genotyping results have been very helpful in allowing them to either select medications their patients are likely to better tolerate, or to adjust doses based on geneotype results and serum levels. Our findings suggest that CYP2D6 and CYP2C19 genotyping provides useful information that help physicians to improve pharmacotherapy for individual patients.

2434/W

Pharmacogenetics of functional variants that influence the warfarin and irinotecan response in the Azorean population (Portugal). *T. Pereirinha[1], M.J. Brilhante[1], C.C. Branco[1,2], R. Cabral[1,2], L. Mota-Vieira[1,2].* 1) Mol Gen & Pathol Unit, Hosp Divino Esp Santo PD, EPE, Azores Islands, Portugal; 2) Instituto Gulbenkian de Ciência, Oeiras, Portugal.

Genetic polymorphisms in genes encoding drug metabolizing enzymes, transporters and targets are known to play an important role in response to therapeutic drugs. Since pharmacogenomic information could predict drug dose and toxic effects, there is a clinical interest in improving strategies for individualized therapy. Here, we analyzed pharmacogenetically relevant genes that influence warfarin (*CYP2C9* and *VKORC1*) and irinotecan (*UGT1A1*) response, in 170 healthy blood donors from the anonymized Azorean DNA bank. Genotyping was performed by real-time PCR and fragment analysis using capillary electrophoresis. For warfarin response, the allele variants CYP2C9*2 (C430T) and CYP2C9*3 (A1075C) presented a frequency of 20.3 and 5.9%, respectively. Five genotypes were observed: CYP2C9*1/*1 (57.1%), CYP2C9*1/*2 (25.3%), CYP2C9*1/*3 (8.2%), CYP2C9*2/*2 (5.9%) and CYP2C9*2/*3 (3.5%). Regarding the *VKORC1* gene (G1639A), we identified 51 (30.0%) wild-type individuals, 96 (56.5%) heterozygous and 23 (13.5%) homozygous for the variant 1639A that corresponds to a frequency of 41.8%. The joint analysis of *CYP2C9* and *VKORC1* revealed that 68 individuals (40.0%) will need intermediate and/or low doses of warfarin if treatment is started, distributed as follows: *i*) 48 (28.2%) cases for intermediate doses (*1*1-AA®11, *1*2-GA®24, *1*3-GA®7, *2*2-GG®3 and *2*3-GG®3 individuals), and *ii*) 20 (11.8%) cases for lower doses (*1*2-AA®7, *1*3-AA®3, *2*2-AA®2, *2*2-GA®5 and *2*3-GA®3 individuals). For irinotecan response, the most relevant variant UGT1A1*28 is associated with low enzymatic activity. Inheritance of this variant was described to be a risk factor for irinotecan toxicity, leading to adverse reactions (myelosuppression or diarrhea). The results demonstrated that Azoreans show a frequency of UGT1A1*28 (30.9%) similar to other European populations. Two other rare variants - UGT1A1*36 and *37 - was also detected with low frequency (0.3%). The presence of these alleles, characteristic of African populations, is in agreement with the genetic makeup of the admixed Azorean population. Currently, we are analyzing other pharmacogenetic variants in order to improve individualized treatment of Azorean patients (lmotavieira@hdes.pt, Funded by the Azores Government, M 1.2.1./I/002/2008).

2435/W

The Serotonin Receptor Gene 5HTR2A and Drug Response in OCD. *M. Richter[1, 2, 3], V. Sinopoli[4], K. Witheridge[1], O. Likhodi[2], E. Burroughs[1], J. Kennedy[2, 3].* 1) Psychiatry, Sunnybrook Health Sciences Centre, Toronto, ON, Canada; 2) Neurogenetics Section, Centre for Addiction & Mental Health, Toronto, ON, Canada; 3) University of Toronto, ON, Canada; 4) McMaster University, Hamilton, ON, Canada.

Introduction: Pharmacogenetics is a valuable tool for reducing potential genetic heterogeneity in complex psychiatric illnesses, while also raising the prospect of personalized medicine in the future. Both these functions may be particularly important in the context of obsessive-compulsive disorder (OCD), a complex, chronic, and frequently severe psychiatric disorder. OCD is typically treated with serotonin re-uptake inhibitors (SRIs) but effectiveness of medication is limited. The serotonin 2A receptor gene (5HTR2A) is believed to be involved in selective serotonin reuptake inhibitor (SRI) response in depression, and thus is an interesting pharmacogenetics candidate in OCD. Method: This study examined retrospective treatment data for N=120 individuals with DSM-IV OCD. The 5HTR2A variants rs6314, rs6313, rs2296972, rs4941573, rs6305, and rs7997012 were genotyped and tested for association with response to any SRI medication (sertraline, fluoxetine, fluvoxamine, paroxetine, citalopram, and clomipramine). Individuals were grouped into those who improved following an adequate trial of one or more of these medications as compared with those who reported “minimal”, “no change” or “worsening” in response to medications tried. Results: No association was identified between the tested polymorphisms and response to any SRI. When considering the SRIs individually, the rs6305 G/G genotype appeared to confer greater likelihood of response to clomipramine (p=1.3 x 10-5). Analysis was suggestive of association between the A allele of rs4941573 and reduced response to fluvoxamine (p=0.052), when individuals who reported response to supoptimal trials were included in the analysis. Conclusions: Further work is needed with larger samples and ideally prospective drug response data. However although inconclusive, these results support a possible role for 5HTR2A and drug response in OCD.

2436/W

Germline Genetic Variation Underlying Resistance to Cisplatin and Carboplatin Treatment in the Yoruba Population. *A.L. Stark[1], H.E. Wheeler[2], W. Zhang[3], P.H. O’Donnell[2], R.S. Huang[2], M.E. Dolan[2].* 1) Dept Human Gen, Univ Chicago, Chicago, IL; 2) Section of Hematology/Oncology, Department of Medicine, Univ Chicago, Chicago, IL; 3) Institute for Human Genetics and Dept of Pediatrics, University of Illinois College of Medicine, Chicago, IL.

Cisplatin and carboplatin are platinating chemotherapeutics used to treat a variety of cancers including testicular, gynecologic, and lung cancer. Interindividual and inter-ethnic variation in cellular sensitivity and clinical response has been observed for both drugs. To elucidate the genetic component contributing to this variation, we investigated cellular susceptibility to these agents in five different HapMap lymphoblastoid cell line populations (CEU I/III, YRI I/III, ASW, ASN, DEN) and found that LCLs derived from the Yoruba (YRI) population were the least sensitive to both drugs (p<0.001). Utilizing 120 unrelated YRI cell lines (phases I and III) of HapMap, we performed a genome-wide association study to identify genetic variants associated with response. At a permissive p-value cutoff of p<0.0001, we found 80 SNPs that associated with both agents in the YRI population, which is significantly more than expected by chance. Forty-one of the 80 SNPs are eQTLs (p<0.0001), demonstrating that over half of the overlap signal represent this functional class. Eight of these 41 SNPs (~20%) are eQTLs with over 10 transcripts suggesting they are master regulators. We are currently performing a functional genome-wide association study to prioritize the gene targets of these master regulators for molecular validation. Identifying germline genetic variation in the Yoruba population allows for the identification of variants underlying resistance to these drugs with the possibility that allele frequency differences explain inter-ethnic differences. This work, ultimately, may lead to a better understanding of resistance to carboplatin and cisplatin treatment.

2437/W

Joint effects of common genetic polymorphisms and regular medication on long-term survival in humans. *S. Ukraintseva, K. Arbeev, L. Arbeeva, A. Kulminski, I. Akushevich, I. Culminskaya, D. Wu, A. Yashin.* Duke University, Durham, NC.

Long-term effects of medication on survival may differ dramatically when genes are taken into account. We studied the effects of ACE and APOE common polymorphisms on long-term survival of individuals regularly treated with drugs that potentially interact with these polymorphisms. We evaluated joint effects of ACE D/I polymorphism and ACE inhibitors, as well as APOE e2/e3/e4 polymorphism and cholesterol-lowering drugs (CHLD), on survival in Framingham Heart Study original and offspring cohorts (10,333 individuals, 53% females). These particular polymorphisms and drugs were selected because of their implication in number of pharmacogenetic and aging studies so that we could hypothesize favorable effects of some gene-drug combinations on longevity. We evaluated age-patterns of survival in following groups of individuals: (i) with ACE D/D, D/I and I/I genotypes; (ii) regularly treated with ACE inhibitors; (iii) combinations of (i) and (ii); (iv) with and without APOE e4 allele; (v) regularly treated with CHLD; (vi) combinations of (iv) and (v). Results. 1) ACE and ACE inhibitors. There was no significant difference in survival among individuals with different ACE genotypes. Regular intake of ACE inhibitors in medical history modestly but significantly ($p<0.01$) improved total survival. When both medication and genotype were taken into account, ACE inhibitors improved survival of D/D and D/I (but not I/I) carriers more substantially compared to situation when drug was considered alone. 2) APOE and CHLD. Individuals with APOE e4 allele had slightly worse survival than those without it. CHLD significantly ($p<0.0001$) improved total survival in both sexes, so that 60% of women taking CHLD survived to age 85 compared to 40% of those not taking it (40% and 20% of men respectively). When both gene and drug were considered, the favorable effect of CHLD on survival was more pronounced for those with e4 allele. Conclusion. When gene-drug interaction is taken into account, favorable effect of ACE inhibitors on survival strengthens for D allele and disappears for I/I genotype carriers, while treatment with CHLD compensates adverse effects of APO e4 allele on survival (possibly providing protection against AD, as some studies suggest). Significant gene-drug interaction effects on long-term survival observed in this study stress importance of pharmacogenetic studies for understanding/revealing possible anti-aging effects of chronic medication in some genotypes.

2438/W

Effect of Genetic Variation in LAT1 and LAT2 on Toxicity in Multiple Myeloma Patients Receiving High-Dose Melphalan and Autologous Stem Cell Transplant. *M.J. White[1], J. Giglia[2,3], C. Holt[1], S. Brandt[2,3], S.M. Williams[1].* 1) Center for Human Genetics Research, Vanderbilt Univ, Nashville, TN; 2) Vanderbilt-Ingram Cancer Center, Nashville, TN; 3) Tennessee Valley VA Healthcare System, Nashville TN.

Multiple myeloma, the second most common cancer of the blood in the United States, is characterized by an overproduction of abnormal plasma cells in the bone marrow. It is frequently treated with high-dose melphalan (HDM) in association with autologous stem cell transplantation. Although HDM is an effective treatment, it has mucositis as a common dose-limiting side effect. Mucositis, or inflammation of the mucous membranes of the digestive tract, causes severe pain and in extreme cases oral ulceration, leading to inability of the patient to ingest food or liquid. In some cases, intravenous feeding (total parenteral nutrition or TPN) is required. The aim of this study was to assess whether genetic variation in two candidate genes, LAT1 (L-type amino acid transporter 1) and LAT2, associates with this adverse drug reaction, so that adequate treatment could be implemented prior to HDM. These two candidates were selected because LAT1 is important in the transport of melphalan into cells and LAT2 has been hypothesized to serve the same role. Patients receiving HDM were recruited at the Vanderbilt University Medical Center, the Nashville Veterans Administration Medical Center, and the Veterans Administration Medical Center in San Antonio, TX. Cases of clinically severe mucositis were defined by requirement for TPN; controls did not require TPN. In total, 87 Caucasian subjects were enrolled, including 49 cases (29 of whom were male) and 38 controls (32 males). Thirty-two tag SNPs were genotyped and 28 passed quality control (8 in LAT1 and 20 in LAT2). One SNP in LAT1 (rs4240803) provided evidence of association in the total data set (OR= 2.48; 95% CI 1.15-5.68 p= 0.015). Association appeared to be stronger in males alone (OR= 3.62; 95% CI 1.32-11.29.68 p=0.006). Haplotype analyses in LAT1 revealed that all significantly associating haplotypes included this SNP, supporting its role in TPN predisposition and, by inference, severe oral mucositis. Results from LAT2 showed this gene was less strongly associated with TPN use following HDM, although some analyses did suggest a weak association with several SNPs in this gene. Our results support a role of LAT1 genetic variation in risk for developing severe oral mucositis following HDM treatment for multiple myeloma, especially in male patients. This suggests that membrane transport of melphalan is a key factor in clinical toxicity, and is subject to genetic variation.

2439/W

Inhaled Corticosteroid Treatment Modulates the Effect of Polymorphisms on Bronchodilator Response in Asthmatics. *A.C. Wu[1,2], B.E. Himes[3,4,5,6], J. Lasky-Su[6], K. Tantisira[6], S.T. Weiss[5,6].* 1) Department of Population Medicine, Harvard Medical School and Harvard Pilgrim Health Care, Boston, MA; 2) Children's Hospital, Boston Division of General Pediatrics, Boston, MA; 3) Harvard-MIT Division of Health Sciences and Technology, Cambridge, MA; 4) Children's Hospital Informatics Program, Boston, MA;; 5) Partners Center for Personalized Genetic Medicine, Boston, MA; 6) Channing Laboratory, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

Inhaled corticosteroids and β2-agonists are the two most commonly used medications for asthma. Single nucleotide polymorphisms (SNPs) appear to influence a patient's response to inhaled corticosteroids and β2-agonists. Because the effect of treatment with inhaled corticosteroids is synergistic with the effect of β2-agonists and a patient's response to both medications is genetically influenced, we hypothesized that inhaled corticosteroids could influence the effect of multiple SNPs associated with bronchodilator response across the genome. Our goal was to assess whether treatment with inhaled corticosteroids modulates the measured association of SNPs with bronchodilator response in a population of subjects with asthma using a genome-wide association approach. Our primary population is composed of subjects from the Childhood Asthma Management Program (CAMP), a clinical trial that followed asthmatic children for four years. Genome-wide SNP genotyping for 561 Caucasian CAMP subjects and their families was performed on Illumina's HumanHap550 Genotyping BeadChip (Illumina, Inc., San Diego, CA). Before frequency and genotyping pruning, there were 516,512 SNPs. SNPs were excluded for missing genotype in more than 5% of subjects, having minor allele frequency (MAF) less than 5%, having Hardy-Weinberg equilibrium p-values among controls less than 0.001. After frequency and genotyping pruning, there were 449,540 SNPs. We conducted a gene by environment analysis in PLINK with inhaled corticosteroid treatment as the environmental exposure and bronchodilator response as the outcome measure. We found that the regions of SNPs that are most significantly associated with bronchodilator response while accounting for inhaled corticosteroid treatment are in chromosomes 19 and 8, with 12 SNPs having a gene by environment interaction p-value of <1E-05. We conclude that treatment with inhaled corticosteroids appears to modify the effect of SNPs on bronchodilator response. We have identified SNPs that appear to modify this effect in the CAMP population. We are replicating these findings in an independent population.

2440/W

Distribution of CCR-5Δ32, CCR2-64I and SDF1-3'A alleles that modulate drug-response and HIV infection among Jordanians. *K.H. Alzoubi[1], O.F. Khabour[2], L.J Abu-Haweleh[2].* 1) Clinical Pharmacy, Jordan University of Science and Technology, Irbid, Jordan; 2) Medical laboratory Sciences, Jordan University of Science and Technology, Irbid, Jordan.

Chemokine receptors are involved in the entry process of the HIV virus into the cells and the subsequent development of AIDS. In this study, genotype distribution patterns of polymorphisms in chemokine receptor genes, CCR5 (CCR5-Δ32), CCR2 (CCR2-64I), and SDF-1 gene SDF1-3'A (the only ligand for CXCR4) that affect drug-response against HIV were evaluated among Jordanians. A total of 540 subjects were randomly selected from all the 12 Jordanian provinces. Polymorphisms were genotyped using PCR sequencing and PCR-RFLP technology. Six individuals were found to carry CCR5-Δ32 allele (0.6%) in heterozygous genotype. The frequencies of CCR2-64I and SDF1-3'A were 17.5 % and 34.2% respectively. No significant difference in the mutant allele distribution of the CCR5 (CCR5-Δ32) and CCR2 (CCR2-64I) polymorphisms was noticed through the different parts of Jordan ($P > 0.05$). However, the genotypes of SDF-1 gene showed significantly different distribution with enriched homozygous mutant genotype in the middle of Jordan ($P < 0.05$).

2441/W

PREVALENCE OF CYP3A4*18, GSTM1 0/0 AND GSTT1 0/0 : A PRELIMINARY STUDY OF GENE POLYMORPHISM AS BIOMARKERS FOR DRUG THERAPY IN FILIPINOS. *C.G. Boado[1], A.P. Baluyot[1], E.A. Banzon[1], M.R. Bernal[1], R.B. Bio[1], J.G. Apostol[1], M.J. Manansala[1], R.M. de Guia[2,3].*
1) Department of Pharmacy, University of Santo Tomas,España, Manila 1015; 2) Department of Biochemistry, University of Santo Tomas,España, Manila 1015; 3) Research Center for the Natural Sciences, University of Santo Tomas,España, Manila 1015.

Introduction: The inter-patient difference in drug metabolism is documented to be attributable to heritable genetic variations in the nucleotide sequence and deletion of genes coding for these DMEs involved in drug transport, metabolism and action. The finding of the existence variant alleles and knowledge about their genotypic/allelic frequency in specific ethnic groups are important to lead to individualized drug dosing and improved therapeutics. This study aimed to detect SNP in CYP3A4 and the homozygous deletion (0/0) of GSTM1 and GSTT1 in a Filipino population. Methodology: One-hundred and forty two Filipino subjects were genotyped of the CYP3A4*18 SNP by using PCR-RFLP with HpaII endonuclease and the GSTM1 0/0 and GSTT1 0/0 by basic PCR with β-globin as internal positive control, all were followed by AGE. Results: The sample DNA pool conformed to the Hardy-Weinberg equilibrium ($\chi 2$=0.01685) based on the gathered genotype frequencies. In the group of 142 sample DNAs, the frequency of the CYP3A4*18 variant allele found in the Filipino population was 0.01056. The percentage of GSTM1 0/0 observed was 64.08%, while that for GSTT1 0/0 was 48.59% Conclusions: Relative to studies done in other countries, the frequency of the variant allele of the CYP3A4*18 using the Fisher Exact Test was not significant higher to the Japanese, Korean and Malaysian studies. The percentage of GSTM1 0/0 observed in Filipinos was significantly higher than Indians, Chinese, Japanese and Koreans and Caucasians while that of the GSTT1 0/0 was not significantly lower than Chinese, Japanese and Koreans. Frequencies of the GSTM1 0/0 and GSTT1 0/0 in this study were higher to that reported previously in the Filipino population.

2442/F

Cloacal Dysgenesis Sequence: elucidating the mechanism of multiple malformations using fetal imaging, genetic testing and autopsy. *A. Robichaux[1], D. Niyazov[2], W. Robichaux[3], A. Kenney[4].* 1) Maternal & Fetal Medicine, Ochsner Clinic Foundation, New Orleans, LA; 2) Dept Pediatrics, Ochsner Clinic Foundation, New Orleans, LA; 3) Dept Pathology, Ochsner Clinic Foundation, New Orleans, LA; 4) Dept Radiology, Ochsner Clinic Foundation, New Orleans, LA.

Cloacal dysgenesis sequence (CDS) is a rare disorder characterized by a phallus-like structure, smooth perineum, and absence of urethral, vaginal, and anal openings. Renal dysgenesis, oligohydramnios and pulmonary hypoplasia are typical associated findings. We present a patient with multiple congenital anomalies which were investigated during the various periods of gestation using ultrasound (US), magnetic resonance imaging (MRI), biochemical and chromosomal studies, and autopsy with gross and microscopic examinations. The proband's parents were 3rd cousins who previously had a child with Zellweger syndrome (ZS). At 16 weeks of their 2nd pregnancy, severe fetal bladder distension was detected on US. Fetal MRI showed oligohydramnios and megacystitis. Clear fluid was aspirated from the bladder and amniotic fluid was mildy decreased. Karyotype and 105K oligo array on amniocytes revealed a normal female. Due to the 25% chance for ZS and one report of ZS with genitourinary anomalies, the amniotic fliud was sent for very long chain fatty acids and plasmalogens which were negative. At 27 weeks, the fetus developed ascites and oligohydramnios. A repeat MRI showed two oval structures of unclear origin, dysplastic kidneys, hypoplastic lungs and dilated sigmoid colon. The proband was delivered at 34 weeks and died shortly after birth. The autopsy showed severe ascites, anorectal atresia, dilated sigmoid colon with meconium and microcalcifications, ambiguous genitalia, urethral stenosis, two large cystic structures, two ovaries, multicystic kidneys and pulmonary hypoplasia. A 135K oligo array on the postmortem tissue was negative. CDS occurs due to a failure of migration and fusion of the urorectal septum with the cloacal membrane thus affecting urogenital and anorectal development. In our proband the urethral stenosis resulted in the bladder outlet obstruction, megacystitis, hydroureters and renal dysplasia. The bladder ruptured and ascites with oligohydramnios ensued, causing pulmonary hypoplasia. The two large cystic structures with ovaries on each side most likely represented a bladder and uterus as one entity which was once reported. The anorectal atresia and dilated meconium-filled sigmoid colon with microcalcifications may have occurred in parallel with genitourinary malformations. In conclusion, we demonstrate how the various diagnostic modalities can be utilized to explore the mechanism of malformations in CDS, which may aid in elucidating its etiology.

2443/F

Aneuploidy Detection by QF-PCR of STR Markers on the Applied Biosystems 3500 Genetic Analyzer. *V. Bansal[1], S. Jordan[2], C. Davidson[1], A. Pradhan[1], A. Felton[1], N. Duxbury[2], S. Higgins[2].* 1) Applied Biosystems, Foster City, CA,; 2) Gen-Probe Life Sciences, Abingdon, UK.

Normal human somatic cells are euploid and contain a diploid (2N) set of autosomes and a pair of sex chromosomes. Cells that do not contain an exact diploid set are termed aneuploid and, therefore, either lack or contain additional chromosomes. Common types of aneuploidy are monosomy (the loss of one sex chromosome) and trisomy, three copies of a given chromosome in a diploid cell. In early pregnancy, samples of amniotic fluid (AF), chorionic villi (CVS), or umbilical cord blood can be analyzed for aneuploidy using the molecular technique quantitative fluorescence PCR (QF-PCR). Analysis of short tandem repeat (STR) markers using QF-PCR is a common strategy employed in clinical research laboratories for the detection of chromosomal aneuploidy. Here we demonstrate performance equivalent between the Applied Biosystems 3130*xl* Genetic Analyzer and the new Applied Biosystems 3500 Genetic Analyzer for the detection of aneuploidy. A reference panel of samples was amplified with reaction mixes from each of the *TrueScience*™ Aneuploidy STR Kits: STR-Plus, STR-XY, STR-13, STR-18, STR-21. The reference panel of 95 samples and a negative control comprised of 45 female samples (47%) and 50 male samples (53%) with gDNA obtained from four different sources CVS (88%), cell-lines (5%), AF (4%), and whole blood (2%). Six aneuploidies were assessed consisting of 18 trisomy 18 samples (19%), 16 trisomy 21 samples (17%), 7 trisomy 21 samples (7%) , 3 Turner syndrome samples (3%) , 1 sample each of Triplo-X and Klinefelter syndromes (1%), and 49 wild type samples (52%). The *TrueScience*™ STR-Plus assay determined the correct genotype for 100% of the samples that passed sizing and reaction quality controls. There was a 100% correspondence between the results obtained on the 3130xl Genetic Analyzer and the new 3500 Genetic Analyzer for the *TrueScience*™ STR-Plus assay. The follow up individual chromosome kits (*TrueScience*™ STR-13, STR-18, STR-21, and STR-XY assays) determined the correct genotype for 100% of the samples that passed sizing and reaction quality controls with a 100% correspondence between the results obtained on the 3130*xl* Genetic Analyzer and the 3500 Genetic Analyzer. The advanced capabilities of the 3500 Series Genetic Analyzers demonstrated performance equivalence with 3130 Series for the detection of chromosomal aneuploidy.

2444/F

Determination of the origin of extra chromosome material on 15p region in an amniotic fluid sample using MFISH techniques. *V. Catala[1,2], C. Garrido[1], Z. Sarrate[3], M. Martin[3], E. Masip[1], E. Cuatrecasas[1], A. Serés-Santamaría[1], F. Vidal[3].* 1) Prenatal Genetics, Barcelona, Catalonia, Spain; 2) Unitat de Biologia Cellular i Genética Médica. Departament de Biologia Cellular, Fisiologia i Immunologia. Facultat de Medecina. Universitat Autònoma de Barcelona, Catalonia, Spain; 3) Unitat de Biologia Cellular. Departament de Biologia Cellular, Fisiologia i Immunologia. Facultat de Biociències. Universitat Autònoma de Barcelona, Catalonia, Spain.

The amniotic fluid sample of the second pregnancy from a 32 years patient was refered to our lab without increased risk indication. The pregnancy was achieved after the transfer of two frozen embryos, coming from a donor sperm ICSI cycle, because the couple has both male and female infertility factors. The previous pregnancy with fresh embryos ended with the birth of a chromosomally normal child. The sample of amniotic fluid obtained by amniocentesis at 16 weeks of gestation, had a normal result after an aneuploidy screening using FISH (Multicolor AneuVysion Probe Kit, Abbott Molecular). The conventional cytogenetic study showed the presence of extra material on the short arms of one chromosome 15 in 72% mosaicism. The extra material was C-banding and Ag-NOR negative, and had no hybridization with D15Z1, D15Z4 and DYZ1 DNA probes (Abbott Molecular). The SpectraVysion MFISH Assay (Vysis) was applied to determine the origin of the extra chromosome 15 material. The results obtained indicated that the small fragment was derived from chromosome 3. Subtelomeric regions probes of chromosome 3 were applied (Abbott Molecular) and an extra signal of the 3p region was observed on the 15p region, which confirmed the result obtained by MFISH. The final karyotype was 46, XX,der(15)t(3;15)(p24.3;p12)[48]/46,XX[19].ish der(15)(D15Z1-, wcp+;,D3S4559+). A fetal blood sample was obtained, and the same translocation between chromosomes 3 and 15 was observed in 96% of the analyzed metaphases. Extensive fetal ultrasound at 20 weeks of gestation revealed a heart disease. The couple decided for the termination of pregnancy. Fetal tissues for cytogenetic analysis were not available.

2445/F

Aneuploid products of conception confer increased risk to subsequent conceptuses. *K.A. Eickholt[1], D.P. Agamanolis[1,2], J.M. Malone[1], R.V. Lebo[1,2].* 1) Department of Pathology, Akron Children's Hospital, Akron, OH; 2) Northeastern Ohio Universities College of Medicine, Rootstown, OH.

Aneuploidy, defined as an abnormal copy number of whole normal chromosomes in one cell, occurs frequently in conceptuses and reduces viability. Culturing chorionic villus cells that remain viable long after other cells in products of conception enabled karyotyping nearly all submitted products of conception samples. The pathologic appearance of the dissected fetal villi did not correlate with the chromosomes as suggested earlier for a more targeted group of viable abnormalities. This study found normal appearing villi in products of conception with 45,X, trisomies of chromosomes 2, 6, 9, 11, 12, 13, 14, 15, 16, 18, 21, 22, and X, triploidy, tetraploidy, and the more complex karyotypes: 48,XX+15,+21, 48,XX+2,+5, 48,XX+16,+21 48,XXX/47,XX+2/46,XX, and 47,XY+15/94,XXYY+15,+15. Furthermore, this study of 345 products of conception revealed that a large proportion of conceptuses with chromosome aneuploidy conferred a previously unreported increased risk of a second aneuploid conceptus to women of any reproductive age. This is in contrast to prior studies that found an increased risk of viable trisomy 13, 18, or 21 in conceptuses with these three trisomies sampled by CVS or amniocentesis, but no increased risk when found in products of conception (Warburton et al. 1980, 2004). Furthermore, our study found that all aneuploidies including monosomy X, the most common trisomy 16, and trisomy 22 confer an increased risk of another aneuploid conceptus. Of interest, a mother with a trisomy 3 POC and a fragile site on band 9q12 subsequently conceived a conceptus with an unbalanced translocation 92,XXXX,t(9;17)(q12;p13) karyotype including a breakpoint involving the fragile site. These results suggest that prudent obstetrical practice would karyotype all products of conception and follow an aneuploid conceptus with an offer to prenatally karyotype any subsequent conceptus regardless of maternal age.

2446/F

Additional Chromosome Copy Number has Minor Influence on Expression of Genes Present on Trisomic Chromosomes in Humans. *J.A. Gebbia, T. Dunn.* CytoGenX, Stony Brook, NY.

Utilizing a high through-put proteomic screen, amniotic fluid was analyzed from patients pregnant with a fetus diagnosed with trisomy 21, 18 or 13 and age-matched, gestational control patients pregnant with a fetus of normal karyotype. Over 800 unique proteins were identified in amniotic fluid revealing a distinct proteomic profile for each trisomy and normal control samples. Most proteins detected to be increased or decreased in trisomic samples, relative to control samples, were found to be encoded on chromosomes other than those present in trisomic form. The presence of an additional chromosome copy of 21, 18, or 13 only effected the expression of a small number of genes present on each respective trisomic chromosome, indicating the expression of most genes present on trisomic chromosomes are not directly influenced by extra copy number. These results suggest only a few genes present on trisomic chromosomes may be responsible for the increased or decreased expression of a cascade of gene products encoded on other chromosomes which cause the phenotypes observed in patients diagnosed with a trisomy.

2447/F

Failure of spermatogenesis in distal Xp nullisomy might be associated with miss-conjunction of sex chromosomes. *Y. Kido[1], S. Sakazume[1], K. Obata[1], N. Murakami[1], Y. Ooto[1], K. Shimojima[2], T. Yamamoto[2], T. Nagai[1].* 1) Deartment of pediatrics, Dokkyo Medical University Koshigaya Hospital, Koshigaya, Japan; 2) Institute for Integrated Medical Sciences, Tokyo Women's Medical University, Tokyo, Japan.

Introduction Generally patients with Xp nullisomy have been known to be infertile, but the precise mechanism of infertility is not uncovered. We experienced 2 patients with Xp nullisomy and determined the breakpoints by STS analysis. Their infertility could not be explained by their deleted genes. We hypothesized that miss-conjunction of their sex chromosomes is associated with their infertility. **Patients** Patient 1 is a 19-year-old male with normal mentality, normal puberty, height of 161 cm (-1.7 SD), hypoplastic mid-face, ichthyosis, hypospadias, and cardiac anomaly (aortic regurgitation). His karyotype by G-banding analysis was 46, XY. Detailed analysis revealed interstitial deletion: the telomeric and centromeric breakpoints were between DXS6837 and DXS6834, between DXS237 and DXS278, respectively. Patient 2 is a 15-month-old boy with mild mental retardation (unable to stand up by himself, no meaningful words, not understanding his own name), height of 68 cm (-3.5 SD), hypoplastic mid-face, ichthyosis, and hypogenitalism (micropenis, hypoplastic scrotum, undescended testes). His karyotype by G-banding analysis was 46,Y,der(X)t(X;Y)(p22.3;q12)mat. **Discussion** Generally patients with Xp nullisomy show various symptoms associated with their karyotypes and many symptoms are explained by deleted genes. Most of all reported patients with Xp nullisomy involving pseudoautosomal region (PAR) were infertile. In contrast, patients with interstitial deletion conserving PAR were fertile. In our patients none of the deleted genes were known to be associated with spermatogenesis. Only *KAL1* gene close to the breakpoints is related to fertility but *KAL1* gene was not deleted in both of them. The main genotypic difference between our two patients was that only patient 1 had PAR. Usually, patients with Xp nullisomy have defect of a pseudoautosomal region and cause miss-conjunction of their sex chromosomes, resulting in oligo- or azoo-spermia. This suggests that patient 1 is fertile and patient 2 is infertile. **Conclusion** The development of spermatogenesis may be related to the existence of PAR.

2448/F

Diagnostic benefit of comprehensive prenatal MLPA testing of fetuses with large NTs. *M.V.E. Macville[1], L.M.H. Houben[1], M.A.A. Lennarts[1], K. Herzberg[1], N.D. Muntjewerff[2], Y.H.J.M. Arens[2], S.G.M. Frints[2], A.B.C. Coumans[3], C. Willekes[3], J.P.M. Offermans[3], D. Veersema[3,4], C.E.M. de Die[2], J.J.M. Engelen[1].* 1) Cytogenetics Unit/Clin Gen, Maastricht Univ Med Ctr, Maastricht, Netherlands; 2) Clinical Genetics, Maastricht Univ Med Ctr, Maastricht, Netherlands; 3) Obstetrics and Gynecology, Maastricht Univ Med Ctr, Maastricht, Netherlands; 4) Diagnostic Center, Eindhoven, Netherlands.

In a case-control cohort of 1576 prenatal samples, 90 (5.7%) presented with a heart-outflow defect or a nuchal translucency (NT) larger than 3.5 mm, including hygroma colli and hydrops. We investigated the diagnostic benefit of testing this cohort with MLPA probe sets P036/P070 and P245 for, respectively subtelomeric imbalances and 21 microdeletion syndromes compared to MLPA P095 alone for common aneuploidies. All samples were karyotyped by GTG-analysis in a routine cytogenetic diagnostic work-up. Subtelomeric imbalances and microdeletions detected by MLPA were confirmed by FISH. Parental blood was available to identify familial copy number variations without clinical consequence. MLPA was performed in duplo on uncultured samples to produce a result in 4-7 days after the invasive procedure. In 6 of 90 samples, one or more MLPA test failed due to inferior DNA quality (6.7%). In the remaining 84 samples, we found 22 aberrant results (26%). Nineteen were detected with MLPA P095 and P036/070 comprising of trisomy 21 (n=9), trisomy 18 (n=4), trisomy 13 (n=2) and monosomy X (n=4). Two were detected by MLPA P036/070 alone, being an autosomal unbalanced translocation and a cryptic terminal deletion 9p. These 21 aberrations were also visible in GTG analysis. One aberration was a microdeletion 9q22.3 that presented with a NT 4 mm as the sole ultrasound finding, that was only detected by MLPA P245 and not by the other methods. FISH and experimental SNP-array analysis further delineated the 9q22.3 deletion. We conclude that prenatal cytogenetic diagnostics benefits from MLPA P036/070 testing with respect to detection of common aneuploidies and subtelomeric imbalances. Targeted testing for microdeletion syndromes may be disputable, although - in a relatively small cohort - it has detected one case that would otherwise not be discovered. The 62 samples with normal MLPA- and GTG-analyses are eligible for a 2.7M SNP-array testing which provides the largest genomic coverage (including subtelomeric regions) for copy number variant detection at this time. In contrast to array-based testing, MLPA testing is fast an cheap. It is easy to implement because it requires equipment that is present in most of today's clinical genomic laboratories. Therefore, in terms of time- and cost-effectiveness, multiple MLPA tests may be utilized preceeding traditional karyotyping to provide a rapid and comprehensive result.

2449/F

Prenatal Diagnosis of 9p Duplication Syndrome. *A. Singer[1], R. Gobozov[1], C. Vikler[2].* 1) Dept Clinical Gen Unit, Barzilai Med Ctr, Tel Aviv, Israel; 2) Institute of Medical Genetics, Wolfson Medical Ctr, Holon, Israel.

In 1970, Rethore' et al. reported the first patients with duplication of 9p as ''trisomy 9p syndrome.'' Since than, a large number of patients (more than 150) have been described with duplication of various segments of 9p. Because of that, the term ''duplication 9p syndrome'' instead of ''trisomy 9p syndrome'' is used. Most of the reported patients had only partial duplication rather than the whole arm duplication of 9p. Clinical findings include growth and mental retardation, ear anomalies, hypertelorism, prominent or globular nose, downturned corners of the mouth, and hand-foot anomalies. It is the one of the most frequent autosomal anomalies with long survival rate. Usually 9p duplication is derived from familial translocation. Only 16 cases have been reported as de novo 9p duplication or partial duplication. Ultrasound imaging during pregnancy of fetuses carrying 9p duplication can be easily missed since dysmorphic features typical to this syndrome are hard to detect.. Thus far there is no report of prenatal diagnosis of 9p duplication. We report a case of a fetus with 9p duplication detected during pregnancy. The proband is a healthy 27 years old woman. NT measured at 12 weeks gestation was 2.2mm. Integrated (first and second trimester) test showed a 1:9100 risk for Down syndrome. Ultrasound at 20 weeks gestation, revealed a 6.9 mm nuchal thickness with no other anomalies. Fetal cardiac echo was normal. Amniocentesis was performed and showed a fetal karyotype 46,XY(9p+). CGH-A analysis of the fetus' DNA diagnosed a 8.5 Mb gain from 9p24.2-9p23. The parents chose to terminate the pregnancy. This is the first case reporting prenatal diagnosis of 9p duplication syndrome associated with increased nuchal thickness.

2450/F

Downs Syndrome: Indian scenario. *S. Komandur[1], R. Chikkala[1], S. Kuruganty[2].* 1) Department of Cytogenetics, Vijaya Diagnostics, Hyderabad, India; 2) Innova Childrens' Hospital, Tarnaka, Hyderabad, India.

In developed countries, prenatal screening for Down Syndrome is a general practice to identify Downs inutero. But in India, prenatal testing is warranted only in cases falling in the high risk group. This leaves less room for options like termination and increased incidence of Downs cases. We, in our clinic evaluated patients of suspected Downs syndrome. Their age ranged between 7 days to 8 years. The maternal age at the time of birth ranged between 20 yrs to 25 yrs. None of the mothers had the screening for Downs prenatally. Karyotyping was performed on 30 GTG banded metaphases for each of these patients. The karyotypes were 47, XY, +21 / 47, XX, +21 / 47, XX, +21, 21p+ / 46, XY, t (21; 21). We conclude that screening of every pregnant woman for Down syndrome must be made as a mandate instead of following it a s a ritual for only high risk groups, to identify Downs inutero. This would leave an informed choice to the couple whether to continue with the pregnancy.

2451/F

An Exon 1 Deletion in OTC Identified Using Chromosomal Microarray Analysis in a Mother and Her Two Affected Deceased Newborns: Implications for the Prenatal Diagnosis of Ornithine Transcarbamylase Deficiency. *F. Quintero-Rivera[1], J. Deignan[1], J. Peredo[2], W.W. Grody[1], B. Crandall[2,4], M. Sims[2], S.D. Cederbaum[2,3,4].* 1) Pathology & Laboratory Medicine; 2) Pediatrics; 3) Human Genetics; 4) Psychiatry,David Geffen School of Medicine at University of California Los Angeles, UCLA, Los Angeles, CA.

Objectives. To describe the outcome of two consecutive pregnancies with a clinical presentation of ornithine transcarbamylase deficiency who initially lacked a molecular diagnosis. To demonstrate the importance of analyzing copy number changes in the management of cases with normal or inconclusive DNA sequencing results. To report the smallest intragenic deletion of the OTC gene. Methods. Oligonucleotide SNP array-based Chromosomal Microarray Analysis (CMA) was performed from the mother's peripheral blood. Polymerase Chain Reaction, PCR, and sequencing were done using DNA extracted from newborn screening blood spots. Results. A 119-kb deletion on Xp11.4 was detected in the phenotypically normal mother including the 5'UTR, exon 1, and part of intron 1 of the OTC gene. PCR identified the same deletion in the DNA from the two deceased male newborns. Amplification and sequencing of the deletion breakpoint in the mother revealed that it was larger than predicted by the CMA results. Conclusions. In patients with a clinical and biochemical presentation of OTC deficiency and negative OTC sequencing, CMA should be performed as a reflex test to look for deletions. PCR confirmation of CMA results should be performed when possible to precisely determine the deletion breakpoints for future use in molecular prenatal diagnosis.

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Siblings with primary congenital glaucoma. *D. Albu, E. Severin, M. Dumitrescu, C. Albu.* Dept Human Genetics, Carol Davila Univ Med & Pharm, Bucharest, Romania.

Usually glaucoma develops in older adults but primary congenital glaucoma (PCG) is observed at birth or in early infancy, and is an important cause of visual loss in children. The aims of the study were to analyze clinical and genetic features of PCG in a family with 2 affected sons and to detect if the unborn child of the pregnant mother inherited the disease-causing mutation. Case presentation: A family of Romany ethnic group with unaffected parents and their 2 sons affected by PCG were investigated clinically and genetically. Ophthalmic examination included: ophthalmoscopy, tonometry, perimetry and gonioscopy. DNA samples from all family members were analyzed for mutations in CYP1B1 gene. The mother is affected by severe myopia. The two sons (aged 9 and 17 years, respectively) are affected by PCG. Age of onset was in the first two month of life for both brothers. The sibs shared similar PCG phenotype and the missense mutation E229K identified in homozygous form in exon 2 from the CYP1B1 gene. The mother being pregnant again asked for genetic counseling. Prenatal diagnosis for PCG was performed and included cytogenetic and DNA analysis. Ultrasound examination of the pregnant woman revealed a singleton pregnancy with enlargement of the eye. Karyotype of the fetus indicated a normal female (46, XX) but DNA analysis confirmed the CYP1B1 mutation. Conclusion: We suggest genetic counseling and prenatal molecular testing for all family cases. This will further help in the prevention of childhood blindness.

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Seroprevalence of Toxoplasma gondii in south Indian women: Urgent Need for Genetic Characterization. *Y.C. Murthy, J. Smita Rao, T. Sunitha, G. Shilpa Reddy, A. Jyothy.* Institute of Genetics and Hospital for Genetic Diseases ,Osmania University, Begumpet, Hyderabad-500016, India.

Statement of Purpose: Toxoplasma gondii represents a serious parasitic infectious threat to humans globally. Especially, pregnant women exposed to T.gondii carry great risk of transmitting the infection to fetus which could lead to severe congenital anomalies or fetal loss. The genetic interaction between this parasite and the human host is believed to play influential role in the generation of potent antibody responses during the process of infection transmission and pathogenesis. In geographically diverse countries like India, the seroprevalence of T.gondii infections is increasing in women with adverse obstetric complaints. Although strong association exists between the magnitude of toxoplasmosis infections and the related genotypes, very little is known about its genetic characterization in this country. Clonal lineages of T.gondii have already been reported from several countries which indicated its great genetic diversity. Till date, no description was made that highlighted the connection between the seroprevalence and the genetic characterization/ genetic diversity of human T.gondii from India. Therefore, the main objective of this study was to determine the seroprevalence of T.gondii in south Indian women with Bad Obstetric History and Repeated Abortions and relate it with its genetic diversity. Method: Between October 2006 and May 2010, 370 serum samples were obtained from women attending the antenatal clinic with complaints of Bad Obstetric History and Repeated Abortions at Institute of Genetics, Hyderabad. They were serologically tested for Ig G and Ig M antibodies specific for T.gondii antigens by commercially available ELISA kit (Euroimmun, Germany). Results: Of the 370 women tested, 95(25.6%) had positive Toxoplasmosis IgG antibodies and 65 (17.5 %)had positive Toxoplasmosis IgM antibodies. Conclusions: This study hypothesizes that increased seropositivity to toxoplasmosis could be due to its genetically varied nature in our geographical region. There is an urgent need for defining the population structure of human T.gondii in India as this could enable to better understand its regional distribution, immunopathogenesis and prevent the congenital defects at the earliest.

2454/F

Cleft Lip and Palate, Annular Pancreas and Multiple Spinal Segmentation Anomalies - A new syndrome? *R. Teitelbaum[1,4], P. Shannon[2], L. Bonafe[3], N. Okun[4], D. Hui[4], D. Chitayat[1,4].* 1) Prenatal Diagnosis and Medical Genetics, Mount Sinai Hospital, Toronto, ON, Canada; 2) Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, ON, Canada; 3) Division of Molecular Pediatrics, Centre Hospitalier Universitaire Vaudois, Switzerland; 4) Department of Obstetrics and Gynecology, Mount Sinai Hospital, Toronto, ON, Canada.

Vertebral segmentation abnormalities are etiologically a heterogeneous disorder. A midline cleft lip and palate is an uncommon finding and usually associated with brain abnormalities. The combination of a midline cleft lip and palate and vertebral segmentation abnormalities, to the best of our knowledge have not been reported. We report a case with these findings pointing towards the possibility of a GDF6 gene dysfunction. The mother was a 33-year-old G5P2L2 of Trinidad/Indian descent and her husband was 32-years-old and of Caucasian descent. The couple was healthy and non-consanguineous. This was their first pregnancy together. The family history was non-contributory. The pregnancy was uncomplicated apart from first trimester bleeding. There was no history of exposure to teratogens. The NT was measured at 12 weeks gestation and was 2.2mm. A detailed fetal ultrasound at 22 weeks gestation revealed midline cleft lip and a suggestion of a cleft palate in a male fetus. The couple elected to terminate the pregnancy and a stillborn fetus was born with normal growth for gestational age. Autopsy showed a large bilateral cleft lip and palate, annular pancreas and multiple vertebral segmentation anomalies. These included duplication of the lateral vertebral elements (right C7), wedge-shaped hemivertebra (C6,C7), accessory ossification centre between the bodies of C7-T1, duplication of left lateral vertebral elements between L1 and L2, eccentric ossification (L1,L2,L3), and 11 ribs on right 12 on left with a large cervical rib. Investigations including chromosome analysis and Microarray analysis were normal and male. The findings in the fetus raised the possible diagnosis of spondylocostal dysostosis spectrum (type 1-4) associated with DLL3, MESP2, LFNG, HES7 gene mutations. However, the spine X-rays were not consistent with this diagnosis. The findings may also be consistent with GDF6 gene mutation. This gene is a member of the bone morphogenetic protein (BMP) family and the TGF-beta super family of secreted signaling molecules. It is required for normal formation of some bones and joints in the limbs, skull, and axial skeleton and gives a variety of phenotypes with vertebral segmentation anomalies, including "kippel-Feil" forms. Results of mutation analysis for this gene are pending.

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Rapid Screening Chromosomal Aneuploidies Using Array-MLPA. *J. Yan[1,2], M. Xu[1], C. Xiong[1], DW. Zhou[1], ZR. Ren[1], Y. Huang[1,2], M. Mommersteeg[3], R. van Beuningen[3], YT. Wang[4], SX. Liao[4], F. Zeng[1,2,5], Y. Wu[1,3], YT. Zeng[1,2].* 1) Institute of Medical Genetics, Shanghai Children's Hospital, Shanghai Jiao Tong University, Shanghai, Shanghai, China; 2) Key Lab of Embryo Molecular Biology, Ministry of Health, and Shanghai Lab of Embryo and Reproduction Engineering, Shanghai, Shanghai, China; 3) PamGene International BV, 's-Hertogenbosch, The Netherlands; 4) Medical Genetic Institute of Henan Province,the People's Hospital of Henan Province, Zhengzhou, Henan, China; 5) Institute of Medical Sciences, Shanghai Jiao Tong University School of Medicine, Shanghai, Shanghai, China.

Cultured karyotype analysis has been considered as a reliable detector of fetal abnormalities. However, its sensitivity depends on the number of cells established in a particular culture. To determine whether array-based multiplex ligation-dependent probe amplification (array-MLPA) can be used in clinical diagnostic settings for screening the common aneuploidies, we designed 116 universal tag-probes covering chromosome 13, 18, 21, X, Y and 8 control autosomal genes. We then performed MLPA and hybridized the MLPA products on 4-well flow-through microarray system. We further determined the chromosome copy numbers by analyzing the relative signals of the chromosome-specific probes. In a blind study of 161 peripheral blood and 12 amniotic fluid drawn samples, we correctly identified 76 of 80 nonmosaic aneuploidy cases, especially 11 amniotic fluid drawn samples and all of the normal controls by array-MLPA. Additionally, we detected two chromosome X monosomy mosaic cases, in which the mosaic rate estimated by the chromosome copy numbers on array-MLPA were basically consistent with the results from the karyotyping. Moreover, we found five sex aneuploidies by array-MLPA, in which the abnormalities could not be clearly identified by the karyotype analysis. Our study demonstrates the success and potential power of array-MLPA in a clinical diagnostic setting for rapid chromosomal aneuploidy screening.

2456/F

Analysis of the Noonan/CFC/Costello syndrome genes in fetuses with abnormal ultrasound findings and a normal karyotype. *H.G. Yntema, W.M. Nillesen, M. Schepens, M. Ruiterkamp-Versteeg, H. Scheffer, B.H.W. Faas, I. van der Burgt.* Dept Human Genetics 417, Radboud Univ Nijmegen Med Ctr, Nijmegen, Netherlands.

Noonan syndrome is an autosomal dominant disorder characterized by short stature, congenital heart defects, distinctive facial features, and a variable degree of intellectual deficits. The disorder is genetically heterogeneous and shows clinical overlap with cardio-facio-cutaneous (CFC) and Costello syndrome. Currently, germline mutations in 10 genes have been described: *PTPN11*, *KRAS*, *SOS1*, *RAF1*, *SHOC2*, *NRAS*, *BRAF*, *MAP2K1*, *MAP2K2*, and *HRAS*. Lee *et al*. (Clin Genet 2009;75:190) recently described *PTPN11* mutations in 9% of fetuses with cystic hygroma and increased nuchal translucency (NT). Because of these findings we offered *PTPN11* testing in a routine DNA diagnostic setting for fetuses with a normal karyotype and an increased NT. Parallel testing for mutations in *KRAS* was performed upon request. This study showed a mutation frequency of 16% in fetuses tested for *PTPN11* and *KRAS* [Houweling *et al*. (2010) Prenat Diagn 30:284]. In our DNA diagnostics laboratory, until now the DNA of 70 fetuses has been investigated for mutations in *PTPN11*, *KRAS*, *SOS1*, and *RAF1*. In 11 fetuses (16%) a de novo mutation has been identified in either *PTPN11* (n=5), *KRAS* (n=1), *SOS1* (n=1), or *RAF1* (n=4). We conclude that in this cohort *RAF1* mutations are as frequent as *PTPN11* mutations. *KRAS* and *SOS1* mutations are less frequent, but still have a mutation frequency of 1.5%. As prenatal diagnosis is dealing with a limited time frame, it is very important to establish a fast DNA diagnostic protocol for small amounts of DNA isolated from uncultured amniocytes. To decide which genes should be included in such a protocol we analysed all 10 Noonan/CFC/Costello syndrome genes in DNA of 78 fetuses with increased NT (>3.5 mm), cystic hygroma, hydrops fetalis or congenital heart disease - with or without ventricular megaly and/or renal abnormalities- and a normal karyotype. Our results suggest that prenatal analysis of all 10 genes should be offered to fetuses with specific ultrasound abnormalities and a normal karyotype. However, since these abnormalities are often detected at 20 weeks of gestation, leaving only a limited time frame for analysis, DNA mostly has to be extracted from uncultured amniocytes, providing only limited amounts of DNA. This makes analysis of all genes very difficult. The results of our studies and the difficulties in establishing a good DNA diagnostic protocol will be discussed.

2457/F

Endoreduplicated Throphoblast cells in cervix of women with normal pregnancies. *A. Amiel[1,2,3], M. Fejgin[1,3,4], P. Tsipouras[5], M. Liberman[3], N. Pery[3], F. Tafas[5], S. Sifakis[6].* 1) Genetics Institute, Meir General Hospital, Kfar Saba, Israel; 2) Bar Ilan University, Ramat-Gan, Israel; 3) Monaliza Medical, Kfar-Saba, Israel; 4) Sackler School of Medicine,Tel Aviv University; 5) Ikonisys Inc, 5 Science Park, New Haven, CT 06511, USA; 6) Department of Obstetrics-Gynecology, University Hospital of Heraklion, Crete, Greece.

Background: Fetal cells represented by the Extra Villous Trophoblast (EVT) obtained from the cervix are important source materials for Non-Invasive Prenatal Diagnosis (NIPD). Fetal cells are present in the uterine cavity from the 5th to the 12th weeks of pregnancy. Objective: In this study we estimated the False Positive Rate (FPR) of aneuploid trophoblast cells in normal pregnancies that appeared in the cervix during the 5-13 weeks of pregnancy. Methods: in the process of assessing the FPR of the procedure, samples were obtained from normal 21 female and 15 male pregnancies. FISH was done with X, Y and two 21 probes. Thirty six normal pregnancies with gestational age from 5-12th week were examined. Results: In the female cases with a mean of 15.2 tetraploid cells per case were found, with a range of 0-52 cells per case (based on the finding of 4 signals for X and 21 chromosomes), while in the male cases a mean number of 2.0 tetraploid cells were found with a range of 0-5 cells per case (based on 4 signals of Y and 21 chromosomes) . Those cells were 2 to 3 times larger than the usual normal cells seeing in the cervix. Conclusion: It is well known in the literature, that EVT tend to form endoreduplication in their nuclei to the ploidy level of 4c-8c DNA. It is of importance that those cells may represent a normal phenomenon of trophoblast cells in the cervix as they appear in normal pregnancies.

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Fragile X prenatal diagnosis of 1,055 pregnancies from 1991-2010. *S. Nolin, A. Glicksman, X. Ding, N. Ersalesi, W.T. Brown, C. Dobkin.* New York State Institute for Basic Research in Developmental Disabilities, Department of Human Genetics, Staten Island, NY.

We have performed fragile X prenatal studies for 1055 pregnancies of women with intermediate, premutation or full mutation alleles from 1991-2010. The studies included 884 chorionic villus and 179 amniotic fluid samples. For 8 pregnancies, both amniocytes and chorionic villi were analyzed due to: unusual findings (3), maternal cell contamination (1), or other technical difficulties (4). The expanded allele was transmitted in 514 (49%) pregnancies with expansion to a full mutation in 221 (20.9%). No maternal age effect was observed in the risk of expansion to full mutation. Five of 53 (9.4%) allele transmissions with 45-49 repeats were unstable as were 12 of 48 (25%) with 50-54 repeats. While most unstable transmissions of intermediate alleles were modest increases of 1 to 4 repeats, a 54 repeat increased to 85, and a 53 repeat decreased to 33 repeats. Contractions in repeat size were observed for 15/514 (2.9%) pregnancies in women carrying alleles of 52 to 150 repeats. The smallest contraction was a loss of two repeats from 54 to 52 repeats and the largest was a loss of 68 repeats from 150 to 82 repeats. Among transmissions of normal maternal and paternal alleles, there were 6 unstable alleles including one normal maternal, 4 normal paternal alleles and 1 intermediate paternal allele. To confirm the prenatal studies DNA from cord blood was analyzed for 19 amniotic fluid and 92 chorionic villus samples. Analyses of the cord blood confirmed all of the prenatal studies which identified 62 normal, 11 intermediate, 27 premutation and 11 full mutation alleles. Among the cases analyzed, one woman with three alleles of 30, 80 and 110 repeats had 3 pregnancies: 1 with a full mutation expansion and 2 with contractions to 57 and 59 repeats. A second woman with three alleles of 23, 49 and 74 repeats transmitted a 49 repeat allele to her fetus. A comparison of prenatal transmissions from women with or without a family history of fragile X suggests greater instability for alleles in families known to have a history of fragile X.

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Phenotype analysis and gene expression profiling in adult offspring in a mouse model of chronic maternal protein deprivation reveals a role for cohesins in developmental programming. *A. Sanchez-Valle[1], B. Sadikovic[1], H. Sangi-Haghpeykar[2], L. Chen[2], M. Fiorotto[3], I. Van den Veyver[1,2].* 1) Molec & Human Gen, Baylor College Med, Houston, TX; 2) Obstetrics and Gynecology, Baylor College Med, Houston, TX; 3) Pediatrics (Nutrition), Baylor College Med, Houston, TX.

Epigenetic changes are thought to underlie the developmental programming of adult-onset metabolic syndrome resulting from a suboptimal intrauterine environment, but the exact molecular mechanisms and identity of altered genes remain incompletely understood. We studied C57BL/6J mice, which are predisposed to obesity and glucose intolerance as a sensitive model for developmental programming. We fed female C57BL/6J dams a maternal low-protein (MLP; 8%) or control diet (20%) starting 4 weeks prior to mating. Litters were culled to 6 on postnatal day 3 (P3) and male pups weaned to lab chow (TD91352) on P21 and single-housed. Offspring were sacrificed at P21and P365 and organs were dissected and snap-frozen. The MLP group had significantly lower body weight at P21 (N=36 MLP; N= 25 controls) and over time (N=17 MLP; N=19 controls) ($p<0.001$). Kidney ($p<0.001$), heart ($p<0.05$), liver ($p<0.01$) weights were also lower at P21 (N=8 MLP; N=6 controls), but not after adjusting for body weight. There was no difference at P365. All absolute hindleg muscle weights and those adjusted for tibia length ($p<0.001$) were lower at P21 in the MLP group (N= 19 MLP; N=8 controls). Standardized weight differences persisted at P365 for soleus ($p<0.01$), gastrocnemius ($p<0.05$) and tibialis anterior ($p<0.05$) (N=8 MLP; N=10 controls). RNA from liver at P365 (n=3 for each diet) was hybridized to Affymetrix Mouse Gene 1.0 ST Arrays. Data was analyzed with Partek Genomic Suites followed by Ingenuity Pathway Analysis. We found 521 upregulated and 236 downregulated genes in the MLP group (one-way ANOVA; $p<0.01$, 1.5 fold change). Genes with the most significantly altered expression include *SMARCA5, ATRX, ARID4b* involved in chromatin regulation; *SMC3, SMC1a, SMC5, SMC6, NIPBL*, and *PCM1* involved in cohesins and chromatin regulation; *TOPI, TOP2B, SMARCA5, MED21*, and *REV3L* involved in transcriptional regulation; and *TMF1, ROCK1, ACP1*, and *ARID4b* involved in adult onset disorders. Quantitative RT-PCR analysis on 10 genes validated our findings. The changes in expression of genes involved in the cohesin complex is a novel finding that suggests a role for altered higher-order chromatin structure as an epigenetic mechanism to stabilize long-term global alterations in transcription. These mechanisms can ultimately be responsible for predisposition to adult onset diseases such as hypertension and diabetes.

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Autophagy is activated in the placenta of pre-eclampsia model mouse. *R. Akaishi[1], T. Yamada[1], I. Furuta[1], M. Morikawa[1], T. Yamada[1], H. Yamada[2], K. Nakayama[3], K. Nakayama[4], T. Yoshimori[5], H. Minakami[1].* 1) Department of Obstetrics; Hokkaido University Graduate School of Medicine; 2) Department of Obstetrics and Gynecology, Kobe University; 3) United Centers for Advanced Research and Translational Medicine, Tohoku University; 4) Department of Molecular and cellular biology, Medical institute of Bioregulation, Kyushu University; 5) Department of Genetics, Osaka University Graduate School of Medicine.

Autophagy is an intracellular bulk degradation system used to recycle the majority of proteins and some organelles. It is activated in response to nutritional deprivation and intracellular stress. Yeast Atg9p is the first integral membrane protein shown to be essential for the cytoplasm to vacuole targeting (Cvt) pathway and autophagy. *ATG9A* and *ATG9B* are identified as human genes homologous of yeast Atg9p. In human adult tissues, *ATG9A* was ubiquitously expressed, whereas *ATG9B* was highly expressed in placenta (trophoblast cells) and pituitary gland. Moreover, *ATG9B* is a vertebrate-specific gene that may have gained critical roles in mammalian-specific developmental events, such as placentation, through rapid evolution. On the other hand, *ATG9B*, is a long variant of *NOS3AS*, regulates *NOS3* post-transcriptionally. The *NOS3* locus has been suggested to be associated with a number of diseases including pre-eclampsia (PE). In this study, we hypothesized *ATG9B* may play critical roles in mammalian-specific developmental events, such as placentation and may have some roles in the mechanism of PE. Mouse placentas were analyzed to investigate the hypothesis of the function of *ATG9B* with related genes. We collected placentas from wild type mice (C57B/L6J: WTm) and PE model mice ($p57^{KIP2}$ knockout mouse: PEm) on 13.5 days post coitum (dpc), 15.5 dpc and 17.5 dpc. We performed quantitative RT-PCR to measure the expression of *Atg9a* and *Atg9b*, *Nos3* and quantified the amount of microtubule-associated protein light chain 3 -II (LC3-II) and Atg9b protein by immunoblot analysis. *Atg9b* proved to increase with advancing gestational days. *Atg9b* was more highly expressed in PEm than WTm. The expressions of *Nos3* did not change during gestation in WTm, while those of *Nos3* were significantly elevated at 13.5dpc and 15.5dpc, but not at 17.5 dpc in PEm. Although the amount of Atg9b protein did not change during gestation in both PEm and WTm, but was significantly higher in PEm than WTm. LC3-II was significantly higher in PEm than WTm. These suggested that *Atg9b* might have a role in maintaining the pregnancy and might have inhibited *Nos3* as *Nos3as* in PEm. The elevated LC3-II suggested that autophagy was activated in PEm placenta. Autophagy was possible to be induced by starvation of PE. Increased Atg9b might have contributed the activation of autophagy in PEm placenta.

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Phenotype-Specific Adverse Effects of *XPD* Gene Abnormalities on Human Fetal and Placental Development: Trichothiodystrophy Versus Xeroderma Pigmentosum Paradox. *A. Dzutsev[1], A. Kumar[2], R. Moslehi[2].* 1) NCI, NIH, Bethesda, MD; 2) Ctr Cancer Genomics, SUNY at Albany, Rensselaer, NY.

Background: Effects of abnormalities in DNA repair and transcription genes in human prenatal life have never been studied. Mutations in *XPD*(*ERCC2*), a gene involved in the nucleotide excision repair(NER) pathway and in transcription, can lead to several different phenotypes such as trichothiodystrophy(TTD), xeroderma pigmentosum(XP) or combination of XP/TTD. Our recent genetic epidemiologic investigation revealed 4.0-fold increased risk of preeclampsia(P=0.0018) and 35-fold increased risk of a severe form of preeclampsia, namely hemolysis, elevated liver enzymes, and low platelets(HELLP) syndrome(P=0.0002) associated with TTD-affected pregnancies (where infant had two mutations) but not in unaffected pregnancies (where the infant was heterozygote or noncarrier). Pregnancies resulting in XP/TTD, also caused by mutations in *XPD*, were not associated with gestational complications. Abnormal placental development may explain the constellation of observed abnormalities; thus we hypothesize that NER/transcription genes play an important role in normal placental development and that besides the fetal genotype, the exact genetic abnormality is also relevant to the postulated mechanism. **Methods**: To test this hypothesis, we conducted a comparison of *XPD*-associated XP(n=48) and TTD(n=42) cases with respect to gestational complications and global gene expression profiles in fibroblasts in a microarray dataset. **Results**: Compared to XP-affected pregnancies, TTD-affected pregnancies were associated with significantly higher incidence of preeclmapsia, preterm delivery, low birth weight(<2500 grams), SGA<10th and <3rd percentile, and NICU hospitalization (P<0.0001 for all comparisons). Furthermore, HELLP syndrome, decreased fetal movement and elevated midtrimester maternal serum hCG levels were only reported among TTD-affected pregnancies with frequencies of 4.2%, 50% and 40%, respectively. Global gene expression profiling of TTD versus normal fibroblasts suggested differential expression of a number of genes affected by mutations in *XPD* with little overlap between XP and TTD. Downregulated pathways in TTD included NRF2-mediated oxidative stress response and progesterone metabolism. **Conclusion**: Our results indicate an important role for XPD during normal human placental and fetal development and highlight the relevance of the exact genetic abnormality to the postulated mechanism which may involve affected oxidative stress response and progesterone metabolism pathways.

2462/F

Replication of a Genome-Wide Association Study of Birth Weight in Preterm Neonates. *K.K. Ryckman[1], E.N.A. Bream[1], J.R. Shaffer[2], E. Feingold[2], S.K. Berends[1], E. Gadow[3], C. Saleme[4], H.N. Simhan[5], D. Merrill[6], C.T. Fong[7], T. Busch[1], B. Feenstra[8], F. Geller[8], M. Melbye[8], M.L. Marazita[2], J.M. Dagle[1], J.C. Murray[1].* 1) Dept Pediatrics, Univ Iowa, Iowa City, IA; 2) Human Genetics, University of Pittsburgh, Pittsburgh, PA; 3) Centro de Educación Médica E Inverstigaciones Clínicas, Buenos Aires, Capital Federal, Argentina; 4) Instituto de Maternidad y Ginecología Nuestra Señora de las Mercedes, San Miguel de Tucumán, Tucumán, Argentina; 5) Obstetrics, Gynecology, and Reproductive Sciences, University of Pittsburgh School of Medicine, Magee-Womens Research Institute, Pittsburgh, PA; 6) Wake Forest University Baptist Medical Center, Wake Forest University School of Medicine, Winston-Salem, NC; 7) Strong Children's Research Center, University of Rochester School of Medicine, Rochester, NY; 8) Epidemiology Research, Statens Serum Institut, Copenhagen, Denmark.

BACKGROUND: A recent genome-wide association study of birth weight by Freathy et al, which included 10,623 European term gestations, showed that the infant CC genotype of rs900400 ($p=2x10^{-35}$) near LEKR1 and CCNL1 and rs9883204 ($p=7x10^{-15}$) in ADCY5 was associated with lower birth weight. However, it is unclear whether these findings are limited to term gestations only or if the same associations are present in preterm infants. OBJECTIVE: To examine associations of maternal and infant genotype with birth weight for rs9883204 and rs900400 in a preterm population. DESIGN/METHODS: TaqMan genotyping was performed on 719 parent-infant trios from two distinct populations. A total of 441 Caucasian trios were recruited from four U.S. sites including Iowa City, Pittsburgh, Rochester and Wake Forest; the other 278 trios were recruited from Argentina. Preterm (delivery at 23-36 weeks gestation) singleton births occurring from spontaneous labor were included. Pregnancies complicated by congenital anomalies, twins, IUGR, artificial reproductive technology, preeclampsia and placenta previa were excluded. Quantitative transmission disequilibrium tests (QTDT) adjusting for infant gender and gestational age were performed in the U.S. and Argentina populations, combined as well as in each population separately. Additionally, linear regression analyses adjusting for infant gender and gestational age were performed with maternal and infant genotypes separately. RESULTS: QTDT in the combined sample revealed that the C allele of rs900400 (LEKR1/CCNL1) was associated with lower birth weight (p=0.005). Separately, rs900400 was associated in the U.S. (p=0.02) but not in Argentina (p=0.24). Linear regression also showed decreased birth weight was associated with the CC genotype of rs900400 in infants but not mothers in the U.S. (p=0.06), Argentina (p=0.04) and combined populations ($p=1.1x10^{-5}$). This result remained significant in the combined population after adjusting for smoking ($p=6.3x10^{-5}$). There were no associations with rs9883204 for the QTDT or linear regression analyses in the combined sample. CONCLUSIONS: We found that rs900400 near LEKR1 and CCNL1, previously associated with birth weight in a term population, is also associated with birth weight in a preterm population. These results indicate the potential importance of this SNP on birth weight irrespective of gestational age. This work is supported by NIH grants: R01 HD-52953 and R01 HD-57192.

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Population-based screening of heterozygous SMN1 deletions in Chinese Han people with Taqman probe based real-time PCR and multiplex ligation-dependent probe amplification. *Y. Chen, MJ. Liu, HB. Cheng, HB. Li, J. Sun, J. Mao, J. Ding, H. Li.* Center for Reproduction and Genetics, Nanjing Medical University Affiliated Suzhou Hospital, Suzhou, China.

Objective: To determine SMN1 gene copy number by an efficient TagMan real-time PCR assay. Methods: 397 subjects were involved in this study: (1) 284 whole blood samples were from randomly selected pregnant women participating in prenatal care in our hospital and three husbands of them; (2) 83 amniotic fluid samples were from pregnant women with high risk of trisomy 21; (3) 30 DNA samples were kindly provided by Peking Union Medical College Hospital in China and Children's Hospital Boston in USA and used for both blinded test and validating this technique platform. The genome DNA of samples were extracted by Qiagen's QIAamp® DNA Mini Kit, and ABI's 7500 real-time fluorescence quantitative PCR system and analysis software were applied in this assay. The reagents involved in this assay, such as probes and primers, were purchased from ABI Company. The SMN1 gene copy number of all samples with ALB using as an internal reference gene was detected according to the standard operating procedures of our lab. Results: Among the first group of subjects, individuals with two copies were 95.07%(270/284), with one copy of SMN1 gene, namely SMA carrier, were 3.17%(9/284), and with three copies were 1.76%(5/284). The corresponding data in the second group were 91.57% (76/83), 7.23% (6/83) and 1.20% (1/83), respectively. The blinded test results were exactly consistent with those provided by the other laboratorys. Conclusion: (1) Established TagMan Real-Time PCR assay to determine SMN1 gene copy number is a simple, economical and efficient technique and has clinical application value; (2) This assay aim to detect SMN1 gene copy number can also apply in prenatal diagnosis or screening; (3) It needs strict quality control for clinical application of this assay.

2464/F

Association between AGTR2 genetic variants and increased risk for congenital abnormalities of the kidney and urinary tract. *H.C. Sarubi[1], L. Bastos-Rodrigues[1], D.M. Debora[2], A.C. Simoes e Silva[2], E. Oliveira[2], L. De Marco[1].* 1) Surgery, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil; 2) Pediatrics, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.

The Renin-Angiotensin System (RAS) plays a critical role in normal nephrogenesis. It has been suggested that, in humans, polymorphisms in genes encoding components of the RAS could cause a spectrum of congenital abnormalities of the kidney and urinary tract (CAKUT), including congenital hydronephrosis. The gene encoding the angiotensin receptor type 2 (AGTR2) is one of the candidate genes proposed for genetic association with CAKUT. During kidney development, angiotensin II functions through its two major receptors, AGTR1 and AGTR2. These receptors belong to the large family of G protein-coupled receptors with seven transmembrane domains and are both highly expressed in the fetal kidney but poorly expressed in most adult tissues. This study was undertaken to explore the role of single-nucleotide-polymorphisms (SNPs) concerning the AGTR2 gene in the development of congenital abnormalities of the kidney and urinary tract. Sixty seven infants with the diagnosis of CAKUT were recruited from a Brazilian reference center. The control population consisted of 89 healthy individuals from the same geographic area. We analyzed three Tag SNPs from the AGTR2 gene (rs1403543, rs3736556 and rs5193) which were genotyped using TaqMan® SNP Genotyping Assays. To account for ethnicity, individual DNA was independently genotyped for a set of 40 biallelic short insertion/deletion polymorphisms (indels) for genomic ancestry analysis. The difference in the proportion of genomic ancestry between the two groups was not significant. After adjustment by the permutation procedure, we found a significant association with the rs1403543 marker [P = 0.0031; OR = 1.98 (1.26 a 3.12)]. In summary, our data suggest that AGTR2 polymorphisms are associated with increased risk for congenital abnormalities of the kidney and urinary tract and are not associated with ethnicity in the Brazilian population.

2465/F

The effect of various antiretroviral therapies on maternal serum aneuploidy screening in HIV positive pregnant women. *S.T. Holmes[1], K. Bajaj[2], L. Reimers[1], S. Klugman[2], P.S. Bernstein[2], R. Wright[2].* 1) Albert Einstein College of Medicine, New York, NY; 2) Department of OB/GYN, Montefiore Medical Center, New York, NY.

Objective: To evaluate differences in maternal serum markers for prenatal aneuploidy/neural tube defect screening in HIV positive women undergoing boosted and non-boosted protease inhibitor therapy. Methods: A retrospective case control study of 64 HIV positive women was conducted stratifying subjects by drug therapies (boosted protease inhibitors (BPI) vs. non-boosted protease inhibitors (NBPI)). Controls were randomly selected, matched for maternal and gestational age, HIV negative women who underwent aneuploidy screen the same month as each case. Women had either 1) first trimester combined screens (FTS) with second trimester alpha fetoprotein levels or 2) second trimester quadruple screen (STS). Results: The sample of HIV positive women receiving boosted protease inhibitor therapy during any portion of their aneuploidy screening (n=17) included 3 subject having FTS, 6 subjects having STS only, and 4 subjects having FTS and second trimester AFP and 4 patients who were on NBPI during the first trimester but were changed to BPI prior to the second trimester AFP. One patient on BPI had a positive FTS, compared to one patient on NBPI. Three of the subjects receiving non-boosted therapy had positive second trimester screens. Among the HIV negative controls, 3 had second trimester screen positive results. Seven HIV positive subjects had amniocentesis, 5 for positive screens (2 for positive first trimester combined screening and 3 for quadruple screening), compared to 1 HIV negative control for positive quadruple screen; the HIV negative control amniocentesis was positive for Down's Syndrome. Conclusion: HIV positive women undergoing boosted protease inhibitor therapy do not appear to have an increased rate of false positive prenatal screenings when compared to women on non-boosted therapy. The overall screen positive rate for HIV positive women, regardless of therapy, seems to be increased when compared to matched controls.

2466/F

ADAM12 in maternal serum prenatal aneuploidy screening: assessment of its screening efficacy in the first and second trimester of pregnancy. *M. Macek Jr[1], D. Springer[2], H. Kluckova[1], R. Vlk[3], I. Spalova[3], P. Calda[4], A. Lashkevich[1], D. Chudoba[1], D. Novotna[1], L. Dvorakova[1], H. Kuzelova[1], S. Vilimova[1,5], M. Simandlova[1], M. Turnovec[1], M. Hladikova[1], M. Macek Sr[1].* 1) Department of Biology and Medical Genetics, 2nd Faculty of Medicine, Charles Univ. Prague, Czech Republic; 2) Department of Biochemistry and Clinical Diagnostics, 1st Faculty of Medicine, Charles Univ. Prague, Czech Republic; 3) Department of Obstetrics and Gynecology, 2nd Faculty of Medicine, Charles Univ. Prague, Czech Republic; 4) Department of Obstetrics and Gynecology, 1st Faculty of Medicine, Charles Univ. Prague, Czech Republic; 5) Sanatorium Pronatal, Prague, Czech Republic.

The aim of the study was to assess the efficacy of ADAM12 prenatal maternal serum aneuploidy screening in the first (1T) and second trimester (2T), i.e. within 9-19 weeks of gestation. ADAM12 levels were measured by DELFIA ADAM12 Research Kit (Perkin Elmer, U.S.A) in frozen sera from controls and from cases with fetal aneuploidies. On average for each day, 30 samples were used for control percentile (P) calculations. A total of 85 sera from pregnancies with autosomal and heterochromosomal aneuploidies were tested in 1T and 2T in order to ascertain the decrease of ADAM12 levels under P25. P50 levels within week 9-19 rose from 235.7 to 1337.8 ng/ml, with an average daily increase of 15.7 ng/ml. Overall, the efficacy of screening by using this marker was not significantly different between 1T and 2T of pregnancy. Positive screening outcome (less than P25), within week 9-19, was for trisomy 21 - 48% (15/35), for trisomy 18 - 57% (4/7), for trisomy 13 -100% (4/4), for triploidy - 89% (8/9), for 47,XXX - 88% (7/8) and for 45,X - 12% (1/8) of cases. The highest decrease from P25 was for trisomy 21, within range P10-25 (22%). In other trisomies the highest degree of positivity was within P1-P5: for trisomy 18 - 29%, for trisomy 13 - 50%, for triploidy - 78% and for 47,XXX - 63%. Marker ADAM12 was not detected in 47,XXY, 47,XYY, including mosaic forms of 45,X pregnancies. Our results indicate an inverse relationship between the degree of phenotypic alteration in autosomal aneuploidies and decrease of ADAM12, i.e. under the P25. It is noteworthy, that the same is true for 47,XXX which is associated with mild phenotypic affection. Our results suggest, that ADAM12 is an important marker for the detection of severe autosomal aneuploidies, including triploidy and 47,XXX in the 1T and 2T of pregnancy. Supported by MZ0FNM2005.

2467/F

The reliability of maternal serum triple screening for the prenatal diagnosis of fetal chromosomal abnormalities in Turkish women. *A. Pazarbasi[1], O. Demirhan[1], A.I. Guzel[1,3], D. Tastemir[1,4], M. Kasap[1], F.T. Ozgunen[2], C. Evruke[2], C. Demir[2], E. Tunc[1], B. Yilmaz[1], S. Kocaturk-Sel[1], D. Onatoglu[1], D. Alptekin[1], S. Koc[1], N. Inandiklioglu[1], O. Ozer[1].* 1) Dept Medical Biol & Genetics, Univ Cukurova, Adana, Turkey; 2) Dept. of Obstetrics and Gynecology, Univ Cukurova, Adana, Turkey; 3) Dept Medical Biol, Univ Rize, Rize, Turkey; 4) Univ Adiyaman, Adiyaman, Turkey.

The purpose of this article is to ascertain the reliability of maternal serum triple marker screening of alpha-fetoprotein, human chorionic gonadotropin and unconjugated estriol for the prenatal diagnosis of fetal chromosomal abnormalities in Turkish women. This screening was preferentially reserved to young patients. Medical records were used to analyze indications of amniocentesis and QF-PCR. Anomaly screening was performed to all patients between 13 and 22 weeks of pregnancy. 1725 pregnancies resulted screen positive to triple test and accepted fetal chromosomal analysis. Chromosomal aberrations observed in 56(3.2%) cases. The 44.6% and 55.3% of the abnormalities were numerical and structural aberrations, respectively. These included twenty cases of inv(9), fourteen cases of trisomy 21, one case of 46,XX/47,XX,+21, two cases of trisomy 18, one case of trisomy 13, one case of 47,XXY, one case 45,X, twelve cases of structural abnormalities and six cases of mosaic or tetraploidy. At a cutoff of between 1:200 and 1:270 the false positive rate increased to 21%, and spesitivity rate of 72%; and sensitivity rate of 44%; without any gains in detecting Down's syndrome. Second trimester triple marker testing is an effective screening tool for detecting fetal Down's syndrome in Turkish women.

2468/F

Characterization of a de novo chromosome abnormality, der(14)(p11.2-;qter), found in the fetus with Dandy-Walker malformation, using oligonucleotide array CGH. *D. Cha[1, 2], S. Shim[2], S. Park[2], J. Park[2], S. Chung[3].* 1) Dept OB/GYN, Kangnam CHA Hosp, CHA Univerity, Seoul, Korea; 2) Genetic Research Laboratory, CHA University; 3) Dept OB/GYN, Bundang CHA Hosp, CHA Univerity, Sungnam, Korea.

A 32-year-old pregnant woman came to hospital for routine 2nd trimester checkup at 21 gestational weeks. Ultrasound examination showed abnormal findings in cerebellum. The fetus was suspected as having Dandy-Walker malformation. Amniocentesis was carried out for fetal chromosome analysis. Fetal karyotype showed that an additional material of unknown origin was identified at the short arm of chromosome 14, 46,XY,der(14)(p11.2;qter). To characterize the unknown material, oligonucleotide array CGH was carried out using the Agilent 44K human genome CGH microarray chip and a gain of 3q25.3-3qter was detected. FISH analysis confirmed the unknown material was originated from chromosome 3q. Both parents showed normal karyotypes. 3q partial trisomy related to Dandy-Walker malformation was presented in previous reports. In this report, we precisely characterized the abnormal chromosome 3 using oligonucleotide array CGH technique and this will be helpful to elucidate the molecular mechanism of Dandy-Walker malformation related to partial trisomy 3q.

2469/F

Prenatal presentations of CHARGE syndrome. *L. Demmer, T. Marino, S. Craigo.* Tufts Medical Center, Boston, MA.

CHARGE syndrome is a multiple anomaly condition characterized by coloboma, heart defects, choanal atresia, retarded growth/development, genital hypoplasia, ear anomalies and deafness. Heterozygous mutations of the CHD7 gene are responsible for most cases. Most previous studies have focused on postnatal diagnosis of CHARGE. We describe two cases, one familial and one sporadic, where anomalies were seen prenatally but the diagnosis not recognized until birth. Case 1: Imaging in the first pregnancy revealed micrognathia, complex cardiac defect, hemivertebra, club foot, Dandy-Walker variant; pregnancy was terminated. Imaging in the next pregnancy revealed micrognathia, complex cardiac defect, hemivertebra, agenesis of corpus callosum, Dandy-Walker variant, and hydrocephalus. Chromosomes and array: normal. Pregnancy was terminated; examination of the fetus revealed prominent forehead and maxilla, large nose and low set ears. Imaging in the third pregnancy revealed an AV canal defect; 3D ultrasound of face and brain MRI were unremarkable. Prenatal karyotype and array:normal. Postnatal exam revealed microphthalmia, hypoplastic semicircular canals and cupped, low set ears. CHD7 Q217X mutation confirmed CHARGE syndrome. Subsequently amniocytes from the 2nd pregnancy revealed the same mutation suggesting gonadal mosaicism. Case 2: Second trimester ultrasound revealed bilateral single lower leg bones, club feet and bowed right forearm bones with a single digit. Prenatal karyotype:normal. Postnatally choanal atresia, microphthalmia, coloboma and deafness were identified. CHD7 c.2999delC mutation confirmed CHARGE. CHARGE syndrome can be variable and complex. While minor limb defects are common, major limb anomalies have only rarely been reported. Apparently isolated major limb defects or isolated cardiac defects, while common to many syndromes, can be the only U/S finding. The classic pattern is difficult to identify prenatally; more focused imaging to detect specific anomalies in the brain, ear, choanae and semicircular canals may be warranted. Prenatal recognition of CHARGE is a worthwhile goal given the seriousness of this condition which includes significant mental, visual and hearing impairment. Increased awareness of the patterns of anomalies seen in CHARGE would allow for CHD7 testing and timely decision-making regarding pregnancy management. In addition, our report underscores the need for counseling regarding the potential for gonadal mosaicism.

2470/F

Evidence review: Fetal gender identification using cell free fetal DNA (cffDNA) in maternal blood. *S.A. Devaney[1], G. Palomaki[2], J.A. Scott[1], D.W. Bianchi[3].* 1) The Genetics & Public Policy Center, Johns Hopkins University, Washington , DC; 2) Women & Infants Hospital, Alpert Medical School of Brown University, Providence, RI; 3) Floating Hospital for Children @Tufts Medical Center, Tufts University School of Medicine, Boston, MA.

Background: Non-invasive prenatal diagnosis, including fetal gender determination, can be an alternative to invasive techniques. In 1997, Lo et al identified circulating cffDNA in maternal blood. Since then a number of cffDNA gender prediction studies have been published. To determine test sensitivity/specificity and identify factors associated with performance, we reviewed the published literature. Methods: Two reviewers (SD, DB) performed independent structured PubMed literature searches with variations of the terms: DNA, cell-free, fetal, gender, prenatal, non-invasive, diagnosis. We excluded reviews and articles not in English. Reviewers extracted data on test performance and methodology. Discrepancies were resolved by discussion. Meta-DiSc, software designed to analyze diagnostic tests, was used. Given the relationship between sensitivity and specificity introduced by the threshold effect, diagnostic odds ratio (dOR) was included. Potential covariates examined were: methodology (conventional PCR, RT-PCR), sample type (plasma, serum, whole blood), publication date (<=2003, >2003), Y chromosome sequence (SRY, DYS14, DYS1) and gestational age (GA) in weeks (<7, 7-12, 13-20, >20). Results: The search yielded 855 publications, 735 were excluded on abstract content, 134 were fully reviewed, and 65 contained primary data suitable for analysis. Nineteen that did not contain >=10 male and 10 female samples were excluded, leaving 46 publications containing 60 data sets representing 3352 male and 2825 female fetuses. For the analysis of GA by week, 29 publications were included due to broad GA ranges in 17. The overall sensitivity was 95.3% (CI 94.5-96.0%), specificity was 98.5% (CI 98.0-99.0%) and dOR was 730 (CI 440-1200). RT-PCR (dOR 1070) was significantly better than conventional PCR (dOR 348) for predicting gender (p=0.03). There were no important differences found for sample type or Y sequence used. In the late first (7-12 wks) and early second (13-20 wks) trimesters, the tests performed equally well (dOR 960 and 710, respectively). Tests performed less well before 7 wks (dOR 91). After 20 wks the sensitivity and specificity of the test was 100% (dOR 4307), but this was based on only 6 data sets. Conclusion: Fetal gender prediction before 7 wks gestation is not reliable. From 7-20 wks, tests utilizing RT-PCR perform better. Multivariate analysis will be done to further clarify the combination of test characteristics that have the highest performance.

2471/F

Unexpected chromosomal rearrangement detected by prenatal CGH array in a fetus with a *de novo* apparently balanced pericentric inversion. *V. Haddad[1,2], B. Benzacken[1], M. Gérard[1], S. Magnier[3], A. Verloes[1], A. Aboura[1].* 1) Department of Medical Genetics, AP-HP-Robert DEBRE University Hospital, Paris, France; 2) Department of Cytogenetics, AP-HP-Antoine BECLERE University Hospital, Clamart, France; 3) Department of Cardiology, AP-HP-Robert DEBRE University Hospital, Paris, France.

Growth retardation, conotruncal heart defect and right renal agenesis were detected in a female fetus by US scan at 24 WG. Amniocentesis performed at 28 WG revealed a *de novo* pericentric inversion 46,XX,inv(3)(p12q26). As a recent study (C.Schult-Bolard and al, 2009) showed that more than 50% of *de novo* inversions are unbalanced, we performed a 180k CGH array (Agilent), which showed no chromosome 3 rearrangement but, surprisingly, disclosed an interstitial 13 Mb deletion at 1q23-25 from 159,917,038 to 173,193,165. The deletion was confirmed by FISH. It occured *de novo*. Interstitial 1q23q25 deletion is characterized by intra uterine growth retardation, short hands/feet.The parents declined termination of pregnancy. The child died at day 3 of cardiac insufficiency. This report illustrates the fact that *de novo* rearrangements may be associated with imbalances located at distance of the breakpoints or even in another chromosome. We suggest that in prenatal diagnosis, array CGH should be used systematically to investigate fetuses witn abnormal phenotype and apparently balanced *de novo* chromosomal rearrangements.

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Identification and characterization of differential DNA methylation for potential use in non invasive prenatal diagnosis of Trisomy 21. *T.J. Jensen[1], T. Zwiefelhofer[1], M. Tang[1], C. Gebhard[3], M. Rehli[3], D. van den Boom[2], M. Ehrich[2].* 1) Research and Development, Sequenom Center for Molecular Medicine, San Diego, CA; 2) Sequenom Inc., San Diego, CA; 3) University Hospital Regensburg, Regensburg, Germany.

Prenatal diagnosis of fetal aneuploidies, including Trisomy 21, is an area of great interest in fetal maternal medicine. Current diagnostic assays use a combination of serum protein markers and ultrasound to identify a high risk group, which require additional confirmatory tests including invasive procedures. These invasive methods have risk since 0.5-2% lead to spontaneous miscarriage. To help reduce this risk, new diagnostic methods are targeting the fetal DNA itself. Fetal DNA is present in the maternal plasma in the form of circulating cell free (ccf) fetal nucleic acids. The fetal fraction comprises only 5-20% of all ccf DNA, thereby presenting a challenge in the analysis. To simplify these methods and increase the ability to utilize ccf DNA for diagnostic purposes, a discriminating factor should be employed that segregates the maternal and fetal ccf nucleic acids, thus allowing analysis of only the fetal component. A potential factor which may allow this segregation is DNA methylation. Maternal buffy coat and matched placenta tissue were used as a surrogate to determine the potential differences in DNA methylation between a woman and her developing fetus. To identify differentially methylated regions (DMRs) in the genome with a focus on chromosome 21, we employed two methodologies: methylcytosine immunoprecipitation (MCIP) coupled with microarray analysis and quantitative methylation analysis of target regions using SEQUENOM's EpiTYPER platform. For the microarray analysis we used two designs: a genome-wide CpG island microarray and a microarray tiling chromosome 21. We also performed targeted methylation analysis of all annotated CpG Islands (356) located on chr 21 using EpiTYPER. In addition, we validated genomic regions that were found to be differentially methylated by the microarrays analysis with EpiTYPER. In summary, we found 4566 regions to be differentially methylated throughout the genome between maternal and fetal DNA. A subset of 316 of these regions is located on chromosome 21. The confirmation of microarray results with quantitative mass spectrometry reveals that 94% of the regions assessed with EpiTYPER were confirmed in a set of 8 paired samples and each of these were also confirmed in a larger set of 65 paired samples. Interestingly we find that the largest methylation differences are found outside of classically defined CpG islands, indicating that those areas deserve special attention when screening for DMRs.

2473/F

An extensive genetic investigation of a translocation Y;11 observed in the antenatal period. *K. Krabchi[1], S. Mai[2], R. Vallente[2], D. Lichtenzstejn[2], O. Samassekou[1], J. Lamoureux[1], M. Bronsard[1], M. Langlois[3], I. Mongrain[3], M.S. Phillips[3], S. Malenfant[4], A. Broussin Ducos[1], C. Bouffard[1], R. Drouin[1].* 1) Division of Genetics, Department of Pediatrics, Faculty of Medicine and Health Sciences, Universite de Sherbrooke, Sherbrooke, QC, Canada; 2) Genomic centre for Cancer Research and Diagnosis, University of Manitoba, Manitoba Institute of Cell Biology, Winnipeg, MB, Canada; 3) Centre de Pharmacogénomique, Institut de Cardiologie de Montréal, Université de Montréal, Montréal, QC, Canada; 4) Centre hospitalier régional de Trois-Rivières, Pavillon Sainte-Marie, Trois-Rivières, QC, Canada.

Turner Syndrome (TS) is a genetic condition defined by the complete or partial absence of X chromosome and the total absence of a Y chromosome. A 35-year-old pregnant woman showed a 1/60 risk after prenatal screening. At 176 weeks, a cytogenetic analysis of amniocytes showed homogeneous monosomy X and the couple decided to continue the pregnancy. Subsequent morphological echography showed the presence of a male foetus. Consequently, other foetal investigations were performed to look for specific male markers. Methodology: Prenatal analysis on amniocytes: express FISH, GTW-banding karyotype and PCR amplification of foetal DNA with SRY specific primers were made. Per-partum analysis on cord blood: conventional and molecular cytogenetic techniques were used to characterize this chromosome rearrangement. Finally, microsatellite markers STS in search of microdeletions of Y (AZFa, AZFb, AZFc) and analysis by microarrays completed the investigation. 3D image analysis of nuclear organization of chromosomes 11 and Y and of telomeres in blood, buccal smears and fibroblasts were performed. Results: A phenotypically normal male child was born at full term with a translocation 45,X,der(11),t(Y;11)(pter®p11.2::q-25®pter). The long arm of the chromosome 11 showed no loss or gain of material up to the subtelomeric area. Analysis by microarrays revealed that the distal 5 Mbp fragment of Yp was translocated to the end of the chromosome 11q. The telomere organization appears regular, chromosome 11 is consistently found at the nuclear edge when it carries the Y translocation. The other chromosome 11 is located more internally. The remainder of chr Y is found in variable nuclear positions. Discussion: Prenatal diagnosis of TS should always be followed by research for SRY sequences. The presence of the arm or of a part of the Yp chromosome translocated to another chromosome gives rise to a relatively normal infertile male phenotype. The segment of the Y translocated to the distal part of the 11q25 represents a Y without centromere and the entire long arm that plays a major role in spermatogenesis. Conclusion: It is important to verify in all monosomic X conceptus for the presence of specific genetic material of the Y chromosome. Systematic complementary genetic investigations and genetic counselling performed by a qualified specialist would efficiently reduce ethical and clinical risk associated with medical pregnancy termination of incorrect TS prenatal diagnosis.

2474/F

Early prediction of preeclampsia using second trimester maternal serum markers including inhibin A. *K. Lee[1], H. Park[2], W. Hahn[2], J. Kang[1], D. Cha[1].* 1) Dept OB/GYN, Kangnam-Gu, Kangnam CHA General Hosp, CHA University, Seoul, Korea; 2) Dept OB/GYN, Bundang-Gu, Bundang CHA General Hosp, CHA University, Seoul, Korea.

The purpose of this study was to determine whether inhibin A and other second trimester serum markers are useful for early identification of pregnant women at risk for preeclampsia. Between January 2005 and March 2009, we analyzed the data of 4764 subjects who underwent second trimester multiple-marker screening for Down syndrome. Serum samples were assayed at 15+0 to 20+6 weeks for maternal serum alpha-fetoprotein (MSAFP), human chorionic gonadotrophin (hCG), unconjugated estriol (uE3) and inhibinA. We reviewed all medical records retrospectively, and assessed the relationships of several markers with preeclampsia using logistic regression analysis. The study sample included 41 patients who developed preeclampsia and a control group consisting of the other 4723 healthy subjects treated between January 2005 and March 2009. There were no significant differences in gestational ages at blood sampling, maternal weights, gravidity and parity between the two groups. However, the mean ages, Apgar scores, gestational age at delivery and neonatal weights were significantly different between the study group and the control group. In the study group, median multiples of median (MoM) values of inhibin A and human chorionic gonadotrophins (hCG), maternal serum alpha-fetoprotein (MSAFP) were elevated significantly, but unconjugated ertriol (uE3) did not differ significantly between the two groups. Inhibin A was among four markers significantly correlated with preeclampsia. Inhibin A and other second trimester serum markers may be useful for early detection of preeclampsia. Inhibin A was in fact the most important predictable marker among the markers we surveyed. The results of this study support those of previous studies, and provide quantified data elucidating the occurrence of preeclampsia. Studies adding maternal history and uterine artery Doppler data at the second trimester may further increase detection rates of preeclampsia.

2475/F

Prenatal imaging of Harlequin ichthyosis. *M. Lefebvre[1], J. Dubois[2], N. Patey[3], F. Audibert[4], V.A. Désilets[1].* 1) Department of Pediatrics, CHU Sainte-Justine, Montreal, Quebec, Canada; 2) Department of Radiology, CHU Sainte-Justine, Montreal, Quebec, Canada; 3) Department of Pathology, CHU Sainte-Justine, Montreal, Quebec, Canada; 4) Department of Obstetrics-Gynecology, CHU Sainte-Justine, Montreal, Quebec, Canada.

Introduction: Harlequin ichthyosis is a life-threatening disorder that is characterised by a thick skin that severely restricts movements and deforms facial features and distal extremities. Numerous complications are associated, particularly neonatal death from respiratory distress, dehydration and susceptibility to infections. It is an autosomal recessive condition, caused by mutations on the ABCA12 gene, on chromosome 2q34. Mutations on this gene prevent the production of a cellular lipid transport protein, explaining the phenotype. Case report: We describe a 28 years old woman, G1, referred for moderate oligohydramnios at 20 weeks of gestation. She had severe persistent oligohydramnios with normal fetal morphology at 24 weeks. Rupture of the membranes was confirmed through intramniotic infusion of indigo carmine. At 31 weeks, a fetal MRI was performed for suspicion of an amniotic bands syndrome on ultrasound. Complex facial malformations with agenesis of the upper lip, absent nasal bridge, retractile pad in front of the eye, supra orbital cutaneous prominence without cerebral malformation, were described. Important physiological, social and esthetical impairments, along with the need for reconstruction surgeries, were expected. Given the severity of the malformations, the couple asked for a pregnancy termination, which was approved by our Ethics Committee. Autopsy showed an 1800 g female foetus with generalized large diamond-shaped plaques of thick, pale and crusted skin, with large fissures and zones of separation. The face showed severe ectropion with bilateral exophthalmia and eclabion with “O-shaped” mouth, without anomalies of the nose and mouth. There were severe contractures of the small articulations bilaterally (wrists, fingers, ankles, toes) along with distal hypoplasia of fingers and toes. All internal organs were normal. Massive hyperkeratosis was seen on skin biopsy. The granulous layer was thin and the epithelium showed a papillomatous aspect. Electronic microscopy is pending. Molecular analysis of the ABCA12 gene on placental tissue reveals a disease-causing mutation (R287X) and two sequence variants (D844G and I1409V), which have not been published yet. Parental studies are pending. The former is a strong candidate for a disease mutation, since it is a non-conservative amino acid change in a position highly conserved. Conclusion: This is the first report of magnetic resonance imaging of a third trimester fetus with harlequin syndrome.

2476/F

Can syndromic macrocephaly be diagnosed in utero? *D. Lev[1,2,8], L. Ben-Sira[5,8], C. Hoffmann[6,8], M. Herrera[9], F. Vinals[10], C. Vinkler[1,2], S. Ginat[4,8], D. Kidron[7,8], T. Lerman-Sagie[2,3,8], G. Malinger[2,4,8].* 1) Inst Medical Genetics, Wolfson Medical Center, Holon, Israel; 2) Fetal Neurology Clinic,Wolfson Medical Ctr, Holon, Israel; 3) Pediatric Neurology Division; Wolfson Medical Center, Holon; 4) Department of Obstetrics and Gynecology Wolfson Medical Center, Holon; 5) Pediatric Radiology Division, Tel-Aviv Medical Center; 6) Neuroradiology Division, Haim Sheba Medical Center, Ramat Gan; 7) Depatment of Pathology, Pinhas Sapir Medical Center, Kfar Saba; 8) Sackler School of Medicine, Tel-Aviv University, Tel-Aviv; 9) Departamento de Ultrasonido, Clínica Sanatorio Alemán, Concepción, Chile; 10) Departamento de Medicina Fetal, Clínica Reina Sofía, Universidad Colsánitas, Bogotá, Colombia.

Objectives To compare between the outcomes of fetuses with apparently isolated macrocephaly and syndromic ones. To present the prenatal findings and postnatal diagnoses in children with syndromic macrocephaly. Methods We reviewed the files of all patients with fetal macrocephaly defined as a HC > 2 SD. All the patients were contacted and their development was evaluated. Results Adequate data was available for 97 patients, in 82 fetuses the macrocephaly was considered isolated (Group A), and in 15 fetuses associated anomalies were identified (Group B). Macrocephaly was diagnosed earlier in Group B patients (28.7 vs. 31.4 weeks , p = 0.028); the head circumference in Group B patients was larger (Z score 3.0 vs. 2.4, p<0.001). Group B CNS associated findings, as demonstrated by US and MRI, included mild ventriculomegaly (7), malformations of cortical development (7), callosal abnormalities (6), overdeveloped sulcation (3), large CSP (3), large subarachnoid space (2), mega cisterna magna (2), large 3rd ventricle (2); and periventricular pseudocyst, open operculum , and vermian dysgenesis (1 each). Syndromic diagnosis was reached in utero in 5 fetuses and after birth in 2. In 8 patients associated malformations were confirmed after birth but a specific diagnosis was not made. In Group A one child was diagnosed as having infantile autism. Conclusions: when fetal macrocephaly is associated with other brain or systemic anomalies the diagnosis of syndromic macrocephaly can be made in utero. Most fetuses with syndromic macrocephaly have a HC more than 2.5 SD above the mean.

2477/F

Pan-Ethnic Diagnosis of Trisomy 21 from Chorionic Villi mRNA Using High Frequency Coding-Single Nucleotide Polymorphism (cSNP) Allele Ratios. *J.A. Tynan[1], C. Deciu[1], L. Cagasan[1], M. Roy[1], J. Clemens[1], D. van den Boom[2], P. Oeth[2].* 1) Sequenom Center for Molecular Medicine, San Diego, CA; 2) Sequenom, Inc., San Diego, CA.

The measurement of allele ratios in coding SNPs (cSNPs) is useful for elucidating differential allele and tissue specific gene expression as it applies to molecular pathologies associated with loss of heterozygosity, gene amplification or loss, tissue transplantation and genetic imprinting. Prior research in prenatal diagnostics used the PLAC4 rs8130833 cSNP allele ratio measurement to detect fetal aneuploidy in mRNA isolated from maternal plasma (Lo et. al., Nat Med 13,218-223, 2007). To explore the expanded clinical viability of this methodology further, we screened a set of cSNPs from placentally expressed transcripts for their ability to detect chromosome 21 trisomy. Transcripts with increased expression in placenta versus maternal peripheral blood mononuclear cells were identified using an Affymetrix Human Exon 1.0 ST Array. SNPs within coding regions of these transcripts and with HapMap minor allele frequency greater than 5% in all populations were then selected. 155 cSNPs were screened, and four multiplexes, each consisting of 17 cSNPs, were developed after filtering assays based on multiple performance metrics. These 4 multiplexes were assessed using chorionic villi mRNA derived from 114 euploid and 11 trisomy 21 placental tissue samples. Heterozygous cSNP allele ratios were calculated from the peak area of single base primer extension products measured by MALDI-TOF mass spectrometry (MassARRAY®). A 10-fold cross-validated Mixture Discriminant Analysis classification algorithm was used to differentiate trisomy 21 positive samples from euploid samples. Two of the 17-plexes independently classified all trisomy 21 and normal samples correctly with complete population coverage; the remaining two multiplexes did not confer complete population coverage. These results demonstrate the ability to use multiplexed cSNP allele ratios in differentially expressed transcripts to distinguish copy number variations between affected and normal individuals for aneuploidy diagnosis from chorionic villi. The use of this strategy may be beneficial to monitor other allele specific gene expression or loci alterations in diverse biological events, for example, as seen with genes in the HER-2 amplification region on chromosome 17 associated with breast cancer.

2478/F

Inherited, presumably non pathogenic, genomic deletion unmasks a rare severe autosomal recessive disorder in a fetus. *H. Yonath[1, 2], H. Reznik-Wolf[1], N. Goldstein[1], Y. Bar-Joseph[1], M. Dushnitzki[1], D. Yagel[2], M. Berkenstadt[1], L. Peleg[1], M. Frydman[1,2], E. Pras[1,2].* 1) Inst Human Gen, Sheba Med Ctr, Ramat Gan, Israel; 2) Sackler Faculty of Medicine, Tel Aviv University, Israel.

Prenatal Chromosomal Microarray was sent due to parental anxiety and increased beta HCG (3.03 MOM). The fetus and father were both found to carry a 224 Kb deletion on 5q21.1. The region includes two genes: *ERCC8* and *NDUFAF2*, the first known to cause Cockayne Syndrome. Concerned by the possible existence of intra-genic mutation on the maternal allele we sequenced both genes in the fetus and detected a homozygous deletion of exon 3 in *ERCC8*. For verification, we performed the following analysis: 1. Amplification of the segment between intron 2 and 4. The normal segment is about 11 Kb long. In the father and controls we could not amplify this segment, while in the mother and fetus a 3Kb fragment appeared. 2. cDNA was extracted from the mother’s peripheral lymphocytes. She was found to have multiple transcripts, as previously described in deletion carriers in *ERCC8* . 3. MLPA of *ERCC8* in the mother disclosed only one copy of exon 3. Although detailed ultrasound studies did not reveal any abnormalities in the fetus, the pregnancy was terminated at 30 weeks. Fibrobalsts and amniocytes from the fetus revealed impaired DNA recovery after 17 hr of UV exposure, thus confirming the diagnosis of Cockayne Syndrome. This is the first diagnosis of Cockayne Syndrome in such a manner. While *denovo* deletions in a prenatal setting strongly suggest a significant medical problem in the fetus, deletions inherited from a healthy parent impose a much greater diagnostic challenge. This case highlights the risk of a deleterious mutation on the second allele in a fetus with an inherited abnormality that includes genes associated with autosomal recessive disorders, and emphasizes the need for genetic counseling and possible evaluation when a deletion or duplication is inherited from a normal parent.

2479/F

Assessing the Combined Significance of SNPs in Candidate Genes for Preterm Birth. *B. Feenstra[1], F. Geller[1], H. Zhang[1], H.A. Boyd[1], K.K. Ryckman[2], J.R. Shaffer[3], J.M. Dagle[2], D.E. Weeks[2], M.L. Marazita[3], E. Feingold[3], J.C. Murray[2], M. Melbye[1].* 1) Department of Epidemiology Research, Statens Serum Institut, Copenhagen, Denmark; 2) Department of Pediatrics, University of Iowa, Iowa City, IA; 3) Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA.

Background: Genome-wide association studies (GWAS) provide a vast body of genetic data and often the initial analysis results in single SNPs reaching genome-wide significance and these results can be replicated swiftly. Some of these SNPs are located near candidate genes, some are in regions where no known gene is close by. It is often the case, though, that GWAS cast doubt on earlier candidate gene studies, which claimed significance observing high risks with moderate sample sizes. Currently, candidacy of genetic regions is usually not in the focus of initial analyses, although sometimes a less stringent significance threshold for SNPs in candidate genes is chosen to prioritize these at the replication stage. With our recent GWAS of preterm birth not resulting in an immediate genome-wide significant finding, we decided to take a closer look at the gene level. Aim: We investigated a broad list of more than 200 genes putatively related to preterm birth in 713 children born at gestational age of less than 37 weeks and 982 control children born at a gestational age from 39 to 42 weeks. These genes are involved in detoxification, infection/inflammation, hormonal regulation, membrane integrity, and hemostasis - all pathways proposed as playing a role in initiating or modifying labor. Results: Set Level Association Testing, a method suggested by de la Cruz et al., was applied, which assesses the significance of SNP sets taking into account the linkage disequilibrium structure between the SNPs. Our sets are based on gene positions +/-50 kb. None of the genes reached the Bonferroni-adjusted significance threshold. However, the order of the genes differs from an order based on minimum p-value in the set of SNPs, warranting further investigation. Conclusions: Several GWAS have identified multiple independent signals in one genetic region, e.g., chromosome 8q24. Set-based approaches provide an interesting method to judge the significance of genetic regions, and SNP sets in candidate genes are an obvious starting point.

2480/F

Methotrexate and Misoprostol Teratogenicity: Further Expansion of the Clinical Manifestations. *C. Kozma, J. Ramasethu.* Department of Pediatrics, Georgetown University Hospital, Washington, DC.

We describe a baby boy who was exposed to Misoprostol and Methotrexate in the first trimester of gestation as a result of a failed medical abortion. He presented with severe growth retardation, skull defects, proptotic eyes, cleft palate, and severe micrognathia. There were bilateral reduction deformities of the upper and lower extremities, missing and hypoplastic ribs, and undescended testicles. He had clinical features of pulmonary hypoplasia with severe persistent pulmonary hypertension and remained ventilator dependent until he expired. An autopsy revealed brain anomalies consistent with arrhinencephaly. Methotrexate is frequently used in conjunction with misoprostol to induce medical abortion, an off-label use as abortifacient. Both of these medications are well-established teratogens and have an X classification during pregnancy. Data from five patients who were exposed to both medications in the first trimester indicate a significant teratogenic risk to the developing fetus. Reported anomalies include growth retardation, absence or hypoplasia of the frontal bones, craniosynostosis, large fontanelle, ocular hypertelorism, short palpebral fissures, wide nasal bridge, malformed and low set ears, and micrognathia. Skeletal anomalies are frequent consisting of syndactyly, mesomelic shortening of the forearms, missing ribs, dislocated hips, and talipes equinovarus. The findings in our case are consistent with the pattern of abnormalities that have been reported in the literature. In addition, our patient had severe pulmonary hypoplasia and brain malformations, anomalies that have not been described previously. This case adds to the further documentation of the teratogenic effects of methotrexate and misoprostol on the developing fetus.

2481/W

New autosomal dominant ataxia loci in Norwegian families. *J. Koht[1,2,5], G. Stevanin[2,3,4], A. Durr[2,3,4], E. Mundwiller[2,3], A. Brice[2,3,4], K.K Selmer[7], W. Carpentier[6], C.M.E. Tallaksen[1,5].* 1) Department of Neurology, Oslo University Hospital, Ullevål, Oslo, Norway; 2) INSERM U975, Groupe Hospitalier Pitié Salpêtrière, Paris, France; 3) 4UPMC Univ. Paris 6, UMR_S975, Centre de Recherche de l'Institut du Cerveau et de la Moelle épinière, CNRS 7225, Groupe Hospitalier Pitié- Salpêtrière, Paris, France; 4) APHP, Groupe Hospitalier Pitié Salpêtrière, Département de Génétique et Cytogénétique, Paris, France; 5) Faculty of Medicine, University of Oslo, Oslo, Norway; 6) P3S platform, Pitié-Salpêtrière Hospital, Paris France; 7) Department of clinical Genetics, Oslo University Hospital, Ullevål, Oslo, Norway.

Introduction: Autosomal dominant cerebellar ataxias are a clinically and genetically heterogeneous group of neurodegenerative disorders primarily characterized by unsteadiness, progressive gait and limb ataxia and dysarthria. Over 30 dominant spinocerebellar ataxias (SCA) are described, of these 16 with known genes. The number of SCA is increasing with SCA1,2,3,6 and 7 as the most common variants. The prevalence of dominant ataxias has been estimated to 4.3 in 100 000 in the Norwegian population. We recently demonstrated that only 8% of the Norwegian families are associated with SCA1,2,3,6 and 7, with many families presenting a mild progressive ataxia. Method: We selected two large 4 generation families (NOR-JON and NOR-THO) from our dominant ataxia cohort and excluded mutations in the SCA1,2,3,6, 7 and 14 genes. Whole genome linkage analysis was performed using the Illumina Linkage Panel of 6090 SNPs and additional microsatellite markers in regions with positive LOD scores. Results: The NOR-JON family displayed a very late-onset, slowly progressive, autosomal dominant cerebellar ataxia. The mean age at onset was 62 years and the first symptoms were unsteadiness and dizziness. All revealed saccadic pursuit and 5 of 7 affected subjects had pyramidal signs. MRI of the cerebrum revealed slight atrophy of the cerebellum. The genome-wide screening analysis identified significant linkage with chromosome 10 markers with a maximum multipoint LOD score of 3.1. Haplotype analysis defined a critical region of 10 cM distinct from other reported SCA loci. The NOR-THO family displayed an early onset, very slowly progressive, autosomal dominant ceebellar ataxia. The mean age of onset was 6 years. All had saccadic pursuit and pyramidal signs. MRI of the cerebrum revealed cerebellar atrophy. This family was linked to markers on chromosome 3 with a maximum bi-point LOD score of 4.02 in a critical region of 23 cM. Conclusion: We describe two Norwegian families with autosomal dominant ataxia with linkage to new loci on chromosome 3 and 10 distinct from other SCA loci. Candidate genes within the critical interval are currently screened for mutations.

2482/W

Variation in GRM7 is associated with multiple features of presbycusis. *D.L. Newman[1,2], R. Parody[3], J. Ohmen[4], L. Fisher[4], D.R. Frisina[2], R.D. Frisina[2,5], R.A. Friedman[4].* 1) School of Biological & Medical Sciences, Rochester Institute Technology, Rochester, NY; 2) International Center for Hearing and Speech Research, National Technical Institute for the Deaf, Rochester Institute of Technology, Rochester, NY; 3) John D. Hromi Center for Quality & Applied Statistics, Rochester Institute of Technology, Rochester, NY; 4) House Ear Institute, Los Angeles, CA; 5) Depts of Otolaryngology, Biomedical Engineering, Neurobiology and Anatomy, University of Rochester School of Medicine and Dentistry, Rochester, NY.

Age-related hearing loss, or presbycusis, is a common condition of the elderly that results in significant difficulties in daily life. Presbycusis is typified by a progressive loss of sensitivity to tones, starting at the highest frequencies, inability to discern between speech sounds, lengthening of the minimum discernable temporal gap in sounds, and a decrease in the ability to filter out background noise. The cause of presbycusis is well established as a combination of environmental and genetic factors. All previous research into the genetics of presbycusis has focused solely on pure tone thresholds. A few loci have been identified based on pure tones as having a likely role in susceptibility to this type of hearing loss; GRM7 is the only gene that has achieved genome-wide significance in a GWAS of age-related hearing loss susceptibility. We examined the association of GRM7 variants identified from the previous study, which was done in a European cohort with Z-scores based on pure-tone thresholds, in our European-American population from Rochester, NY (N=725), using a variety of phenotypes related to presbycusis. Principle Components Analysis was used to define factors to test against genotypes using MANOVA. We found that GRM7 was associated not only with the component related to pure tone thresholds, but also with the independent components of gap detection and hearing in noise.

2483/W

Clinical and genetic characterization of a new childhood leukodystrophy: Tremor-Ataxia with Central Hypomyelination (TACH). *G. Bernard[1], I. Thiffault[2], T. Martine[1], M.L. Putorti[1], I. Bouchard[3], M. Sylvain[3], S. Melançon[4], R. Laframboise[3], P. Langevin[3], J.-P. Bouchard[5], M. Vanasse[6], A. Vanderver[7], G. Sébire[8], B. Brais[1].* 1) Research Center CHUM-Notre-Dame, Montreal, Quebec, Canada; 2) Montreal Neurological Institute, Montreal, Quebec, Canada; 3) CHUL, Centre Mère-Enfant, Quebec, Quebec, Canada; 4) Montreal Children's Hospital, Montreal, Quebec, Canada; 5) CHA, Enfant-Jésus Hospital, Québec, Québec, Canada; 6) Ste-Justine Hospital, Montreal, Quebec, Canada; 7) Children's National Medical Center, Washington DC, USA; 8) CHUS, Sherbrooke, Quebec, Canada.

PURPOSE: Leukodystrophies are a heterogeneous group of degenerative disorders characterized abnormal white matter on brain imaging. The clinical manifestations include upper motor neuron signs, developmental and/or cognitive regression with or without other neurological signs. It is estimated that up to 40 percent of patients with leukodystrophy are left without a precise diagnosis despite extensive investigations. Here, we present the clinical features and the locus of a new form of childhood-onset leukodystrophy: Tremor-Ataxia with Central Hypomyelination (TACH). METHOD: We have recruited a group of eight (1 deceased) French-Canadian children affected by a childhood-onset leukodystrophy of unknown cause. All cases have ancestors originating from the same regions of Quebec province known for genetic founder effects. We completed a detailed clinical evaluation of each patient and performed a genome scan with the Illumina Hap310 or Hap610 SNP chip on seven affected individuals and at least one non-affected family member. RESULTS: All cases presented with motor regression and were found to have upper motor neuron signs, as well as cerebellar ataxia, tremor, dysarthria, and dysmetria. Cognition seemed to be relatively preserved. Homozygosity mapping uncovered a shared chromosomal region of 3.98 Mb not previously associated with a leukodystrophy. CONCLUSION: This study presents the clinical description of a new childhood-onset leukodystrophy and the mapping of its original chromosomal locus. Identification of the mutated gene will allow physician to provide a precise diagnosis and genetic counseling to the patients and their families. It will also improve our understanding of neurodegeneration in leukodystrophies and hopefully, provide potential therapeutic targets.

2484/W

Homozygosity in 1q44 presenting with microcephaly and corpus callosum abnormalities. *H. Carlson-Donohoe[1], T. Markello[2,3], C. Tifft[2,3], G. Golas[2,3], D. Adams[1,3], K. Fuentes-Fajardo[3], W.A. Gahl[1,2,3].* 1) Medical Genetics Branch, National Human Genome Research Institute, Bethesda, MD; 2) Office of the Clinical Director, National Human Genome Research Institute, Bethesda, MD; 3) Undiagnosed Diseases Program, National Institutes of Health, Bethesda, MD.

Single copy deletions in 1q44 are associated with agenesis of the corpus callosum and microcephaly. Despite extensive mapping and sequencing in the critical deletion region no gene has been validated as the cause of this phenotype in humans, although a similar phenotype in mice is caused by a recessive deletion of the *AKT3* gene. We analyzed this region as part of a genome-wide SNP array survey in a family with a single affected 6 year old male. The proband was admitted to the NIH Clinical Center through the Undiagnosed Diseases Program presenting with progressive global brain volume loss, agenesis of the corpus callosum, and developmental regression. Illumina Omni1-Quad SNP chip results revealed a single 4.7 Mb region of anomalous continuous homozygosity in 1q44 beginning with rs4658571. This region of homozygosity was not seen in any other family member, including two unaffected siblings who were determined to have the opposite parental haplotypes in the 1q44 region on both copies of chromosome 1. The total fluorescent hybridization intensity was normal throughout the region, excluding any deletion greater than a few kilobases. Analysis of the rest of chromosome 1 demonstrated no segmental uniparental disomy. Capillary sequencing of the exons of *AKT3* and the ultra conserved sequences uc.45 and uc.46 were normal on both chromosomes. We plan to sequence the remaining 4.7 Mb using regional capture technology and Next-Gen Sequencing to detect all coding and non-coding mutations. Finding a homozygous mutation in this patient may provide insight into the mechanism of the microcephaly and corpus callosum phenotype seen in the 1q44 deletion syndrome.

2485/W

Combination of "benign" CNVs predisposing to Autism Disorder in two affected brothers. *C. Castronovo[1], D. Rusconi[1], L. Larizza[1,2], M.T. Bonati[3], P. Finelli[1,4].* 1) Laboratorio di Citogenetica Medica e Genetica Molecolare, Istituto Auxologico Italiano, Milano, Italy; 2) Divisione di Genetica Medica, Dipartimento di Medicina, Chirurgia e Odontoiatria, Facoltà di Medicina e Chirurgia, Ospedale San Paolo, Università degli Studi di Milano, Milano, Italy; 3) Ambulatorio di Genetica Clinica, Istituto Auxologico Italiano, Milano, Italy; 4) Dipartimento di Biologia e Genetica per le Scienze Mediche, Università degli Studi di Milano, Milano, Italy.

Autism Disorder (AD) is a complex neurodevelopmental disorder characterized by impairment of social interaction, communication and repetitive behavior. De novo and inherited submicroscopic Copy Number Variations (CNVs) are emerging as major category of genetic risk for AD, accounting for about 10-20% of cases. We report on two brothers - 12 and 5 years of age - affected from AD and classified as high-functioning and fully autistic, respectively, by using ADI/ADOS scales. High resolution array-CGH analysis identified a few heterozygous inherited microrearrangements, shared by the siblings and reported as polymorphic CNVs in healthy people: a 252 kb deletion at 16p11.2 and two duplications at 17q21.31, 31 and 129 kb in size, which target the same sequence, one on each allele. The 16p11.2 deletion was inherited from the mother - suspected to bear it in a mosaic condition - as well as the largest duplication at 17q21.31, whereas the smallest derived from the father. We suggest that the shared CNVs, singly or combined, may be good candidate loci for AD. Both the microduplications in 17q21.31 interrupt the KIAA1267 gene, encoding for a hypothetical protein specifically expressed in the brain. CNVs affecting the same locus have been already described in autistic patients but in a heterozygous state. In our two siblings we found both alleles affected in a compound heterozygous state, likely resulting in the lack of protein expression. The CNV at 16p11.2 targets the same region disrupted by a de novo t(7;16)(p22.1;p11.2) carried by an autistic patient. This deletion is located proximally to SLC6A10 gene, paralogous to SLC6A8 on Xq 28 which has been found mutated in individuals with mental retardation, with or without autism. SLC6A10, a member of transporter protein family responsible for the uptake of neurotransmitters, is expressed in brain with a possible function in neurodevelopment. In order to understand the pathogenic role in AD of the identified CNVs, we are planning further investigations to: i) confirm the disruption of KIAA126 alleles through the two different intragenic duplications; ii) verify by BAC i-FISH the mosaicism of 16p11.2 deletion in the mother which may almost in part explain the contrasting phenotypes; iii) study the possible creatine transport perturbation by biochemical screening (creatine/creatinine dosage) as well as further SLC6A8 anomalies on Xq28, which may contribute to the phenotype onset.

2486/W

Association of myocardial infarction-associated SNPs with ischemic stroke: a meta-analysis of three European Caucasian populations. *Y. Cheng[1], C.D. Anderson[2,3,4], M. Nalls[5], A. Biffi[2,3,4], L. Cortellini[2,3,4], N.S. Rost[2,3,4], K.L. Furie[2,3], T.G. Brott[6], R.D. Brown, Jr.[7], D. Hernandez[5], M. Matarin[5], J.W. Cole[8], O.C. Stine[1], J.R. O'Connell[1], S.J. Kittner[8], J. Rosand[2,3,4], J.F. Meschia[6], B.D. Mitchell[1].* 1) Department of Medicine, University of Maryland, Baltimore, MD; 2) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA; 3) Department of Neurology, Massachusetts General Hospital, Boston, MA; 4) Program in Medical and Population Genetics, Broad Institute, Cambridge, MA; 5) National Institute of Aging, Bethesda, MD; 6) Department of Neurology, Mayo Clinic, Jacksonville, FL; 7) Department of Neurology, Mayo Clinic, Rochester, MN; 8) Department of Neurology, University of Maryland, Baltimore, MD.

Background — Stroke is the third leading cause of death in the United States. Ischemic stroke, which is caused by the blockage of blood flow to the brain, shares many common risk factors with coronary artery disease. We hypothesized that genetic variants associated with myocardial infarction (MI) are also associated with the risk of ischemic stroke.

Methods — Eleven previous identified MI-associated SNPs were evaluated for their associations with ischemic stroke in 2,441 cases and 3,189 controls from three European Caucasian case-control studies, including the Genetics of Early Onset Stroke (GEOS) study, Ischemic Stroke Genetics Study (ISGS) and the Massachusetts General Hospital (MGH) study. The previously identified MI-associated SNP, or a surrogate SNP in high LD ($r^2 > 0.8$) with it, was genotyped by the Illumina or Affymetrix GWAS platform used in the respective studies. Imputed genotypes were used in the ISGS study. A genotype risk score was also computed for each individual by summing the number of MI-risk alleles across each of the 11 loci. Logistic regression, adjusted for age, sex, study-specific covariates, was performed within each study to obtain study-specific betas and p-values under an additive model. Meta-analysis of the three studies was conducted using both a sample-size weighted and an inverse variance weighted approach as implemented in the METAL software program.

Results — Despite having 80% power to detect odds ratios of 1.12 - 1.19 (at allele frequencies = 0.10 - 0.50) in the combined sample, none of the 11 MI-associated SNPs were significantly associated with ischemic stroke in any of the studies individually or in the meta-analysis. However, the genotype risk score was significantly associated with risk of ischemic stroke, with each additional MI-risk allele associated with a 1.04-fold increase in the odds of stroke (95% CI: 1.01-1.07; meta-analysis P =0.006).

Conclusions — None of the individual MI-associated SNP was significantly associated with ischemic stroke in this study of European Caucasians. However, these previously identified MI risk alleles increased stroke risk on average by 4% per allele.

2487/W

P53 gene Arg72Pro polymorphism and Sporadic Amyotrophic Lateral Sclerosis. *E. Ergul[1], M.D. Sozuguzel Ozel[1], A. Sazci[1], H.A. Idrisoglu[2].* 1) Medical Biology and Genetics, Faculty of Medicine, University of Kocaeli,Umuttepe, 41380, Kocaeli, Turkey; 2) Department of Neurology, Faculty of Medicine, University of Istanbul, Capa, 34280,Istanbul, Turkey.

Amyotrophic lateral sclerosis (ALS) is a devestingly progressive and eventually fatal neurodegenerative disorder affecting motor neuron cortex, brain stem and spinal cord, and leading to muscle wasting, weakness and profound paralysis.To examine an association between p53 gene Arg72Pro (rs1042522) polymorphism and sporadic amyotrophic lateral sclerosis (SALS), we genotyped 394 Turkish SALS patients and 439 matched healthy controls by a PCR-RFLP technique. We obtained a statistically significant association between female SALS patients with 72AP genotype and female healthy controls (X2=4.102; P=0.043). Consequently it appears that p53 Arg72Pro polymorphism is associated with SALS in a gender specific manner.

2488/W

Vascular endothelial growth factor -2578C/A polymorphism and amyotrophic lateral sclerosis. *Y.T. Esengul[1], E. Ergul[1], M.D. Sozuguzel Ozel[1], A. Sazci[1], H.A. Idrisoglu[2].* 1) Medical Biology and Genetics, Faculty of Medicine, University of Kocaeli,Umuttepe, 41380, Kocaeli, Turkey; 2) Department of Neurology, Faculty of Medicine, University of Istanbul, Capa, 34280,Istanbul.

Amyotrophic lateral sclerosis(ALS) is a exceptionaly progressive and eventually fatal neurodegenerative disorder. Motor neurons of the motor cortex, brainstem, and spinal cord are affected, leading to muscle wasting, weakness and profound paralysis. The incidence and prevalence of ALS are 1-2 and 4-6 per 100 000 people each year in the world. Vascular endothelial growth factor (VEGF) plays a critical role in the induction of angiogenesis.- Mice with a subtle deletion of the hypoxia response element in the VEGF promoter, causing reduced VEGF expression, leading to adult-onset motor neuron degeneration, reminiscent of ALS. Studies in human gave conflicting results. We genotyped 385 SALS patients and 312 healthy controls to show whether VEGF -2578C/A(rs699947) polymorphism was implicated in SALS by using a PCR-RFLP method. In conclusion, There was no association between VEGF -2578C/A polymorphism and SALS(X2=0.533; P=0.766).

2489/W

Very High-Density Linkage Survey of Schizophrenia and Bipolar Disorder Families Reveals Genetic Overlap and Implicates Novel Regions and Genes. *A. Fanous[1,2,3], F. Middleton[4], R. Amdur[1,2], B. Maher[3], H. Medeiros[5], K. Gentile[4], J. Knowles[5], M. Azevedo[6], M. Pato[5], C. Pato[5].* 1) Washington VA Medical Center, Washington, DC; 2) Georgetown University School of Medicine, Washington, DC; 3) Virginia Commonwealth University School of Medicine; 4) Upstate Medical University, Syracuse, New York; 5) University of Southern California, Los Angeles, California; 6) Psychiatry Service, Ponta Delgada, Azores, Portugal.

Background: Recent family and GWAS studies strongly suggest shared genetic risk factors for schizophrenia (SZ) and bipolar disorder (BP). Linkage studies have not been rigorously used to test this hypothesis. Methods: Fifty-one families with at least one sibpair concordant for either SZ or psychosis (PSY, which includes either SZ or psychotic BP) were genotyped using the Affymetrix 50K Xba SNP array. NPL and LOD scores for SZ, BP, and PSY were calculated in Merlin. Empirical significance was calculated using 1000 gene-dropping simulations. Chromosomes with loci linked to BOTH SZ and BP were counted. Empirical significance of joint linkage was determined by a) the number of simulated BP scans having the same number of loci jointly linked with the real SZ scan, and b) the number of simulated SZ scans having the same number of loci jointly linked with the real BP scan. Results: For SZ and PSY, several regions previously linked in this and other samples were suggestively linked. For BP, chromosome 1p36 (11.54-15.71 MB) achieved significance with a LOD of 3.51; as did chromosome 11p (89.32-90.15 MB) with an NPL of 4.15. The 95% CI of the latter corresponded extremely closely to the physical coordinates of DLG2, which encodes postsynaptic density protein PSD-93. Four chromosomes had loci at which both SZ and BP had NPL≥1.98 (empirical P=.01 using simulated SZ scans; .07 using simulated BP scans). Conclusions: Using higher-density marker maps than used in any previous SZ and BP linkage study, we observed a) significant linkage to 1p36, which was not observed using sparser maps; b) for the first time we know of, significant linkage of any psychiatric disorder (BP) implicating only one gene (DLG2); and c) confirmation of genetic overlap between SZ and BP.

2490/W

Association study of TCI scale and genetic variants of DRD4, DAT1 and 5HTT with bipolar disorder in the Japanese. *L. Gotoh, N. Oribe, A. Takata, H. Mitsuyasu, H. Kawasaki, S. Kanba.* Dept Neuropsychiatry, Kyushu Univ, Fukuoka, Japan.

Bipolar disorder (BD) is a common, severe, chronic, and life-threatening psychiatric illness where patients alternate between episodes of depression and mania. Detailed pathophysiology of BD is still unclear, but family, twin and adoption studies consistently indicate a strong genetic component. Therefore, a number of genetic studies of BD have been conducted. In particular, genes related to the serotonin and dopamine neurotransmissions were regarded good candidate genes for BD. Furthermore, some reports suggested that several dimensions of temperament and character inventory (TCI) scale correlated with BD. TCI scale is constructed with 7 dimensions including 4 temperaments which was involved in several monoaminergic neurotransmissions in the CNS and 3 characters. It was reported that BD patients showed the low self-directedness (SD) score and high harm-avoidance (HA) and self-transcendence (ST) scores of TCI and that the genetic variants affected the scores of TCI. Therefore, we performed an association study of BD or TCI score and some genetic variants (serotonin transporter (5HTT); 44-bp insertion/deletion polymorphism in the promoter region (SERTPR) and variable number of tandem repeats (VNTR) in second intron (SERT-in2), dopamine D4 receptor (DRD4); 48-bp VNTR in exon3 and dopamine transporter (DAT1); 40-bp VNTR in 3' UTR). Fifty Japanese BD patients diagnosed using the Structured Clinical Interview for DSM-IV (SCID) and 50 Japanese normal controls (NC) were included in this study. All subjects were given informed consent before blood collection strictly based on the ethical regulations of Kyushu University. These genetic variants were genotyped by electrophoresis after PCR amplification and association analyses were performed. As a result, comparing of genetic variants and disease, SERTPR indicated statistically significant difference between BD and NC, whereas other genetic variants showed no differences. In comparison of genetic variants and TCI scores, only DAT1-VNTR showed significances in Extravagance score (NS3; a subscale of novelty-seeking (NS)) and in analysis of disease and TCI scores, the HA and SD scores indicated significant differences. Thus, it was able to hypothesize that the genetic variants which were analyzed in this study, affected TCI scores and pathophysiology of BD respectively but not concurrently. Further analysis with more samples and genetic variants is now in progress.

2491/W

The role of *Chrna6* and *Chrnb3* genes in alcohol behaviors using mouse models. *N. Hoft[1], H. Kamens[1], J. Miyamoto[1], M. Ehringer[1,2].* 1) Inst Behavioral Genetics, Univ Colorado, Boulder, CO; 2) Department of Integrative Physiology, Univ Colorado, Boulder, CO.

Genes encoding the α6 and β3 nicotinic acetylcholine receptor subunits (*CHRNA6* and *CHRNB3*) have been associated with early subjective response to nicotine (Zeiger et al, 2008; Ehringer et al, 2010) and nicotine dependence (Bierut et al, 2007; Saccone et al, 2007; Hoft et al, 2009). It is well-known that tobacco and alcohol use behaviors are highly co-morbid (Kendler et al, 2007), and functional evidence from pharmacology and animal models (Cardoso et al, 1999; Zuo et al, 2004; Butt et al, 2004) supports the hypothesis that nicotinic receptors may be a common site of action for these two drugs in the dopaminergic system. We recently found evidence for association between the *CHRNB3/A6* gene cluster and alcohol behaviors in humans (Hoft et al, 2009). Furthermore, we have performed in vitro assays showing that variations (SNPs) in the promoter region of the human *CHRNB3* gene may lead to differences in gene expression (Ehringer et al, 2010). We are now engaged in testing knock-out (KO) mice, which lack either the *Chrna6* or *Chrnb3* gene. Specifically, we are examining whether the KO animals show differences in voluntary alcohol consumption (two-bottle preference), sensitivity to alcohol (loss of righting reflex and acute functional tolerance), or alcohol induced ataxia (a balance test). Preliminary data suggest that the *Chrnb3* KO mice may drink less alcohol than their wild-type littermates, while differences in animals lacking *Chrna6* are less clear. For the sensitivity measures, preliminary data suggest that female mice lacking the *Chrna6* gene take significantly longer to recover righting reflex ($p<0.03$) indicating greater sensitivity than their wild-type littermates. Similarly the *Chrnb3* KO animals show a trend toward being slightly more sensitive to alcohol in that it took longer for them to recover from loss of righting (return of righting reflex) than their wild-type littermates. These results complement the human genetic studies, providing support for the *CHRNB3/A6* genes as potential contributors to the co-morbidity of alcohol and tobacco behaviors. This work was supported by NIH grants AA015336 (ME), DA017637 (NH), and AA017889 (NH, JM, HK).

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Genome-wide gene expression and DNA methylation analyses of significantly discordant monozygotic twins for cognitive ability. *K. Kobayashi[1], M. Furukawa[1], C. Yu[1], C. Shikishima[2], J. Sese[3], H. Sugawara[4], K. Iwamoto[5], T. Kato[4], J. Ando[6], T. Toda[1].* 1) Div Mol Brain Sci & Neurol, Kobe Univ Grad Sch Med, Kobe, Japan; 2) Keio Adv Res Ctr, Keio Univ, Tokyo, Japan; 3) Dept Comput Sci, Ochanomizu Univ, Tokyo, Japan; 4) Lab Mol Dynam Mental Disord, RIKEN BSI, Saitama, Japan; 5) Dept Mol Psychiatry, Grad Scl Med, Univ Tokyo, Tokyo, Japan; 6) Fac Letters, Keio Univ, Tokyo, Japan.

It has been suggested that intelligence is a multifactorial trait and is influenced by not only environmental but also genetic factors. Although the concordance rate in monozygotic (MZ) twins is much higher than in dizygotic twins, some MZ twins exhibit significant discordance in intellectual levels. The discordance could be caused by epigenetic differences between twins or de novo genomic mutations that occurred in either sib. Epigenetic differences are not accompanied with the changes of genomic sequence, so that gene expression analysis is a promising predictor of discordance-associated genes that are influenced by the epigenome. To investigate the genetic factors for intelligence, we extracted total RNA from lymphoblastoid cell lines of six pairs of MZ twins with significantly discordant intelligence quotient (IQ) and performed gene expression analysis by using Affymetrix gene expression microarrays. The expression data were normalized for each pair with Robust Multichip Average method and analyzed with Gene Set Enrichment Analysis method to identify differentially expressed gene sets between the sibs. As a result, we found that some gene sets related to immunological response tend to be up-regulated in the sibs with lower IQ. Next, we also conducted the DNA methylation analysis of the same twin pairs using the genome DNA derived from lymphocytes with Affymetrix human promoter array and Tiling Analysis Software. Quantile normalization and wilcoxon signed-rank test were performed for each pair to detect significantly methylated regions in one side of the sibs. On the candidate methylated regions identified by TAS, we could not find remarkable differences in methylation status between the sibs by bisulfite genomic sequencing. Further studies will be required to identify genes associated with intelligence.

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Evidence for an association of the *NOS1* and *NOS3* genes with stroke susceptibility. *H. Manso[1,2,3], T. Krug[1,4], J. Sobral[1,2,3], I. Albergaria[2], G. Gaspar[2], M. Correia[5], R. Taipa[5], M.R. Silva[6], J.P. Gabriel[6], G. Lopes[5], I. Matos[7], J. Jiménez-Conde[8], R. Rabionet[9], J.M. Ferro[10], S.A. Oliveira[1,4], A.M. Vicente[1,2,3].* 1) Instituto Gulbenkian de Ciência, Oeiras, Portugal; 2) Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisbon, Portugal; 3) Center for Biodiversity, Functional & Integrative Genomics; 4) Instituto de Medicina Molecular, Lisbon, Portugal; 5) Serviço de Neurologia, Hospital Geral de Santo António, Porto, Portugal; 6) Serviço de Neurologia, Hospital de São Pedro, Vila Real, Portugal; 7) Serviço de Neurologia, Hospital Distrital de Mirandela, Mirandela, Portugal; 8) Neurology Unit, IMIM-Hospital del Mar, Barcelona, Spain; 9) Center for Genomic Regulation (CRG-UPF) and CIBERESP, Barcelona, Spain; 10) Serviço de Neurologia, Hospital de Santa Maria, Lisbon, Portugal.

Stroke is a leading cause of death and significant disability in western countries. It is a complex disease thought to result from the interplay between well-established environmental/life-style risk factors and genetic risk factors, still unknown for the most common forms of stroke. Clinical conditions, such as hypertension and atherosclerosis, also increase the risk of stroke. Several lines of evidence suggest that nitric oxide (NO) plays a role in hypertension and can have anti-atherosclerotic actions by regulating platelet function, leukocyte adhesion and diapedesis, by preventing vascular smooth muscle cells proliferation and by inhibiting LDL oxidation. The synthesis of NO is catalyzed by nitric oxide synthases (NOS) expressed in various tissues, encoded by the *NOS* genes. In the present study we investigated the role of *NOS1* and *NOS3*, which encode the neuronal and endothelial NOS, respectively, in stroke risk. 37 *tag* SNPs in *NOS1* (12q24.2-q24.31) and 5 *tag* SNPs in *NOS3* (7q36), were genotyped and tested for association with stroke risk in a population sample of 551 ischemic stroke patients and 530 control subjects. Logistic regression analysis was carried out to adjust for significant stroke risk factors: age, gender, hypertension, diabetes and ever smoking. 13 SNPs and 8 haplotypes in *NOS1* and 2 SNPs in *NOS3* were associated with stroke risk ($0.0002<P<0.0464$ and $0.0117<P<0.0379$, respectively); 4 SNPs and 1 haplotype in *NOS1* and 1 SNP in *NOS3* remained significant after correction for multiple testing. SNP imputation in and around the *NOS1* gene identified 7 additional SNPs associated with stroke risk ($P<0.01$). Preliminary *NOS1* sequencing results, for 7 exons including regions with the most significant associations, revealed one exonic synonymous alteration and two intronic base changes affecting donor and acceptor splice sequences. The significance of these sequence changes is under assessment. We further investigated the occurrence of epistasis (non-additive genetic interaction) between *NOS1* and *NOS3* variants, as it is expected to occur in complex disorders like stroke where genotype/phenotype correlations are non-linear. Using the multifactor dimensionality reduction method, we found no evidence for epistasis between *NOS1* and *NOS3*, indicating an independent effect of both genes. The present findings confirm the previous stroke association of *NOS3* and show novel evidence implicating *NOS1* gene variants in the susceptibility to ischemic stroke.

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Identification of *FOXP1* deletions in three unrelated patients with mental retardation and significant speech and language deficits. *G. Rappold[1,16], J. Kapeller[1,16], N. Rivera-Brugués[3], U. Moog[4], B. Lorenz-Depiereux[3], S. Eck[3], M. Hempel[5], J. Wagenstaller[3], A. Gawthrope[6], A.P. Monaco[6], M. Bonin[7], O. Riess[7], E. Wohlleber[8], T. Illig[9], C.R. Bezzina[10], A. Franke[11], S. Spranger[12], P. Villavicencio-Lorini[2], W. Seifert[2,13,14], J. Rosenfeld[15], E. Klopocki[2], T.M. Strom[3,16], D. Horn[2,16].* 1) Dept. Human Molecular Genetics, Inst. of Human Genetics, University of Heidelberg, Heidelberg, Germany; 2) Institute of Medical Genetics, Charité, University Medicine of Berlin, Germany; 3) Institute of Human Genetics, Helmholtz Zentrum München, German Research Center for Environmental Health, 85764 Neuherberg, Germany; 4) Institute of Human Genetics, University of Heidelberg, Germany; 5) Institute of Human Genetics, Technische Universität München, 81675 Munich, Germany; 6) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford OX3 7BN, UK; 7) Department of Medical Genetics, Institute of Human Genetics, University of Tübingen, Germany; 8) Institute of Human Genetics, Rheinische Friedrich-Wilhelms-University, Bonn, Germany; 9) Institute of Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, 85764 Neuherberg, Germany; 10) Heart Failure Research Center, Department of Experimental Cardiology, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands; 11) Institute for Clinical Molecular Biology, Christian-Albrechts-University zu Kiel, Germany; 12) Praxis für Humangenetik, Bremen, Germany; 13) Cologne Center for Genomics, Universität zu Köln, Köln, Germany; 14) Faculty of Biology, Chemistry, and Pharmacy, Free University of Berlin, Germany; 15) Department of Audiology and Phoniatrics, University Medicine of Berlin, Germany; 16) These authors contributed equally to this work.

Mental retardation affects 2-3% of the population and shows a high heritability. Neurodevelopmental disorders that include pronounced impairment in language and speech skills occur less frequently. For most cases, the molecular basis of mental retardation with or without speech and language disorder is unknown due to the heterogeneity of underlying genetic factors. We have used molecular karyotyping on 1523 patients with mental retardation to detect copy number variations (CNVs) including deletions or duplications. These studies revealed three heterozygous overlapping deletions solely affecting the *forkhead box P1 (FOXP1)* gene. All three patients had moderate mental retardation and significant language and speech deficits. Since our results are consistent with a *de novo* occurrence of these deletions, we considered them as causal although we detected a single large deletion including *FOXP1* and additional genes in 4104 ancestrally matched controls. These findings are of interest with regard to the structural and functional relationship between FOXP1 and FOXP2. Mutations in *FOXP2* have been previously related to monogenic cases of developmental verbal dyspraxia. Both *FOXP1* and FOXP2 are expressed in songbird and human brain regions that are important for the developmental processes that culminate in speech and language.

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The Gabbr1 and Gabbr2 genes are involved with addictive behavior: a study in different phenotypes of ethanol consumers. *A.F. Ribeiro[1], D. Correia[2], S. Chiavegatto[3], R. Boerngen-Lacerda[2], A.L. Brunialti-Godard[1].* 1) Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil; 2) Universidade Federal do Paraná, Curitiba, Paraná, Brazil; 3) Instituto do Coração, São Paulo, São Paulo, Brazil.

Studies involving humans and animals have shown the modulation of ethanol intake behavior mediated by GABAB receptor agonist (baclofen). The Gabbr1 and Gabbr2 genes codify two proteic subunits, GB1 and GB2, that compose the GABAB receptor. Presynaptically, the GABAB receptor inhibits dopaminergic, GABAergic, and glutamatergic systems, and alterations in these systems are well known to be associated with addictive behavior. In an attempt to understand the participation of GABAB in alcoholism, we used non-inbred, Swiss mice exposed to an addiction model based on a three-bottle free-choice paradigm (water, 5% v/v ethanol, and 10% v/v ethanol) consisting of four phases: acquisition (AC), withdrawal (W; first aversive stimulus), reexposure (RE), and quinine-adulteration (AD; second aversive stimulus). Based on their individual ethanol intakes, mice were divided into three groups: addicted (A; preference for ethanol during AC and no reduction in consumption in AD), non-addicted heavy-drinker (HD; preference for ethanol during AC and reduction after AD), and non-addicted light-drinker (LD; preference for water during all phases). For the molecular analysis, mRNA was quantified using real-time polymerase chain reaction in four dissected brain regions: prefrontal cortex, hypothalamus, hippocampus, and striatum. In the prefrontal cortex of animals in Group A, we found high Gabbr1 and Gabbr2 transcript levels, with significant Gabbr1 transcript levels compared with Groups C (control), LD, and HD. In the hippocampus of animals in Group A, Gabbr2 mRNA levels were significantly lower compared with Groups C, LD, and HD. In the striatum, we found a significant increase in Gabbr1 transcription compared with Groups C, LD, and HD. No differences in Gabbr1 or Gabbr2 transcript levels in the hypothalamus were observed among groups. We found molecular differences on transcript levels of the Gabbr1 and Gabbr2 genes in cerebral areas related to drug taking only mice behaviorally classified as addicted, suggesting that these genes contribute to ethanol addiction. Moreover, it was possible to visualize the dynamic of the answers of the Gabbr1 and Gabbr2 genes within different brain areas that are important for maintaining addictive behavior.

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The impact of cytochrome P450 polymorphisms on verbal memory in HIV infected and at risk women. *E.E. Sundermann[1], L.H. Rubin[2], J.R. Bishop[3], K.M. Weber[4], M.H. Cohen[4], H. Crystal[5], V. Valcour[6], E.T. Golub[7], R. Karim[8], K. Anastos[9], C. Liu[10], P.M. Maki[1, 2].* 1) Psychology, University of Illinois, Chicago, Chicago, IL; 2) Psychiatry, University of Illinois, Chicago, Chicago, IL; 3) Pharmacy Practice, University of Illinois, Chicago, Chicago, IL; 4) The Core Center at Stroger Hospital of Cook County, Chicago, IL; 5) State University of New York Downstate Medical Center, Brooklyn, NY; 6) WIHS Center at the University of California, San Francisco, San Francisco, CA; 7) Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 8) WIHS Center at the University of Southern California, Los Angelos, CA; 9) Medicine, Montefiore Medical Center and Albert Einstein College of Medicine, Bronx, NY; 10) District of Columbia WIHS Center, Washington D.C.

Objective: Polymorphisms on genes encoding the cytochrome 450 enzyme (CYP1A1 and CYP1B1) influence estrogen catabolism and have been associated with mood and menopause symptoms. Estrogen impacts certain cognitive abilities, including verbal memory. Although verbal memory can vary in relation to estrogen polymorphisms, particularly in African-American women, the relationship between certain CYP genes and verbal memory is unknown. We examined CYP genes in relation to verbal memory among a sample of women enrolled in the Women's Interagency HIV Study (WIHS). Methods: Participants included primarily African American, premenopausal and perimenopausal women from the WIHS who consented to genetic testing and completed the Hopkins Verbal Learning Test (HVLT). Functional SNPs on the CYP1A1 (rs2606345) and CYP1B1 (rs1056836) genes were examined due to their previous association with estrogen-related clinical outcomes in a healthy population. Linear regression was used to test the association between CYP SNPs and verbal memory scores (HVLT immediate and delayed recall trials) after adjusting for relevant covariates under an additive model. Results: The sample included 412 HIV-infected and 198 HIV-uninfected women (age range 23-57, 71% African-American). In unadjusted analyses, the CYP1A1 SNP was associated with both immediate ($p<0.05$) and delayed ($p<0.001$) recall scores, and the CYP1B1 was associated with delayed recall scores ($p<0.05$). In analyses adjusted for age, depression, HCV, race, menopause status, educational attainment and illicit drug use, only the CYP1A1 SNP was associated with verbal memory; the CC genotype was associated with lower delayed recall compared to the AA genotype ($\beta=0.09$, $p<0.05$). When analyses were restricted to African American women only, the relationship between the CYP1A1 CC genotype and poorer verbal memory became stronger ($\beta= 0.11$, $p<0.001$). No significant interactions were observed between HIV serostatus and genotype group. HIV serostatus independently predicted verbal memory; HIV+ women performed significantly worse than HIV- women on immediate recall trials. Conclusion: Both HIV and CYP1A1 CC genotype are independent risk factors for poorer verbal memory performance. The effect of the CYP1A1 SNP on verbal memory was most pronounced among African American women. Findings suggest that changes in estrogen signaling, by way of a CYP1A1 polymorphism, impacts verbal memory, in addition to mood and menopause symptoms.

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Characterization of the Neuroglobin promoter and its response to amyloid beta in vitro. *M. Szymanski[1], R. Wang[2], S. Bassett[2], D. Avramopoulos[1,2].* 1) Inst Gen Med, Johns Hopkins Univ, Baltimore, MD; 2) Psychiatry, Johns Hopkins Univ, Baltimore, MD.

Alzheimer's disease (AD) is a late onset dementia with significant heritability. Brain pathology findings include plaques containing amyloid beta (A-beta) aggregates, a peptide suspected to be directly involved in the observed neuronal cell death. We previously reported strong linkage on chromosome 14q to AD using the presence or absence of co-morbid hallucinations as a covariate. Upon follow-up we reported that the gene encoding neuroglobin (NGB) contains SNPs with significant associations to AD and an expression profile in the temporal lobe that is consistent with a role in the disease risk. NGB is a vertebrate globin mainly expressed in neurons, and is shown by many studies to play a neuroprotective role. NGB expression is induced by hypoxia and ischemia in vitro and overexpression protects cells against hypoxia, ischemia, oxidative stress and from the toxic effects of A-beta in vitro and in vivo. Previously we showed that NGB RNA levels in the temporal lobe are reduced with advancing age and in females, both risk factors for AD. We also found that NGB expression is higher in AD affected brains, consistent with upregulation in response to the disease process. We have explored the NGB promoter region through constructs driving a luciferase reporter gene transfected into neuronal-like cells. We identified potential enhancer elements between -306 and -1142 and potential repressor elements between -1124 and -1568 from the transcription start site. Based on our results and the literature we hypothesized that A-beta likely upregulates NGB expression, perhaps through promoter elements present in our constructs. We tested our hypothesis on our longest reporter construct, treating transfected cells with a toxic fragment of A-beta, including amino acids 25 to 35. We found significant upregulation of the reported expression upon A-beta exposure, increasing with concentration and with time. Our results support previous observations on postmortem tissue and the hypothesis that Aβ induces NGB expression. We speculate that this is a defense mechanism and might be inadequate in AD patients. Further exploration of the mechanisms underlying the response to A-beta and the regulation of NGB might lead to targets for pharmacological intervention enhancing the NGB mediated neuroprotection against AD and stroke.

2498/W

Rare mutations in ^{ANG} confer large risk for ALS and Parkinson's disease. *M. van Es[1], P.W.J. van Vught[1], H.J. Schelhaas[2], P.M. Andersen[3], C. Klein[4], J.J. van Hilten[5], P. Heutink[6], E. Cuppen[7], P.I.W. de Bakker[8], T. Gasser[9], A.C. Ludolph[10], W. Robberecht[11], R.A. Ophoff[12], J.H. Veldink[1], J.E. Landers[13], B.P. van de Warrenburg[2], L.H. van den Berg[1].* 1) Dept Neurology, Univ Medical Ctre Utrecht, Utrecht, Netherlands; 2) Department of Neurology, Donders Instute for Brain, Cognition and Behaviour, Center for Neuroscience, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands; 3) Institute of Clinical Neuroscience, Umeå University Hospital, Umeå, Sweden; 4) Section of Clinical and Molecular Neurogenetics at the Department of Neurology, University of Lübeck, Lübeck, Germany; 5) Department of Neurology, Leiden University Medical Center, Leiden, The Netherlands; 6) Department of Clinical Genetics, Section of Medical Genomics, VU University Medical Centre, Amsterdam, The Netherlands; 7) Hubrecht Institute for Developmental Biology and Stem Cell Research, Cancer Genomics Center, Royal Netherlands Academy of Sciences, Utrecht, The Netherlands; 8) Program in Medical and Population Genetics, Broad Institute of Harvard and MIT, Cambridge, MA, USA; 9) Department for Neurodegenerative Diseases, Hertie Institute for Clinical Brain Research, University of Tübingen, and German Center for Neurodegenerative Diseases, Tübingen, Germany; 10) Department of Neurology, University of Ulm, Ulm, Germany; 11) Department of Neurology, University Hospital Leuven, University of Leuven, Leuven, Belgium; 12) UCLA Center for Neurobehavioral Genetics, Los Angeles, USA; 13) Department of Neurology, University of Massachusetts School of Medicine, Worcester, USA.

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, is a fatal neurodegenerative disorder characterized by progressive wasting and weakness of limb, bulbar, and respiratory muscles. A candidate gene study suggested that mutations in angiogenin (*ANG*) are associated with ALS. However, subsequent studies have produced conflicting results. In this study we sought to elucidate the role of *ANG* in ALS by performing large sequencing experiments and incorporating data from previous studies into the analysis. We demonstrate an excess of rare mutations in ALS patients with $P = 1.62 \times 10^{-8}$ and OR = 27.37. Several patients carrying *ANG* mutations also demonstrated signs of Parkinson's disease (PD). Epidemiologic studies have also that relatives of ALS patients are at an increased risk for PD (RR = 3.0). We therefore explored the possibility that *ANG* may also predispose to PD. Statistical analysis showed an excess of rare mutations in PD with $P = 3.67 \times 10^{-4}$ with OR = 13.45. This study firmly establishes that *ANG* is involved in ALS and highlights the discovery of a novel risk gene for PD.

2499/W

Inactivation of fibroblast growth factor binding protein 3 causes anxiety-related behaviors associated with attenuated phosphorylation of extracellular signal-regulated kinase in the orbitofrontal cortex. *Y. Yamanaka[1], A. Kitano[1], K. Takao[2], A. Prasansuklab[3], T. Mushiroda[3], K. Yamazaki[4], T. Abe[5], N. Iwata[6], T. Miyakawa[2], Y. Nakamura[7], T. Nakahata[1], T. Heike[1].* 1) Graduate School of Medicine, Kyoto University, Kyoto, Japan; 2) Genetic Engineering and Functional Genomics Unit, Kyoto University, Kyoto, Japan; 3) Laboratory for International Alliance, RIKEN Center for Genomic Medicine, Yokohama, Japan; 4) Laboratory for Genotyping Development, RIKEN Center for Genomic Medicine, Yokohama, Japan; 5) Laboratory for Animal Resources and Genetic Engineering, RIKEN Center for Developmental Biology, Kobe, Japan; 6) Department of Psychiatry, Fujita Health University, Toyoake, Japan; 7) Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

The neurobiological mechanisms of emotional modulation and the molecular pathophysiology of anxiety disorders are largely unknown. The fibroblast growth factor (FGF) family has been implicated in the regulation of many physiological and pathological processes, which include the control of emotional behaviors. The present study examined mice with a targeted deletion of the fgf-bp3 gene, which encodes a novel FGF-binding protein, in animal models relevant to anxiety. To define the behavioral consequences of FGF-BP3 deficiency, we evaluated fgf-bp3-deficient mice using a number of anxiety-related behavioral paradigms that provide a conflict between the desire to explore an unknown area or objects and the aversion to a brightly lit open space. The fgf-bp3-deficient mice exhibited alterations in time spent in the central area of the open-field arena, were less active in the lit areas of a light/dark transition test, and had a prolonged latency to feed during a novelty-induced hypophagia test. These changes were associated with alterations in light-induced orbitofrontal cortex (OFC) activation in an extracellular signal-regulated kinase (ERK) pathway-dependent manner. These results demonstrate that FGF-BP3 is a potent mediator of anxiety-related behaviors in mice and suggest that distinct pathways regulate emotional behaviors. Therefore, FGF-BP3 plays a critical role in the regulation of emotional states and in the development of anxiety disorders and should be investigated as a therapeutic target for anxiety disease in humans.

2500/W

Clinical Case Report of KIF1A Duplication in Idiopathic Autism. *F. Bartel[1], A. Dwivedi[1], S. Cathey[2], B. DuPont[1].* 1) Dept Cytogenetics, Greenwood Gen Ctr, Greenwood, SC; 2) Charleston Clinical Office, Greenwood Gen Ctr, N. Charleston, SC.

A 3 yr old male with idiopathic autism, tall forehead and mildly hyperextensible elbows was referred for genetic testing. Molecular testing for Fragile X syndrome was normal. Subsequent high resolution microarray analysis using the Affymetrix Genome-Wide SNP 6.0 array identified a 47 kb duplication within the KIF1A (kinesin family member 1A) gene. Quantitative PCR data for confirmation of the duplication, family study, and transcript expression will be presented. To our knowledge this is the first report implicating KIF1A in idiopathic autism. These results also suggest a possible contribution of KIF1A to the autistic behaviors observed in some patients with monosomy 2q37.

2501/W

Replication study of association of the DPP6 gene with Primary Progressive Multiple Sclerosis (PPMS). *P. Brambilla[1], F. Esposito[1,2], G. Giacalone[1,2], M. Sorosina[1], M. Rodegher[2], R. Capra[3], A. Ghezzi[4], D. Galimberti[5], E. Scarpini[5], H. Westerlind[6], V. Martinelli[2], J. Hillert[6], G. Comi[1,2], F. Martinelli Boneschi[1,2].* 1) Institute of Experimental Neurology (INSPE), San Raffaele Scientific Institute, Milan, MI, Italy; 2) Department of Neurology, San Raffaele Scientific Institute, Milan, MI, Italy; 3) Department of Neurology, Spedali Civili, University of Brescia, Brescia, Italy; 4) Multiple Sclerosis Study Center, Hospital of Gallarate, Gallarate (VA), Italy; 5) Department of Neurological Sciences, “Dino Ferrari” Center, University of Milan, IRCCS Ospedale Maggiore Policlinico, Milan, Italy; 6) Department of Neurology, Karolinska University Hospital, Huddinge, Stockholm, Sweden.

Objectives: It is still unclear whether the clinical course and severity of MS is driven by genetic factors. A whole-genome association study (WGA) has been performed on 200 Italian patients affected with PPMS, which represents a rare clinical course affecting about 10-15% of MS patients and it is characterized by a progressive accumulation of irreversible neurological symptoms from the onset of the disease. Results of WGA study identified a novel locus, the dypeptidil-peptidase 6 (DPP6) gene. Methods: We carried out a case-control association study on 244 Italian PPMS cases and 225 age- and sex-matched controls (discovery sample) to better explore the locus by genotyping 19 single nucleotide polymorphisms (SNPs) on DPP6 gene using the TaqMan technology (Applied Biosystems®). 13 SNPs have been selected using a tagging approach to cover the region around two SNPs (rs6956703 and rs11767658), originally associated to PPMS in the WGA study. 5 SNPs have been selected as coding SNPs and as present in the 5’region, and one SNP because found to be associated with amyotrophic lateral sclerosis. The same genotyping technology was used to explore the association of the locus in 179 PPMS and 198 controls from Northern Europe (replication sample). PLINK and Haploview softwares were used for the data analyses. Results: 6/19 SNPs (rs2046748, rs4960555, rs11976061, rs10244476, rs1532090 and rs11767658) showed a significant difference in allelic frequencies ($p<0.05$ after Bonferroni correction) between cases and controls in the discovery sample. None of them have been confirmed to be associated in the replication sample. An haplotype association analysis has been performed in the discovery sample confirming the results on individual SNPs. Additional analyses on haplotype distribution in replication sample and approaches of meta-analyses are ongoing. Conclusions: 6/19 SNPs were found to be associated with Italian PPMS, confirming the original results of WGAS, but none of them was confirmed in the replication sample of North Europe origin, suggesting that the original association was a false positive signal or a phenomenon restricted to South European populations. Additional experiments are ongoing to make a final statement on the relationship between DPP6 and PPMS and MS in general.

2502/W

Next-generation pooled sequencing identifies new rare variants implicated in late-onset familial AD. *C. Cruchaga[1], G. Haller[1], S. Chakraverty[1], K. Mayo[1], FL. Vallania[2], RD. Mitra[2], A. Goate[1], NIA-LOAD/NCRAD Family Study Consortium.* 1) Department of Psychiatry, Washington University, St. Louis, MO, USA; 2) Department of Genetics, Washington University, St. Louis, MO, USA.

Familial Alzheimer disease (FAD) is characterized by an early onset (<60 years) and is inherited as an autosomal dominant trait with high penetrance. Mutations in three genes, APP, presenilin 1 (PSEN1), and presenilin 2 (PSEN2), have been reported to cause FAD. To date 173 mutations in PSEN1, 26 in APP and 14 in PSEN2 have been identified in FAD cases. In addition, mutations in progranulin (GRN), and microtubule-associated protein tau (MAPT) which cause frontotemporal dementia, can have a clinical presentation indistinguishable from AD. The frequency of mutation in these genes in late-onset AD (LOAD) families has been less well studied. We have used second generation sequencing technology to screening APP, PSEN1, PSEN2, MAPT and GRN for pathogenic mutations in 450 LOAD families with 4 or more affected members, recruited by the NIA Late Onset Alzheimer's Diseases Genetics Initiative. We have found 2 known pathogenic mutations in PSEN1 affecting 8 families, 2 mutations in PSEN2 affecting 13 families, 1 mutation in MAPT affecting 1 family and 3 mutations in GRN affecting 5 families. In total 6% of the families carried a known pathogenic mutation. We have also found 22 new non-synonymous and splice variants in an additional 35 families (7.8% of the total families). Preliminary results indicate that some of the new mutations, such as N660Y in PSEN1, A556T in MAPT and a splice variant (c.1181-1G>T) in GRN, segregate with disease status. On the other hand, we found that the non-synonymous change R62H in PSEN2, previously reported but with unclear pathogencity, does not segregate with disease status but modifies age at onset (P = 1.2 ×10-3, n=21) and shows epistasis with APOE ε4 (P-value for the epistasis (APOE*SNP) = 0.01. These results demonstrate that approximately 10% of LOAD families with 4 or more affected individuals carry pathogenic variation in known dementia causing genes. Furthermore, mutations in MAPT and GRN were just as common in these families as mutations in PSEN1, APP and PSEN2, highlighting the need to screen these genes in clinically diagnosed family cohorts in addition to the FAD genes. Lastly, most of these densely affected families do not carry mutations in the known genes strongly suggesting that novel LOAD genes with high penetrance remain to be identified.

2503/W

EPISTASIS IN ADRENERGIC GENES CONFERRING RISK FOR SUICIDAL BEHAVIOUR AND SCHIZOPHRENIA. *V. De Luca, R.P. Souza, J. Strauss, J.L. Kennedy.* Dept Neurogenetics, Univ Toronto, Toronto, ON, Canada.

A large body of evidence suggests that suicidal behaviour is associated with altered noradrenergic neurotransmission. There are nine adrenergic receptor genes (α1A, α1B, α1D, α2A, α2B, α2C, β1, β2, β3). Our hypothesis is that the gene encoding for the adrenergic receptor genes might contain genetic variants conferring increased risk for suicidal behaviour in schizophrenia. In order to test this hypothesis, we genotyped two adrenergic receptors α2B and α2C in a cohort of 600 schizophrenics in which 21% attempted suicide. No association between suicide attempt and the Ins/del polymorphisms in ADRA2C and ADRA2B genes was found, however these two genes have a significant homology in the polymorphic regions suggesting that epistasis can be present between these two genes. Haplotype analysis for the two genes, revealed no association between suicide attempts and haplotype distribution. As we tested for epistasis between ADRA2C and ADRA2B genes, we found no significant interaction in conferring risk for suicide attempts. These results suggest that α2 adrenergic receptors may not influence suicidal behaviour in patients with schizophrenia.

2504/W

Identification and functional characterization of three novel alleles for the serotonin transporter-linked polymorphic region. *E.A. Ehli[1,4], Y. Hu[2], T. Lengyel-Nelson[1], J.J. Hudziak[3], G.E. Davies[1,2,4].* 1) Avera Institute for Human Behavioral Genetics, Sioux Falls, SD; 2) South Dakota State University, College of Pharmacy, Brookings, SD; 3) University of Vermont, College of Medicine, Burlington, VT; 4) University of South Dakota, Sanford School of Medicine, Department of Psychiatry, Sioux Falls, SD.

A promoter polymorphism in the serotonin transporter gene (5-HTTLPR) has been reported to confer relative risk for phenotypes (depression/anxiety) and endophenotypes (amygdala reactivity). In this report, we identify and characterize three rare 5-HTTLPR alleles not previously described in the human literature. The three novel alleles were identified while genotyping 5-HTTLPR in an ADHD clinical population. Two of the novel alleles are longer than the common 16-repeat long (L) allele (17 and 18 repeats) and the third is significantly smaller than the 14-repeat short (S) allele (11 repeats). The sequence and genetic architecture of each novel allele is described in detail. We report a significant decrease in the expression between the XL17 (17r) allele and the LA (16r) allele. The XS11 (11r) allele showed similar expression with the S (14r) allele. A 1.8 fold increase in expression was observed with the LA(16r) allele compared to the LG (16r) allele which replicates results from earlier 5-HTTLPR expression experiments. In addition, transcription factor binding site (TFBS) analysis was performed using MatInspector which showed the presence of different putative TFBSs between the novel alleles and the common L (16r) and S (14r) alleles. Our findings argue for at least four functionally different SERT promoters.

2505/W

Mutations in the TSGA14 gene in families with autism spectrum disorders. *E. Korvatska[1], A. Estes[2], J. Munson[3], G. Dawson[4], L. Bekris[5], R. Kohen[3], C.-E. Yu[5], G. Schellenberg[6], W.H. Raskind[1,3].* 1) University of Washington, Medicine, Division of Medical Genetics, Seattle, WA; 2) University of Washington, Speech and Hearing Sciences, Seattle, WA; 3) University of Washington, Psychiatry and Behavioral Sciences, Seattle, WA; 4) Autism Speaks Foundation; University of North Carolina, Department of Psychiatry, NC; 5) University of Washington, Medicine, Division of Gerontology and Geriatric Medicine, Seattle, WA; 6) University of Pennsylvania, Department of Pathology and Laboratory Medicine, Philadelphia, PA.

Linkage to 7q has been robust genetic finding in familial autism. A previous scan of multiplex families with autism spectrum disorders found a linkage signal of genome-wide significance at D7S530 on 7q32. We searched a candidate imprinted region at this location for genetic variants in families with positive linkage scores. Using exon resequencing, we identified three rare potentially pathogenic variants in the TSGA14 gene, which encodes for a centrosomal protein. Two of the variants were missense mutations (c.664C>G; p.P206A and c.766T>G; p.C240G) that changed conserved residues in the same protein domain; the third variant (c.192+5G>A) altered splicing, which resulted in a protein with an internal deletion of 16 residues and a G33D substitution. These rare TSGA14 variants are enriched in the affected subjects (6/348 patients versus 2/670 controls, Fisher's exact two tailed p= 0.022). This is the first report of a possible link of a gene with a centrosomal function with familial autism.

2506/W

Acute to Chronic Pain Transitions (ACPT) After Peripheral Nerve Injury: Genomic Insights. *R.C Levitt[1,2,3], Q. Li[1], Y. Zhang[1], R.W Morris[1,2], E.S. Fu[1].* 1) Anesthesiology, Miller School of Medicine, University of Miami, Miami, FL; 2) Hussman Institute of Genomics, Miller School of Medicine, University of Miami, Miami, FL; 3) Miami Veterans Healthcare System, Miami, FL.

Background: Acute postoperative pain is followed by persistent pain in up to 50% of patients undergoing surgical procedures. It is now generally accepted that the development of chronic pain depends on nerve injury and is a complex heritable trait influenced by multiple genes, gene-environment, and gene-gene interactions. We hypothesize that susceptibility to ACPT after peripheral nerve injury, along with the resistance to therapy and rehabilitation, are causally associated with polymorphic genes, and that these genes and the pathways can be identified using functional genomics. In this study, using mechanical pain responses after peripheral nerve injury in mice, we have identified genetic loci associated with the ACPT over time. **Methods:** The chronic constriction injury of the sciatic nerve (CCI) model in mice was used to evaluate the ACPT (C57BL6/J, DBA2/J, A/J, NZW/LacJ, SJL/J, AKR/J, 129S1/SvLmJ, CAST/EiJ, FVB/NJ, BALB/cJ, CBA/J, C3H/HeJ, n= 5-8 males each). Withdrawal thresholds to mechanical pain (von Frey filaments to affected hindpaw) were measured on Days 1, 7, 14 and 21 after CCI. Haplotype association mapping (HAM) was conducted using snpBrowser (http://compgen.unc.edu/?page_id=17.) This method depends on the dataset of 7.8M single nucleotide polymorphisms (SNP) in these stains. HAM uses ANOVA to analyze association with the phenotype, using contiguous 3 SNP windows. In this exploratory study, a significant level of P<0.0001 (-logP=LOD 4.0) was used to select SNP associated with mechanical responses. **Results:** Baseline mechanical responses differed between inbred strains. Thresholds after CCI as compared with Baseline indicate A/J and NZW/LacJ had the least change in mechanical threshold after CCI; AKR/J, BALB/cJ, and DBA/2J showed the greatest ACPT. Heritability estimates averaged about 50%, with an estimated 3.5 genes underlying the ACPT. HAM results identify four separate chromosomal loci associated with differences in mechanical response on Day 1 and 7 after CCI. **Discussion:** Highly reproducible differences between strains for the ACPT demonstrate that this neurobehavioral phenotype is heritable. Preliminary HAM mapping of quantitative trait loci (QTL) contributing to the ACPT through Day 7 suggest the ACPT is likely to be oligogenic. Our continuing studies may help identify gene candidates underlying susceptibility to neuropathic pain and explain resistant to treatment and rehabilitation, and lead to new therapeutic approaches.

2507/W

Haplotype-Based Study of the Association of Alcohol Metabolizing Genes with Alcohol Dependence in Four Ethnically Diverse Populations. *J. LIU[1], Z. Zhou[1], C. Hodgkinson[1], Q. Yuan[1], P. Shen[1], C. Mulligan[2], A. Wang[1,2], R. Gray[2], A. Roy[3], M. Virkkunen[4,5], D. Goldman[1], M. Enoch[1].* 1) National Institute on Alcohol Abuse and Alcoholism, Rockville, MD; 2) Department of Anthropology, University of Florida,Gainesville, FL 32610; 3) Mental Health and Behavioral Science, Department of Veterans Affairs New Jersey Health Care System, East Orange; 4) Institute of Clinical Medicine, Department of Psychiatry, University of Helsinki, Finland (MV); 5) Kellokoski Hosapital, Kellokoski, Finland (MV).

Background: Ethanol is metabolized by two rate limiting reactions: alcohol dehydrogenases (ADH) convert ethanol to acetaldehyde, subsequently metabolized to acetate by aldehyde dehydrogenases (ALDH). Approximately 50% of East Asians have genetic variants that significantly impair this pathway and influence alcohol dependence (AD) vulnerability. We investigated whether variation in alcohol metabolism genes might alter the AD risk in four non-East Asian populations by performing systematic haplotype association analyses in order to maximize the chances of capturing functional variation. Methods: Haplotype-tagging SNPs were genotyped using the Illumina GoldenGate platform. Genotypes were available for 40 SNPs across the ADH genes cluster and 24 SNPs across the two ALDH genes in four diverse samples that included cases (lifetime AD) and controls (no Axis 1 disorders). The case, control sample sizes were: Finnish Caucasians: 232, 194; African Americans: 267, 422; Plains American Indians: 226, 110; Southwestern American (SW) Indians: 317, 72. Results: In all four populations, as well as HapMap populations, five haplotype blocks were identified across the ADH gene cluster: (1) ADH5-ADH4; (2) ADH6-ADH1A-ADH1B; (3) ADH1C; (4) intergenic; (5) ADH7. The ALDH1A1 gene was defined by four blocks and ALDH2 by one block. No haplotype or SNP association results were significant after correction for multiple comparisons; however several results, particularly for ALDH1A1 and ADH4, replicated earlier findings. There was an ALDH1A1 block 1 and 2 (extending from intron 5 to the 3' UTR) yin yang haplotype (haplotypes that have opposite allelic configuration) association with AD in the Finns driven by SNPs rs3764435 and rs2303317 respectively, and an ALDH1A1 block 3 (including the promoter region) yin yang haplotype association in SW Indians driven by 5 SNPs, all in allelic identity. The ADH4 SNP rs3762894 was associated with AD in Plains Indians. Conclusions: The systematic evaluation of alcohol metabolizing genes in four non-East Asian populations has shown only modest associations with AD, largely for ALDH1A1 and ADH4. A concentration of signals for AD with ALDH1A1 yin yang haplotypes in several populations warrants further study.

2508/W

Synaptic vesicles related genes and migraine predisposition. *J.L. Neto[1], C. Lemos[1], J. Pereira-Monteiro[1,2,3], J. Sequeiros[1,3], A. Sousa[1,3], I. Alonso[1].* 1) UnIGENe, Institute for Molecular and Cell Biology (IBMC), Porto, Portugal; 2) Neurology Service, Hospital Santo António (HSA), Porto, Portugal; 3) Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Porto, Portugal.

Migraine is a neurological disorder clinically characterized by recurrent headache episodes associated with other symptoms such as sensorial hypersensitivity or nausea. This disorder affects approximately 15% of the general population and has a significant genetic component, although the genes responsible for common migraine susceptibility have not yet been identified. There is evidence that the neurotransmitter systems play a pivotal role in common migraine pathophysiology. Recently, in Portuguese and Catalonian populations an association between *STX1A* and increased migraine predisposition was found. *STX1A* encodes Syntaxin 1A, a component of the SNARE complex, involved in the synaptic vesicles' membrane fusion and subsequent neurotransmitter release. A case-control association study is being conducted, using the Portuguese sample where association with *STX1A* was previously ascertained, composed of 188 unrelated migraineurs and 287 migraine-free controls, age and ethnically-matched with cases. 110 tagging SNPs are being studied with total coverage of common variation (MAF>10%) in genes encoding proteins related with the synaptic vesicles' machinery. The SNPs' frequencies in the European population were obtained resorting to the Hapmap , and Haploview v4.1 was used to plot LD patterns and select the tagging SNPs. The SNPs in study were divided in sets, and are being genotyped in five 22-plex multiplex SNaPshot assays. SNaPshot is a powerful, consistent, easy to use, high throughput technique specifically used to genotype SNPs. Results are being analyzed with Genemapper v4.0 software. Genotyping of all 475 individuals for 22 of the 110 SNPs is currently in its final stage; a preliminary association analysis is expected very soon. When all SNPs from a gene are genotyped, haplotypic association is also going to be analyzed in order to assess the possible existence of a susceptibility haplotype. Epistasis, often neglected in complex traits' studies, will be taken in consideration and the presence of genetic interactions, based on functional relationships, will be analyzed to assess their role in migraine predisposition. The acquirement of knowledge about association between genetic polymorphisms and migraine susceptibility is crucial because it can be useful as a starting point for more in depth studies, and the determination of genetic risk factors for migraine can bring dazzling advantages in migraine diagnosis and treatment.

2509/W

Dopamine Transporter Genotype Predicts Cognitive Flexibility in Healthy Control and Methamphetamine-Dependent Subjects. *E.L. Nurmi[1], D. Ghahremani[1], S.A. Wilson[1], J.T. McCracken[1, 3], E.D. London[1, 2, 3].* 1) Department of Psychiatry and Biobehavioral Sciences, University of California Los Angeles, Los Angeles, CA; 2) Department of Molecular and Medical Pharmacology, University of California Los Angeles, Los Angeles, CA; 3) The Brain Research Institute, University of California Los Angeles, Los Angeles, CA.

Cognitive flexibility is crucial for adaptive responding to environmental stimuli. Deficits in cognitive flexibility may be seen as behavioral rigidity, indexed by perseverative responding on "reversal learning" tasks that require response adjustment when contingencies of the task change. Studies in animal models and human subjects have shown that dopamine (DA) neurotransmission in the striatum and prefrontal cortex mediates reinforcement learning and the integration of negative feedback during reversal learning. In addition, serotonin in the orbitofrontal cortex may contribute to cognitive flexibility, possibly by reducing interference from salient stimuli. As an initial assessment of the contribution of genetic variation to cognitive flexibility, we employed a deterministic reversal learning task (Ghahremani et al, Cerebral Cortex, in press) and compared performance (reversal errors) among carriers and non-carriers of the dopamine transporter (DAT1 or SLC6A3) 9-repeat allele as well as the long promoter allele of the serotonin transporter (HTT or SLC6A4). Because of the purported association of cognitive control deficits with substance abuse disorders, we included a group of methamphetamine-dependent (MA) subjects (n=36) in addition to a healthy control (HC) group (n=35) and tested for an effect of MA abuse and interactions of MA abuse with genotype. The findings to date indicate that 9R carriers (n = 35) of DAT 1 exhibit poorer reversal learning than those without (n = 34) the 9R allele (mean errors ± SD in HC and MA groups: 1.66 ±1.16 and 1.86 ±.610 for 9R+; 1.28 ± 0.562 and 1.55± 0.971 for 9R-). The effect of DAT1 genotype was statistically significant (p=.042) whereas the main effect MA and the interaction of MA with genotype were not, nor were comparisons on the basis of HTT-LPR genotype (p=.123). Expression of DAT1 is modulated by the number of repeats in the 3'UTR VNTR, with the 9 repeat allele reducing transporter expression. These findings are consistent with a central role for intrasynaptic DA and its transporter modulated reuptake in cognitive flexibility in humans. Ongoing investigations will comprehensively screen candidates and correlate genotypes, in vivo dopamine binding, and behavior to further explore these mechanisms. Supported by NIH grants P20 DA022539 and R01 DA020726 (EDL), M01 RR00865 (UCLA GCRC) and endowments from the Thomas P. And Katherine K. Pike Chair in Addiction Studies and Marjorie M. Greene Family Trust (EDL).

2510/W

Vitamin D metabolic pathway genes and risk of multiple sclerosis in Canadians. *S. Orton[1], S.V. Ramagopalan[1], L. Handunnetthi[1], M. Chao[1], M.R. Lincoln[1], F. Moultrie[1], N. Perrier[1], A. Para[1], A.D. Sadovnick[2], G.C. Ebers[1], Canadian Collaborative Study Group.* 1) Wellcome Trust Centre for Human Genetics and Dept Clinical Neurology, University of Oxford, Oxford, United Kingdom; 2) Department of Medical Genetics and Faculty of Medicine, Division of Neurology, University of British Columbia; Vancouver, Canada (ADS).

Background. Multiple sclerosis (MS) is determined by interactions between genes and environment and the influence of vitamin D adequacy has been proposed. Previous studies have shown that serum 25-hydroxyvitaminD (25(OH)D) levels are genetically influenced. Polymorphisms in vitamin D pathway genes are candidates for association with MS susceptibility. Methods. MS patients (N=1364) and their unaffected first-degree relatives (N= 1661) were ascertained through the Canadian Collaborative study. Seventy-one SNPs, across four genes [vitamin D receptor (VDR), 1-alpha-hydroxylase (CYP27B1) enzyme, vitamin D binding protein (DBP), 24-hydroxylase (CYP24)], were genotyped and tested for association with MS susceptibility using TDT. Secondary analyses included stratification for HLADRB*15, differences in parental allele frequencies and parent of origin analysis. Results. We found no significant association of vitamin D pathway genes with MS susceptibility after correction for multiple comparisons. However, SNPs selected for previously positive associations with MS and/or 25(OH)D levels showed marginally significant results, including distorted transmission of the VDR Fok1 variant (rs2228570, p=0.03) in DRB15-negative patients and differences in maternal versus paternal allele frequency of DBP SNPs (rs4588, p=0.02 and rs7041, p=0.03). Interpretation. The findings do not directly connect vitamin D metabolism genes to MS susceptibility, despite a large sample size. We cannot exclude small effects and the results are consistent with the involvement of previously reported SNPs in VDR and DBP genes.

2511/W

Whole Genome aCGH Analysis for CMT and Related Peripheral Neuropathies. *D. Pehlivan[1], C. Gonzaga-Jauregui[1], F. Zhang[1], J.R. Lupski[1,2,3].* 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Pediatrics, Baylor College of Medicine, Houston, TX; 3) Texas Children's Hospital, Houston, TX.

Charcot-Marie-Tooth (CMT) and related peripheral neuropathies represent a heterogeneous group of genetic disorders of the peripheral nervous system with an estimated frequency of 1 in 2500 individuals. Over the last two decades, about 30 genes and 10 linkage regions have been identified in which mutations cause CMT. Still for ~10% of cases the causative genes and mechanism remain unknown. Copy number variations (CNVs) are an important cause of CMT cases as duplication of PMP22 gene region represents 70% of CMT1 cases. Therefore, we hypothesized that copy number changes involving other CMT genes might be found amongst CMT patients in which the molecular genetic etiology is unknown. We designed Agilent Custom 8x60K comparative genomic hybridization (CGH) array to detect copy number variations in all CMT genes, CMT linkage regions and neuropathy related genes. We selected probes spanning 47 genes including 10 kb of flanking regions and 13 CMT candidate regions to be interrogated. Thus far, 96 patients with CMT with unknown molecular cause were already analysed. In total, 39 CNVs were detected. 31 out of 39 were unlikely to be the cause of the disease as they overlap with variants found among the normal population. Eight CNVs varying in size were selected as potential disease-associated candidate rearrangements. Breakpoints of those candidate CNVs were further refined using long-range PCR and sequencing; Chromosomal aberrations were in linkage regions for 6 patients and in one of the CMT gene regions for 2 patients. For cases with rearrangements in linkage regions, function of the gene is not associated with CMT phenotype in 4 cases. For the remaining 2 cases, both were Alu mediated deletion and the function of the gene may be associated with the CMT phenotype. For aberrations in CMT gene regions, one patient had a duplication in the MPZ gene and the other patient had a deletion in PMP22 region. These results indicate that CNVs might be a relevant cause of CMT in patients with unknown molecular genetic etiology.

2512/W

Serotonin transporter gene variants (5HTTLPR and STin2) and susceptibility to depressive and anxiety symptoms in undergraduate students in Bogotá Colombia. *C.S. Perea[1], A. Castañeda[2], R. Sebastian[1], K. Peña[1], B.A. Otalora[1], H. Groot[1], Y. Gomez[2], M.C. Lattig[1].* 1) Laboratorio de Genetica Humana Departamento de Ciencias Basicas Universidad de los Andes Bogota - Colombia; 2) Departamento de Psicologia Universidad de los Andes Bogota - Colombia.

The functional 5HTTLPR and the STin2 variations located in the serotonin transporter gene (SERT, SLC6A4, 5HTT) have been associated with hyper reactivity to stress and therefore with vulnerability to develop higher than normal anxiety and depression symptoms in young adults in several populations. We thought to examine the influence of the short/long (s/l) 5HTTLPR variant and the (12/10/9) STin2 variants on susceptibility to develop depression and/or anxiety symptoms in undergraduate freshman and sophomore students from the Universidad de los Andes, Bogota - Colombia. Psychological tests (BDI-II, IDER, STAI) were used to evaluate depression and anxiety traits and genotype analysis of the s/l 5HTTLPR variants and the 12/10/9 STin2 variants were performed. A total of 348 students participated in the study (female 177, male 171). We classified the individuals in five different groups according to the psychological tests; controls for depressive traits (n=277, 82.9%), controls for anxiety traits (n=241, 76.7%), individuals with depressive traits (n=57,17.1%), individuals with anxiety traits (n=73, 23.3%), and individuals with both depressive and anxiety traits (n=38). Regarding the allele frequencies, the individuals were categorized in the following groups; individuals with the 5HTTLPR short allele (52.3%), 5HTTLPR long allele (47.7%), STin2.12 allele (67.3%), STin2.10 allele (32.2%), and STin2.9 allele (0.54%). Genotype frequencies are as follows: 5HTTLPR s/s (26.4%), 5HTTLPR s/l (51.9%), 5HTTLPR l/l (21.7%), STin2 12/12 (46.8%), STin2 12/10 (39.9%), STin2 10/10 (12.2%), and STin2 12/9 (1.1%). In order to determine the effect the 5HTTLPR and STin2 variants on the susceptibility to develop depressive or anxiety traits we performed non parametric multivariate analysis (SSPS 18). Our results indicate that neither 5HTTLPR and STin2 alleles, either acting alone or together, are strong determinant factors in the vulnerability to develop depressive and/or anxiety symptomatology in our population of study; suggesting that additional gene variants and environmental factors are influencing these behavioral traits as has been shown in several studies. Although we did not find any association, it is important to note that this is the first report of the STin2 genetic frequencies in the Colombian population.

2513/W

The Association Analysis of COMT, GABA, and SCL6A4 Genes With Autism Spectrum Disorders in the CHARGE Study. *L. Qi[1], Y.H. Lo[2], L. Schmidt[3], R.J. Schmidt[1,4], I.N. Pessah[4,5], I. Hertz-Picciot[1,4], F. Tassone[3,4].* 1) Dept. Public Health Sciences, Univ California, Davis, Davis, CA; 2) Dept. Biostatistics, Univ. Michigan, Ann Arbor, MI; 3) Dept. Biochemistry and Molecular Medicine, Univ California, Davis, Davis, CA; 4) MIND Institute, Univ California Davis Medical Center, Sacramento, CA; 5) Dept. Molecular Biosciences, School of Veterinary Medicine, Univ California, Davis, Davis, CA.

We have studied three genes, catechol-O-methyl transferase (COMT), gamma-aminobutyric acid (GABA) A receptor, and SCL6A4 (serotonin transporter) in children participating in the CHARGE (Childhood Autism Risks from Genetics and the Environment) Study. The CHARGE study is a large, ongoing case-control investigation of Northern California children aged 2-5 years with clinically confirmed diagnoses of: autism (AU), autism spectrum disorders (ASD), developmental delays, or typical development (TD). In this study, we genotyped 15, 16, and 7 single nucleotide polymorphisms (SNPs) located within the COMT, GABA, and SCL6A4 genes, respectively, in 104 children with AU and 130 TD controls. Significant genetic associations were observed for 5 COMT, 3 GABA, and 1 SCL6A4 SNPs. Haplotype-specific association analysis revealed that the SNP3/SNP4/SNP5/SNP6 and SNP10/SNP11/SNP12 haplotypes within the COMT gene, and the SNP3/SNP4/SNP5/SNP6 and SNP6/SNP7/SNP8/SNP9 haplotypes within the GABA gene were significantly associated with autism (p-values were 0.021 and 0.048 for the COMT haplotypes, and 0.004 and 0.014 for the GABA haplotypes, respectively). Our study suggests the possible involvement of the COMT and GABA genes in the susceptibility to autism. Future replications are warranted before definitive conclusions can be drawn.

2514/W

Fine-scale mapping of a schizophrenia susceptibility locus on chromosome 13q32-34. *L. Rodriguez-Murillo[1], B. Xu[1], J.L. Roos[4], G.R. Abecasis[5], J.A. Gogos[2, 3], M. Karayiorgou[1].* 1) Department of Psychiatry, Columbia Univ, New York, NY; 2) Department of Physiology and Cellular Biophysics, Columbia Univ, New York, NY; 3) Department of Neuroscience, Columbia Univ, New York, NY; 4) Department of Psychiatry, University of Pretoria, Pretoria, RSA; 5) Department of Biostatistics, Michigan University, Ann Arbor, MI.

Schizophrenia is a chronic psychiatric disease characterized by psychotic symptoms as delusions and hallucinations, apathy, altered emotional reactivity, and cognitive deficits as impairments in executive function, attention and working memory. Heritability is estimated to be up to 80% for first degree relatives. Schizophrenia, like other common diseases, is multifactorial in nature with complex genetic etiologies. It is likely that both common and rare alleles contribute to the risk of schizophrenia, although the relative impact of each remains unknown. Previously, we performed a genome-wide linkage scan in South Africa Afrikaners (a founder population of European descent) with microsatellites spaced 2 cM on average across the genome and obtained strong evidence for linkage on chromosome 13q32-34. We could not find any rare or common CNV that accounts for the linkage signal within this region. The goal of the present study was to look for common variants associated with schizophrenia located within the linkage region. To this end, we genotyped 415 Afikaner families with at least one member with schizophrenia for ~700 SNPs localized within the 13q32-34 linkage region, and performed a family-based association analysis. A few SNPs showed nominally significant association and were followed up in a US family sample. Replication and meta-analysis identified one SNP within 13q33.3 as associated with schizophrenia. However, further analyses will be necessary to understand the contribution of this genetic variant to schizophrenia.

2515/W

Amyloid pathway-based candidate gene analysis of [^{11}C]PiB-PET in the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort. *S. Swaminathan[1,2], L. Shen[2,3], S.L. Risacher[2], S. Kim[2,3], K. Nho[2,3], J.D. West[2], T. Foroud[1], K.K. Yoder[2], S.G. Potkin[4], M.J. Huentelman[5], D.W. Craig[5], W.J. Jagust[6,7], C.A. Mathis[8], M.W. Weiner[9,10], A.J. Saykin[1,2], Alzheimer's Disease Neuroimaging Initiative (ADNI).* 1) Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN; 2) Center for Neuroimaging, Department of Radiology and Imaging Sciences, Indiana University School of Medicine, IN; 3) Center for Computational Biology and Bioinformatics, Indiana University School of Medicine, Indianapolis, IN; 4) Department of Psychiatry and Human Behavior, University of California, Irvine, CA; 5) Neurogenomics Division, The Translational Genomics Research Institute, Phoenix, AZ; 6) Helen Wills Neuroscience Institute, University of California Berkeley, Berkeley, CA; 7) Lawrence Berkeley National Laboratory, Berkeley, CA; 8) Department of Radiology, University of Pittsburgh, Pittsburgh, PA; 9) Departments of Radiology, Medicine and Psychiatry, University of California, San Francisco, CA; 10) Department of Veterans Affairs Medical Center, San Francisco, CA.

Amyloid neuroimaging provides *in vivo* data on plaque formation in those with or at risk for Alzheimer's disease (AD). This method provides an opportunity to examine the influence of genetic variation within the amyloid pathway in plaque deposition during early stages of AD. We studied 103 participants from the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort with available [^{11}C]PiB, which binds with high affinity to Aβ, and genotype data (Illumina Human610-Quad BeadChip). Standardized uptake value ratio (SUVR) measures of PiB binding in anterior cingulate, frontal cortex, parietal cortex and precuneus, have been shown to be good indicators of amyloid deposition and we used the average baseline SUVR across the four regions as a quantitative phenotype. Candidate genes identified from a search for "amyloid" on Gene Ontology and present in the AlzGene database (a collection of previously studied AD genes), were included in the analysis. SNPs in the untranslated regions, exons and introns of these genes with a minor allele frequency ≥ 0.20 were chosen. 275 SNPs in 16 genes remained after filtering and were subjected to a set-based test in PLINK software with age as a covariate, and without and with *APOE* ε4 status as a covariate. The set method takes into account linkage disequilibrium between SNPs and calculates an empirical p-value for each gene correcting for number of SNPs within each gene tested. Two candidate risk genes were identified: the known risk factor SNP (rs429358) in the *APOE* (Apolipoprotein E) gene and a SNP (rs7551288) in *DHCR24* (24-dehydrocholesterol reductase or seladin-1) gene (empirical p = 0.022 and 0.052 without and with *APOE* ε4 status as a covariate, respectively). *DHCR24* is thought to confer resistance against Aβ- and oxidative stress-induced apoptosis, and effectively inhibits the activation of caspase-3, a key mediator of the apoptotic process. The identified SNP appears to have an additive effect: higher number of minor alleles correlate with lower average SUVR values, supporting the hypothesis that this gene may play a neuroprotective role against Aβ deposition. Our initial results suggest that further investigation of the role of *DHCR24* in AD is warranted.

2516/W

Is there a common evolutionary background for music and language faculties? - Analysis of dyslexia candidate genes in Finnish families tested for musical aptitude. *L. Ukkola[1], K. Salo[1], P. Onkamo[2], P. Raijas[3], K. Karma[4], I. Järvelä[1,5].* 1) Dep Med Gen, Univ Helsinki, Helsinki, Finland; 2) Dep Biological and Environmental Sciences, University of Helsinki, Helsinki, Finland; 3) Sibelius Academy, DocMus Department, Helsinki, Finland; 4) Sibelius Academy, Department of Music Education, Helsinki, Finland; 5) Laboratory of Molecular Genetics, Helsinki University Central Hospital, Helsinki, Finland.

Musical aptitude is a cognitive function of the human brain. Common evolutionary background of music and language recognition has been long speculated, based for example on partially overlapping brain regions in PET studies. Moreover, low scores in a test for musical aptitude, Karma Music test (KMT), have been shown to reliably predict dyslexia. Here phonological awareness, a basic natural property not dependent on learning and defective in dyslexia could be understood as poor auditory structuring ability measured by KMT. Previously, we have carried out a genome-wide linkage analysis for musical aptitude in multigenerational families tested for musical aptitude using auditory structuring ability test (KMT) and Carl Seashores test for pitch (SP) and for time (ST). Evidence for linkage was obtained for several loci, including DYX6, a candidate locus for dyslexia, on chr. 18q.

In order to understand the neurobiological basis of music in human evolution and communication we analyzed previously reported risk alleles of a set of dyslexia susceptibility genes: *KIAA0319, DCDC2, DYX1C1, and C2ORF3*. Several SNPs were chosen in these loci and genotyped in multigenerational families (N=395). Family-based association analyses (FBAT and QTDT) were used for analyzing the data. Preliminary results point to SNPs in *KIAA0319* and *DCDC2*, which are located next to each other on chr. 6 with the Seashore Pitch test, with p-value 0.02. Currently, more families (total N=600) are being genotyped, thus, refined results will be presented in the meeting.

2517/W

Autism and/or Intellectual Disability in Children with 16p13.11 Microduplication: report on 4 New Cases. *A. Battaglia[1,2], A. Novelli[3], L. Bernardini[3], R. Tancredi[1], R. Igliozzi[1], E. Santocchi[1], A. Capalbo[3], M.C. Digilio[4].* 1) Dev Neurosciences, Stella Maris nst/Univ Pisa, Pisa, Italy; 2) Division of Medical Genetics, Dept. Pediatrics, University of Utah, Salt Lake City, UT, USA;; 3) Ospedale CSS-IRCCS, San Giovanni Rotondo e Istituto CSS Mendel, Roma, Italy; 4) Ospedale Bambino Gesù-IRCCS, Roma, Italy.

Autism and intellectual disability (ID) are often associated, suggesting that these conditions are etiologically related. Recently, array-based comparative genomic hybridization (array-CGH) has identified submicroscopic deletions and duplications as a common cause of ID, and as risk factors for autism spectrum disorders (ASDs). Here we describe 4 patients (3 females, 1 male) from four unrelated families, with autism or ID in whom array-CGH disclosed a dup 16p13.11. The smallest overlapping duplicated region started to14,956,252 and ended at 16,157,108, including 11 RefSeq genes (PDXDC1, NTAN1, RRN3, MPV17L, C16orf45, KIAA0430, NDE1, MYH11, C16orf63, ABCC1, ABCC6). The duplication was confirmed in all cases by RT-PCR on MYH11 gene. The same analysis extended to parents demonstrated in 3 cases the maternal and in 1 case the paternal segregation of the CNV. One female patient had autism (through ADOS, ADI) and the other 3 had ID. Dup16p13.11 was associated with FMR1 premutation in the autistic female; and with an expansion of the promoter region of FMR2 and with a del 12p11.23 in two of the other three ID patients. Minor dysmorphic features were present in all. Our report suggests that dup16p13.11 represent recurrent genomic imbalances which predispose to autism and/or ID.

2518/W

Autism, Mental Retardation, and Cognitive-Behavioral Features of Children with Subtelomeric Deletions. *G. Fisch[1], P.D. Grossfeld[2], R. Falk[3], J. Youngblom[4], R. Simensen[5], A. Battaglia[6].* 1) NYU Colleges of Dentistry & Nursing, New York, NY; 2) School of Medicine, University of California, San Diego; 3) Cedars Sinai Hospital, Los Angeles, CA; 4) Dept of Biology, California State University, Turlock, CA; 5) Greenwood Genetics Center, Greenwood, SC; 6) Dept. of Neurology, University of Pisa, Pisa, Italy.

Until recently, cognitive-behavioral features of children with subtelomeric deletions have not been systematically evaluated. The aim of our study was to provide an update of our assessment cognitive-behavioral features of children with 4 different subtelomeric deletions, using a comprehensive neuropsychological assessment battery. Our assessment battery consisted of 5 standardized measures of cognitive ability [Stanford-Binet 4th Ed: SBFE], adaptive behavior [Vineland Adaptive Behavior Scale: VABS], emotionality and temperament [Child Behavior Checklist: CBCL], attentiveness/hyperactivity [Conners Parent Rating Scale: CPRS], and autism [Child Autism Rating Scale: CARS]. We examined 43 children, ages 4-20 years, with deletion 2q37 [n=8], invdupdel(8p23) [n=7], deletion 11q25 [Jacobsen syndrome: JBS; n=11], or 4p16 [Wolf-Hirschhorn syndrome: WHS; n=19], from 9 sites in the US, the UK and Europe. We found 13/43 (30%) of our sample with CARS scores ≥ 30 who could be diagnosed as autistic. Attention deficits and hyperactivity [ADHD] were also noted in 25/43 [58%] of the sample of children assessed. Cognitive ability ranged from low-normal to severe ID. Children with JBS had significantly higher cognitive abilities, while those with WHS were significantly lower. Adaptive behavior was also significantly higher among children with JBS. Cognitive ability and adaptive behavior profiles were statistically significantly different among the groups. We conclude that cognitive-behavioral profiles and the risk of developing autism differ among children with dissimilar subtelomeric deletions. Moreover, differences in cognitive-behavioral profiles support a model of different gene-brain-behavior pathways producing ID.

2519/W

The association of brain-derived neurotrophic factor haploinsufficiency and autism symptoms in WAGR syndrome. *S.R. Fuhr[1], C. Golden Williams[2], A. Thurm[2], S.M. Brady[1], M.D. Lee[1], Y.C. Zheng[1], S.E. Swedo[2], J.C. Han[1].* 1) NICHD, National Institutes of Health, Bethesda, MD; 2) NIMH, National Institutes of Health, Bethesda, MD.

Purpose: WAGR syndrome (Wilms tumor, aniridia, genitourinary anomalies, mental retardation) is a rare genetic disorder caused by heterozygous chromosome 11p13 deletions of variable size. Due to its role in brain development and function, we hypothesized that haploinsufficiency of brain-derived neurotrophic factor (BDNF), a gene that is often deleted in patients with WAGR syndrome, may play a role for autism spectrum disorder (ASD) development in this population. **Methods:** Twenty patients with WAGR syndrome (10F/10M, age 6-37y) were recruited to the NIH through the International WAGR Syndrome Association. ASD symptoms were assessed using Autism Diagnostic Interview-Revised (ADI-R, parent interview, N=20), Autism Diagnostic Observation Schedule (ADOS, direct behavioral observation, N=15), and clinical judgment of doctoral level psychologists. Deletion boundaries were determined by array comparative genomic hybridization. Prevalence of ASD was compared by Fisher's exact test. **Results:** The deletion sizes ranged from 2.9 to 15.1 Mb. Thirteen subjects had heterozygous *BDNF* deletion (+/-) while 7 had intact *BDNF* (+/+). All but one subject met criteria for mental retardation, ranging from mild to severe. Visual impairment ranged from legal blindness to complete lack of vision. Using ADI-R, 7 out of 13 *BDNF*+/- subjects met "ever" ASD criteria compared to none out of 7 *BDNF*+/+ subjects [54% (95% CI: 25-80%) vs. 0% (95% CI: 0-41%), p=0.04]. However, using ADI current codes, ADOS, and clinical judgment, only 1 out of 9 *BDNF*+/- subjects met ASD criteria (with 1 other also meeting criteria only on the ADI-R) compared to none out of 6 *BDNF*+/+ subjects [11% (95% CI: 0-48%) vs. 0% (95% CI: 0-45%), p=1.00). **Conclusion:** Because WAGR syndrome includes visual impairment, mental retardation, and serious medical illness in early childhood, current functioning may be more reliable than ADI-R "ever" codes in assessing autism symptoms in this population. Further exploration of cognitive and/or visual impairments is needed to validate the observation of an association between *BDNF* haploinsufficiency and a history of autism symptoms. (This research was supported by the Intramural Research Program of the NICHD and NIMH, NIH.).

2520/W

High frequency of abnormalities in genetic copy number analysis in 245 cases of combined schizophrenia and epilepsy. *L. Stewart, A. Hall, S. Kang, C. Shaw, A. Beaudet.* Baylor College of Medicine, Houston, TX.

Recent studies have identified copy number variants (CNVs) in many neuropsychiatric disorders, including intellectual disability, autism, epilepsy, schizophrenia, and bipolar disorder. Remarkably, certain deletions can be associated with any combination of these phenotypes. We hypothesized that individuals with more than one of these phenotypes might be more likely to have a CNV as a cause of their disabilities. We selected 245 individuals with both schizophrenia and epilepsy from the NIMH Human Genetic Initiative collection. Specifically excluded from the study cases were individuals with seizures from: antipsychotic drugs, electrolyte imbalances, infection/fever, and trauma. DNAs were analyzed using array comparative genomic hybridization (CGH). Clinical arrays, used once previously in a CLIA laboratory, were stripped and re-used to screen these samples. The array used is a 180K Agilent oligonucleotide array designed to detect common clinical abnormalities, including exon-by-exon coverage for over 1700 genes known to be relevant or potentially relevant to various phenotypic disabilities. All potentially clinically significant abnormalities were confirmed by an independent array or other method. We identified the following changes: 3 del 22q11.2, 3 del 1q21.1, 2 dup CHRNA7, 2 dup 15q11-q13, 1 del NRXN1, 1 del15q13.3, 1 del 9p24.3, 1 dup7q11.23 and 1 del 16p13. A similar number of NIMH control DNA samples were analyzed using the same array platform. We eliminated controls who reported by questionnaire a history suggestive of major depressive episode, generalized anxiety disorder, alcohol and/or drug dependence, or obsessive compulsive disorder. Only one abnormality potentially known to be associated with phenotypic effects (dup 22q11.2) was detected in the control population. We identified 13 abnormalities of highly likely clinical significance not counting the two dup CHRNA7 in the 245 cases with combined schizophrenia and epilepsy for a frequency of 5.3 percent. Many variants of uncertain significance were observed in cases and controls, and further validation and characterization are in progress. These data support the hypothesis that the frequency of identifiable genetic abnormalities in cases with combined schizophrenia and epilepsy is greater than for either phenotype alone. These samples should also be enriched for other genetic abnormalities, including point mutations that would lead to the combined phenotype of schizophrenia and epilepsy.

2521/W

Exon-Focused CGH Array for X-Linked Mental Retardation. *P. Bayrak-Toydemir, M. Priest, T. Lewis.* ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT.

BACKGROUND: Mental retardation (MR) is estimated to effect 1-3% of the population. Given the excess of males observed in large cohorts of patients, defects of genes on the X chromosome are believed to account for greater than 25% of moderate and severe forms and almost 50% of mild forms of mental retardation. There are more than 90 genes on the X chromosome which have been related to MR. However, in other genes copy number changes of variable sizes ranging from single exon of a gene to deletions/duplications of many genes might also cause X-linked MR (XLMR). METHODS: To explore the role of X-chromosome gene deletions and duplications XLMR, a custom 720K NimbleGen CGH array was designed. 1737 transcripts were identified in the USCS Genome Browser and compiled into 954 composite transcripts to account for genes with multiple transcripts. The array contains probes spanning the length of the chromosome, covering the 7722 exons of the 954 transcripts with tiled 60mer probes at 15 bp spacing. Exons represent 2.6Mb, or just 1.7%, of the X chromosome yet 24% of the probes are concentrated in these regions. Probes are placed at a mean interval of 140 bp in introns and 1kb 5' and 3' of each transcript, accounting for 49% of the probes. Probe density in intergenic regions is at a mean spacing of 525 bp. Genomic DNA of nine individuals with known variations were hybridized against sex-matched controls and results compared against the known variations. RESULTS: Nine patient samples harboring deletions or duplications ranging in size from 1.6 Mb to 1186 bp were analyzed. Three samples with contiguous multiple gene deletions were identified using a 10X averaging window in the SignalMap2.5 software. Deletions and duplications in the four exon and two single gene samples were identified using a 1X-averaging window. Results were concordant with previous studies. CONCLUSIONS: A high-density custom X-chromosome array has been designed for detecting copy number changes ranging from large genomic regions to variations within a single gene. Currently 215 (22%) of the genes on the X chromosome are known to be involved in XLMR, however roughly 40% of the protein coding genes on the X chromosome are expressed in the brain; in principal XLMR could result from copy number changes in any of these genes. Our custom designed exon-focused X chromosome array includes coverage of all known XLMR genes and potential XLMR genes with high sensitivity and specificity.

2522/W

High Resolution aCGH Reveals Rare Structural Variation in Autism Candidates. *D.J. Hedges[1], P.L. Whitehead[1], B. Butler[1], H.N. Cukier[1], K. Hamilton[1], J.M. Jaworski[1], L. Nathanson[1], R. Martinez[1], S.L. Hauser[2], J. Oksenberg[3], J. Lee[1], M.L. Cuccaro[1], J.R. Gilbert[1], M.A. Pericak-Vance[1].* 1) Dr. John P. Hussman Institute fro Human Genomics, University of Miami Miller School of Medicine, Miami, FL; 2) Department of Neurology, School of Medicine, University of California, San Francisco, CA; 3) Institute for Human Genetics, School of Medicine, University of California, San Francisco, CA.

In order to detect copy number variation (CNV) events in autistic and control individuals at sub-10kb resolutions typically not achievable with SNP-based arrays, we employed a candidate gene strategy using array-based comparative genomic hybridization (aCGH). Candidate loci were chosen based on their relationship to biological pathways implicated in autism. We placed particular emphasis on genes involved in Gamma-aminobutyric acid (GABA) processing and signalling. GABA is the major inhibitory neurotransmitter in the brain, acting primarily via the GABA receptors (GABR). Multiple lines of evidence, including alterations in levels of GABA and GABA receptors in autistic patients, indicate that the GABAergic system may be involved in autism etiology. Thus, we examined GABA-related pathways, along with genes from additional biological pathways implicated in autism, for structural variants. All autism cases were idiopathic with a minimum of comorbid conditions and dysmorphic features. Our initial high resolution (custom targeted Agilent 244k) screen of 66 loci (43 GABA-related and 23 additional autism candidate loci) within 150 idiopathic autism cases and 150 age and race-matched controls identified several hundred potential structural variations. A subset of these variants were exclusive to (or over-represented within) autism cases compared to control individuals. CNV loci were prioritized for confirmation and additional analyses based on frequency distribution among cases and controls, as well as predicted impact on gene structure, expression, and/or regulation. Validation assays were performed on 29 priority loci using TaqMan CNV assays (Applied Biosystems). The specific individuals harboring the variants were tested, along with 170 case and 170 matched control individuals that were not part of the initial aCGH screen. Insertions and deletion events were confirmed to be present at 14 loci, including GABBR2, JAKMIP1, SLC38A3, Neuroligin 4Y, Neuroligin 2, and ABAT. Four events below 10kb in size were detected via aCGH and independently confirmed. These results provide further evidence that biologically important structural variation exists below the effective resolution typically provided by standard SNP-based arrays. Our data highlight that the continued heavy reliance on GWAS array data for CNV discovery will fail to provide a complete picture of biologically relevant structural variation.

2523/W

MAGI1 copy-number variation in bipolar affective disorder and schizophrenia. *R. Karlsson[1,2], L. Graae[1], M. Lekman[1,3], D. Wang[4], R. Favis[4], T. Axelsson[5], D. Galter[1], A. Carmine Belin[1], S. Paddock[1].* 1) Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden; 2) Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden; 3) Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden; 4) Johnson & Johnson Pharmaceutical Research and Development LLC, Raritan, NJ; 5) Department of Medical Sciences, Molecular Medicine, Uppsala University, Uppsala, Sweden.

Bipolar affective disorder (BPAD) and schizophrenia (SZ) are common and devastating diseases of the human brain that have consistently been shown to be highly heritable. After many years of ambiguous genetic research results, recent large-scale genetic studies have been very encouraging and revealed common variants that contribute to disease risk, as well as several rare disease-associated copy-number variants (CNVs). It is, however, remarkable that to date no variation has been identified that shows segregation with disease in larger pedigrees, which is to be expected for rare yet highly penetrant disease loci.

We identified a ca. 200kb deletion in the first intron of the MAGI1 gene that showed complete segregation with disease in a BPAD pedigree, with six of six affected individuals carrying the mutation. Additional support for involvement of the MAGI1 gene in psychiatric disease was derived from further analysis of 47 BPAD pedigrees (N=270), sporadic cases from clinical trials (1,123 BPAD, 3,192 SZ and 377 schizoaffective [SA]) and publicly available datasets (2,698 BPAD, 4,095 SZ). Seven additional CNVs over 100kb were identified in MAGI1 in these materials, while just two CNVs over 100kb were identified in the same gene in 14,907 control samples (association p-value = 0.05; Fisher's exact test, two-sided alternative hypothesis).

The MAGI1 gene encodes a post-synaptic scaffolding protein that interacts with a multitude of molecules that have been identified in the molecular pathology of BPAD and SZ, such as neuroligin, beta-catenin, the neuregulin receptor ERBB4 and glutamate receptors. In addition, we found further supporting evidence for a role of MAGI1's close relative MAGI2 in psychiatric disease. Our findings together with previous studies suggest involvement of MAGI1 in BPAD and SZ etiology, support the notion of a genetic overlap between the disorders, and may contribute to our understanding of underlying molecular mechanisms.

2524/W

***CHRNA7* duplication - Could this common copy number variant represent a risk factor for neurodevelopmental and neuropsychiatric phenotypes?** *C.P. Schaaf, S. Mahadevan, R. Person, P. Szafranski, P. Stankiewicz, A.L. Beaudet.* Molecular & Human Genetics, Baylor College of Medicine, Houston, TX.

Copy number variants of 15q13.3 have been associated with a wide variety of neurodevelopmental and neuropsychiatric phenotypes, including intellectual disability, autism spectrum disorder, schizophrenia and epilepsy. *CHRNA7*, encoding for the alpha7 subunit of the neuronal nicotinic acetylcholine receptor, which is highly expressed in brain, has been proposed as the causative gene for the majority of neurodevelopmental phenotypes in the 15q13.3 microdeletion and microduplication syndromes. Four main classes of copy number variants at the 15q13.3 locus have been reported: Large (1.5-1.6 Mb) deletions encompassing six genes, including *CHRNA7*; the reciprocal large (1.5-1.6 Mb) duplications; and the smaller (430-680 kb) *CHRNA7* deletions and duplications. While the respective deletion syndromes have variable expressivity of phenotypes, penetrance of all reported cases to date is >80%. The respective microduplications and their associated potential for increased dosage of alpha7 subunit of nicotinic acetylcholine receptors are of uncertain clinical significance at present. We ascertained individuals with *CHRNA7* duplications that were referred to the Baylor Genetics Laboratories for high-resolution array-CGH. We present detailed clinical data of 13 probands (ages 2-12 years) and extensive pedigree analyses thereof. All probands display developmental delay/intellectual disability of variable severity. Other common clinical findings include muscular hypotonia (8/13) and neuropsychiatric phenotypes such as autism spectrum disorder (6/13), anxiety disorder, disruptive behavior disorder and bipolar disorder (each 1/13 among probands). The study of 735 parents of patients with autism spectrum disorder from the Simons Simplex Collection reveals a frequency of 7/735 (1/105) for the *CHRNA7* duplication, which is higher than the frequency reported for the general population (1/170). Transmission Disequilibrium Testing reveals an increased inheritance of the duplicated allele to affected children (5 vs. 3) as compared to unaffected children (2 vs. 5).Preliminary findings might suggest some pathological significance of *CHRNA7* duplications with modest penetrance, but until larger numbers of samples are analyzed, the *CHRNA7* duplication remains of unknown significance.

2525/W

Analysis of Neuropsychiatric Copy Number Variants in 22q11.2 Deletion Syndrome. *M.B. Sheridan[1], P. Goldenberg[1], M. Xie[2], X. Gai[2], J.C. Perin[2], M. Bowser[1], A. Hacker[1], D. McDonald-McGinn[1], E.H. Zackai[1,3], R. Gur[4], T.H. Shaikh[5], B. Morrow[6], B.S. Emanuel[1,3].* 1) Division of Human Genetics; 2) Bioinformatics Core, Children's Hospital of Philadelphia, Philadelphia, PA; 3) Department of Pediatrics; 4) Department of Psychiatry, University of Pennsylvania School of Medicine, Philadelphia, PA; 5) Department of Pediatrics, University of Colorado, Denver, CO; 6) Department of Genetics, Albert Einstein College of Medicine, Bronx, NY.

22q11.2 deletion syndrome (22q11DS) is the most common microdeletion syndrome, occurring in ~1:4,000 births. It is associated with a spectrum of neuropsychiatric disorders, including schizophrenia (SCZ) which is present in 23-30% of adults with 22q11DS. This is significantly increased over the 1% population prevalence of SCZ. We hypothesize that 22q11DS patients who develop SCZ have copy number variants (CNVs), other than the 22q11 deletion, that affect genes critical to neurobiological and neurodevelopmental pathways. The Affymetrix SNP 6.0 platform was used to identify CNVs in 208 22q11DS patients. CNV analysis was performed with Partek Genomics Suite, as well as in-house software. We focused primarily on larger CNVs that were detected by both approaches and represented by more than 50 probes, as they are less likely to be false positives. Validations are ongoing using FISH and/or real-time quantitative PCR. Not surprisingly, the 22q11 deletion is the largest CNV detected in each patient with an average length of 2,617,625 bp. 1,784 additional CNVs were detected in the 208 patients studied. The majority of these (87.3%) were either common CNVs or did not contain genes. Nevertheless, several of the large CNVs were rare, contained genes that have been previously associated with neuropsychiatric phenotypes, and were not detected in healthy controls in available CNV databases. These included an 870kb duplication involving *GRM3* in a 9 year-old patient who has deficits in emotion recognition, typical of SCZ patients. *GRM3* encodes a metabotropic glutamate receptor and is a candidate SCZ gene that may have epistatic interactions with catechol-O-methyltransferase (*COMT*), a gene in the 22q11 deleted region. Another 22q11DS patient was found to carry a ~1.5Mb deletion at 16p13.11. Deletions and reciprocal duplications in this region have been associated with SCZ and mental retardation. The behavioral phenotype of this individual is currently not known, as evaluation of this group of patients through brain-behavior studies is ongoing. Thus, although the entire cohort of patients has not been extensively screened for neuropsychiatric disorders, CNVs were detected in some that affect genes or chromosomal regions that have been implicated in neuropsychiatric diseases. These preliminary observations support our hypothesis that there may be other CNVs or alterations within the genomes of 22q11DS patients that predispose to neuropsychiatric phenotypes.

2526/W

Analysis of phenotype and microarray detected genome dosage changes in autism. *M. Smith[1], P.L. Flodman[1], K. Osann[1], M.C. Moore[1], J.J. Gargus[1], M. Simon[2], D. Wallace[2].* 1) Dept Pediatrics, Univ California, Irvine, Irvine, CA; 2) MAMMAG, Dept. of Biological Chemistry, Univ California, Irvine, Irvine, CA.

In our study of autistic probands recruited locally we carried out autism diagnosis using ADOS and ADI instruments. Probands underwent dysmorphology and neurological examinations and cognitive and language evaluations. We undertook microarray analyses using AFFYMETRIX 6.0 SNP arrays in 51 probands. We identified 3 groups: probands with CNVs >1mb in size (10 individuals), those with CNVs >100kb but less than 1mb (12 individuals); 29 with no copy number changes found using defined search parameters (20 markers, 100kb segments no more than 60 percent overlap with known variants). We determined that the gender ratio of subjects in each of these three groups was significantly different (Chi square p=0.03). In the group with copy number variants greater than 1mb the male:female ratio was 1:1.In the group with copy number variants greater than 100k but less than 1mb, the male:female ratio was 3:1. In the group with no copy number variants the male:female ratio was 8.7:1. Among those with no CNVs or CNVs >100kb, but less than 1mb lower IQ was associated with more severe autism as evidenced by higher CS scores (r=-0.34, p=0.03). In the group with large CNV >1Mb, IQ scores were significantly lower and were not correlated with CS scores, suggesting this group constitutes a separate category. In 2 siblings with autism we identified an unbalanced chromosome translocation that led to duplication of 2pter-p25.2 and deletion of CACNA1C and other genes in 12pter-12p13. The siblings differed in the degree of severity of impairment and SNP genotyping combined with copy number analysis (Birdsuite algorithm) revealed that they had each inherited different homologs of chromosomes 12 and 2 from the non-translocation carrying parent. We identified a number of large rare chromosome abnormalities in regions of the genome reported to be associated with autism, other neurobehavioral abnormalities and epilepsy by other investigators, these included CNVs greater than 1 Mb in size in 15q11-q13.3, 15q13-q14 and 15q24. In addition we found rare copy number variants around or >100kb that impacted specific genes previously noted in other studies to be altered in autism, e.g. NRXN1,and Xp11.2 genes ASMTL, ASMT, and ZNF57 gene on Xp11.3. We identified CNVs in THSD7A and THSD7B that encode subunits of thrombospondin 1 that interacts with neuroligin 1. In two subjects we identified deletions in KCNH8 that encodes a potassium channel gene involved in ion and neurotransmitter conductance.

2527/W

Genetic and epigenetic analyses of monozygotic twins discordant for bipolar disorder. *H. Sugawara[1,2], K. Iwamoto[3], M. Bundo[3], N. Adati[4], J. Ueda[1], I. Kusumi[5], Y. Okazaki[6], J. Ishigooka[2], T. Kojima[4], T. Kato[1].* 1) RIKEN Brain Science Institute, Wako, Japan; 2) Department of Psychiatry, Tokyo Women's Medical University, Tokyo, Japan; 3) Department of Molecular Psychiatry, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; 4) Computational Systems Biology Research Group, Advanced Computational Sciences Department, RIKEN Advanced Science Institute, Kanagawa, Japan; 5) Department of Psychiatry, Hokkaido University, Sapporo, Japan; 6) Tokyo Metropolitan Matsuzawa Hospital, Tokyo, Japan.

Although concordance rates in monozygotic (MZ) twins in bipolar disorder and schizophrenia are very high, they are not 100%. Because MZ twins have been regarded as having identical genomes, these facts suggest the importance of environmental or epigenetic factor for the onset of mental disorders. In fact, considerable epigenetic differences between MZ twins have been reported by several groups. On the other hand, there are also reports of genetic differences between MZ twins such as point mutations and microsatellite repeat length. In addition, difference of copy number variations between MZ twins has recently been discovered. Therefore, both genetic and epigenetic factors may play a role in the discordance of the onset of diseases between MZ twins.

We have been searching for both genetic and epigenetic differences between MZ twins discordant for bipolar disorder. For genetic analysis, we performed in-depth CNV analysis of the lymphoblastoid cell lines derived from two pairs of discordant MZ twins using Agilent 244K CGH and 488K CNV arrays. We could not detect significant CNV differences between the MZ twins except for the immunoglobulin-associated regions. In addition, we are performing whole exome sequencing to explore the genetic differences between the MZ twins at a single base pair resolution. For epigenetic analysis, we used Methylcollector Ultra Kit for the enrichment of CpG methylated DNA, and compared genome-wide DNA methylation patterns of promoter regions between the MZ twins using Affymetrix Human Promoter Array. We performed bisulfite PCR sequencing analysis to confirm the differences. We found a number of regions showing DNA methylation differences between the MZ twins. Some of them were confirmed by bisulfite PCR sequencing analysis.

Possible pathophysiological role of the both genetic and epigenetic difference between the MZ twins is a matter of interest in our future study.

2528/W

Apolipoprotein E polymorphisms and sporadic amyotrophic lateral sclerosis. *M.D. Sozuguzel Ozel[1], E. Ergul[1], A. Sazci[1], H.A. Idrisoglu[2].* 1) Medical Biology and Genetics, University of Kocaeli, Faculty of Medicine, Umuttepe, Kocaeli, Turkey; 2) Department of Neurology, Faculty of Medicine, University of Istanbul, Capa, 34280, Istanbul, Turkey.

The apolipoprotein E (apoE) gene located on chromosome 19 in human plays a critical role in predisposition to late onset Alzheimer's disease and other human neurodegenerative disorders. In the present study, for the first time in the Turkish population, we carried out the analysis of genetic association of apoE gene variants in 392 (244 male (62.24%), 148 female (37.76%);)sporadic amyotrophic lateral sclerosis patients (ALS) aged from 20 to 75 years (53.56±13.780) and 366 (225 male (61.48%); 141(38.52%) female;healthy controls aged from 20 to 75 years(53.54±16.403). In order to do genotype analysis, a polymerase chain reaction- restriction fragment length polymorphism procedure was employed. The distribution of ε2, ε3, and ε4 alleles was 7.53%, 84.57% and 7.90% in SALS and 7.92%, 84.02%, and 8.06% in controls respectively. There was no allelic association between APOE gene and SALS in overall population.However individuals with ApoE2/4 genotype showed significant protection against SALS (χ2=7.062; P=0.008; OR=0.101; 95%CI= 0.013, 0.805). When we analyzed the data according to gender, there was no association between APOE gene and SALS in males(χ2=5.649; P=0.342). Whereas the ε3 allele was associated with SALS in females(χ2=5.978;P=0.014; OR=8.842; 95%CI=1.091-71.634). APOE 2/4 genotype provided protection against SALS in females(χ2=6.431;P=0.011). Consequently APOE gene shows association with SALS in Turkey on a gender specific manner.

2529/W

Recurrent disruption of *HIP1* identified in patients with epilepsy, learning difficulties, mental retardation, and neurobehavioral abnormalities. *M. Bartnik[1] *, M.B. Ramocki[2,3] *, P. Szafrański[4], Z. Xia[4], G.S. Miller[5,6], E. Szczepanik[7], J.G. Coldwell[5], C.I. Akman[2], P.I. Bader[8], S.-H.L. Kang[4], C.A. Bacino[4], A. Patel[4], E. Bocian[1], S.W. Cheung[4], T. Mazurczak[1], P. Stankiewicz[1,4].* 1) Dept of Medical Genetics, Institute of Mother and Child, Warsaw, Poland; 2) Dept of Pediatrics, Section of Pediatric Neurology and Developmental Neuroscience, Baylor College of Medicine, Houston, TX; 3) Texas Children's Hospital, Houston, TX; 4) Dept of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 5) Children's Medical Center at Hillcrest, Tulsa, OK; 6) University of Oklahoma, Tulsa, OK; 7) Dept of Neurology, Institute of Mother and Child, Warsaw, Poland; 8) Northeast Indiana Genetic Counseling Center, Fort Wayne, IN. *These authors contributed equally.

In a few patients with an unusual association of Williams-Beuren syndrome (WBS) and infantile spasms, larger sized 7q11.23 deletions extending distally have been described. *MAGI2* (*membrane-associated guanylate kinase inverted-2*), mapping ~ 3.5 Mb distal to the WBS common deletion region, has been proposed to be responsible for infantile spasms. However, not all reported patients with deletions of *MAGI2* developed infantile spasms and to date, no point mutations have been identified in *MAGI2*. Further, at least two reported WBS patients with a 7q11.23 deletion leaving *MAGI2* intact have been diagnosed with infantile spasms. We report 10 individuals from five unrelated families with epilepsy, learning difficulties, mental retardation, and neurobehavioral abnormalities, who segregated a microdeletion between the WBS region and *MAGI2*. In four families, a recurrent ~ 1.1 Mb deletion likely resulted from nonallelic homologous recombination between flanking large complex low-copy repeats. A smaller sized deletion (~ 180 kb) in the fifth family enabled us to narrow the critical region to one gene, *HIP1* encoding Huntington interacting protein 1. HIP1 regulates AMPA-type (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) glutamate receptor trafficking in the central nervous system through its function in clathrin-mediated endocytosis. Mice with targeted mutation in the *Hip1* gene (*Hip1*$^{-/-}$) develop a neurological phenotype characterized by failure to thrive, tremor, and gait ataxia by 3 months of age. Five previously described patients with HIP1 deletions manifested severe MR but not epilepsy, and a parent with *HIP1* deletion in one of our families is reportedly normal, indicating incomplete penetrance. Magnetic resonance imaging of the brain was abnormal in one affected individual. We also have identified a reciprocal ~ 1.1 Mb microduplication in a patient with neurobehavioral abnormalities, including bipolar disorder and ADHD. Our collective data suggest that haploinsufficiency of *HIP1* is responsible for both focal and generalized epilepsies, which are associated with a broad range of cognitive dysfunction (cognitively normal to mild learning difficulties to severe intellectual disability) and neurobehavioral abnormalities, including inattention, hyperactivity, impulsivity, and aggression. Supported in part by grant R13-0005-04/2008 from the Polish Ministry of Science and Higher Education.

2530/W

An investigation into the causes of Landau-Kleffner Syndrome. *J. Conroy[1], J. Casey[1], N. Shah[1], R. Regan[1], M. Morgan[2], K. Collins[3], B. Parry-Fielder[3], S.A. Lynch[4], M. King[2], S. Ennis[1,4].* 1) Sch Med & Med Sci, Univeristy College Dublin, Dublin 4, Ireland; 2) Department of Neurology, Children's University Hospital Temple Street, Dublin, Ireland; 3) The Royal Children's Hospital, Melbourne, Australia; 4) National Centre for Medical genetics, Dublin, Ireland.

Landau Kleffner Syndrome (LKS) is a rare epileptic disorder of childhood of unknown aetiology (OMIM 245570). It is characterised by acquired receptive and expressive aphasia, epileptic seizures, and is associated with characteristic focal/multifocal spikes or spike and wave discharges which are continuous during sleep. Many children also exhibit behavioural disturbances that overlap those displayed by individuals with autism. Some individuals experience a complete recovery of language aphasia, but approximately 50% remain with severe residual language deficits. This study comprises 2 sets of discordant twins (one twinset has previously been reported by Feekery et al, 1993) and an additional 3 affected individuals. As the etiology of LKS remains unclear, a broad investigation was undertaken. All samples were genotyped on the Illumina 1M SNP array. CNV analysis was performed using PennCNV. *De novo* post-twinning CNV frequency could be 5% on an individual basis, and 10% per twinning event (Bruder et al, 2008). CNVs were analysed in a two step strategy. Firstly CNVs were compared in each affected twin to its unaffected twin. Then, all CNVs in the remaining individuals were compared to the "discordant CNVs" to identify if a common gene was affected. No single causative CNV was detected in all of the 5 affected individuals. Methylation differences were assessed using the Human methylation27 Illumina array. Again, the methylation analysis was performed using a two step strategy. Firstly, those methylation differences (Delta Beta values ≥ or £ 0.34, and Diff Score ≥ 32) between the discordant twin pairs were catalogued. In total there were 135 probes which were differentially methylated between affected and unaffected twin. Two overlapped (in PEG10 and CASP2). The additional 3 samples were compared to the unaffected twins and 14 control samples. The above 2 probes did not show differential methylation. When all LKS samples were compared to all controls, no significant autosomal differential methylation was detected. Using exome sequencing, possible patient specific mutations leading to the development of LKS were sought. Both discordant twin pairs, and 2 of the 3 remaining samples were sequenced (performed by GATC Biotech). Again, the analysis of variants began with identifying those mutations that are discordant between twins, and then analysis was extended to the 2 remaining affected individuals. These results will be presented at this meeting.

2531/W

Array CGH detection of clinically relevant copy-number changes in epilepsy. *M.B. Ramocki[1,3], S.R. Lalani[2,3], Z. Xia[2], C. Shaw[2], S.-H.L. Kang[2], C.A. Bacino[2,3], A. Patel[2], J.R. Lupski[2,3,4], S.W. Cheung[2], A.L. Beaudet[2,3,4], P. Stankiewicz[2].* 1) Department of Pediatrics, Section of Pediatric Neurology and Developmental Neuroscience, Baylor College of Medicine, Houston, TX; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 3) Texas Children's Hospital, Houston, TX; 4) Department of Pediatrics, Baylor College of Medicine, Houston, TX.

Copy-number variation (CNV) is a well-documented cause of syndromic epilepsy. Nonrecurrent CNVs disrupting *SCN1A*, *LIS1*, *MEF2C*, and *CDKL5*, or duplicating MECP2, and recurrent deletions in 15q11.2, 15q13.3 (*CHRNA7*), 16p13.11, 16p11.2, 16p12.1, and 1q21.1 cause generalized or focal epilepsy. We hypothesized that retrospective query of clinical chromosomal microarray analyses (CMA) performed on patients with epilepsy at Texas Children's Hospital would provide a rich source of information for gene identification and lend insight into the prevalence of previously identified CNVs in epilepsy. Out of ~2000 local patients referred for CMA between 2/04 and 3/10 with referring clinical indications of epilepsy, seizure, or infantile spasms, we identified 182 (9%) cases with nonpolymorphic CNVs. Upon extensive chart review, 36 cases were excluded because an epilepsy diagnosis could not be confirmed. Of the remaining 146 cases, 41 (28%) were genetically diagnostic based on previously reported CNVs associated with epilepsy including: Angelman syndrome (6), unbalanced translocations (5), 16p11.2 deletion (3), 1p36 deletion (3), 1q21.1 deletion (3), 1q21.1 duplication (2), *MECP2* duplication (2), *SCN1A* deletion (2), 15q11.2 (BP1-BP2) deletion (2), 15q13.3 duplication (2), Williams-Beuren region duplication (1), 9q34.3 deletion (1), 14q11.2 deletion (1), 15q13.3 deletion (1), 16p13.11 duplication (1), 17q21.31 deletion (1), 22q11.2 deletion (1), Xp22.31 deletion (1), *CDKL5* deletion (1), mosaic deletion of LIS1 (1), and XYY (1). We also identified 20 novel microdeletion (14) and microduplication (6) CNVs less than 1 Mb in size that harbor potential dosage-sensitive epilepsy causative or susceptibility genes. Microdeletions include chromosomes 2q34 (*ERRB4*), 3q21.2 (*PTPLB*), 7p11.2 (*GBAS*), 7p15.2 (*HOXA1*), 7q11.23 (*HIP1*), 8q24.22 (*KCNQ3*), 9q34.2 (*SLC2A6*), 11q12.2 (*MS4A* gene family), 11q22.3 (*GRIA4*), 15q26.1 (*KIF7*), 18q12.3 (*RIT2*), 19q13.12 (*ZNF* gene family), Xq21.31 (*CPXCR1*), and Xq28 (*VAMP7*); and microduplications include chromosomes 3p24.3 (*KCNH8*), 5p13.2 (*GDNF*), 8p21.2 (*NEFL*), 14q12 (*FOXG1*), 17p13.2 (*UBE2G1*), Xp22.32 (*NLGN4X*) (2 cases), and Xp11.22 (*PHF8*). Our results further confirm the pathogenic role of both recurrent and nonrecurrent submicroscopic CNVs in the etiology of epilepsy and demonstrate the usefulness of our approach to the identification of novel epilepsy genes. Accumulating data support the clinical use of CMA in the genetic diagnosis of epilepsy.

2532/W

MOLECULAR ANALYSIS OF THE SCN1A GENE IN SOUTHERN ITALIAN PATIENTS WITH SEVERE MYOCLONIC EPILEPSY OF INFANCY. *F. Rocca[1], F. Annesi[1], G. Provenzano[1], E.V De Marco[1], P. Tarantino[1], V. Greco[1], D. Civitelli[1], V. Scornaienchi[1], G. Tortorella[2], G. Incorpora[3], A. Labate[4], A. Gambardella[1,4], G. Annesi[1].* 1) Inst Neurol Sci, National Res Council, Mangone, Italy; 2) Departement of Neurosciences, Psychiatry and Anaesthesiology University of Messina, Messina, Italy; 3) Pediatric Unit, Hospital " Civile - Paternò Arezzo", Ragusa, Italy; 4) Institute of Neurology, University Magna Graecia, Catanzaro, Italy.

Severe myoclonic epilepsy of infancy (SMEI), or Dravet syndrome, is a severe autosomal dominant epileptic encephalopathy mainly caused by de novo mutations in the voltage-gated sodium channel gene (SCN1A). Most of these mutations are nonsense or frameshift; missense mutations are also common. Recent studies have already reported that about 12% of mutation-negative SMEI patients have microchromosomal abnormalities involving SCN1A. However, the rates of detection of SCN1A mutation in SMEI patients range widely in the different populations. To investigate the frequency of SCN1A mutations in our population, we analyzed 19 SMEI patients (10 males and 9 females) from Southern Italy, who fulfilled the strict clinical definition of SMEI.Mutation analysis was performed by direct sequencing. The genomic anomalies were screened using multiplex ligation-dependent probe amplification (MLPA) and confirmed by Gene dosage experiment.Six different heterozygous coding variants were found in 6 unrelated SMEI cases (31.5%): 4 novel mutations (Thr1289Ile, 3840insT, IVS7+4delA, Glu1021X) and 2 previously described (IVS24-2A/G, Ser1505X). Using MLPA assay we identified a deletion of exons 1-25 in one of the 13 patients without SCN1A point mutations (8%). The 3840insT and IVS24-2A/G mutations were de novo; the parents of the other patients were not available.The frequency of SCN1A mutations is lower in our SMEI patients in comparison to other populations. Our results confirm the importance of screening the coding regions with both direct sequencing and a quantitative method and suggest that further genetic studies are needed to determine the causative mutations and genes involved in the remaining SCN1A-negative patients.

2533/W

Analysis of Autism Spectrum Disorder Families Reveals Association with Inflammatory Gastrointestinal Disease Loci. *S.J. Walker[1], P.S. Ramos[2], C.D. Langefeld[2].* 1) Institute for Regenerative Medicine, Wake Forest Univ, Winston Salem, NC; 2) Dept Biostatistical Sciences, Wake Forest Univ Health Sciences, Winston Salem, NC.

Autism spectrum disorders (ASDs) comprise a heterogeneous collection of affected developmental features centered on deficits in cognition, communication, social acuity, and coupled with repetitive, stereotyped patterns of behavior. In addition to the core deficits, affected children (currently estimated to be 1 in 110 in the U.S.) also display a wide range of concurrent medical conditions. One frequently cited co-morbid finding in ASD children is chronic gastrointestinal (GI) symptoms. To determine if the GI symptoms observed in these ASD children share unique genetic risk factors with known inflammatory diseases of the bowel, we performed a family-based association analysis between gene variants implicated in GI inflammatory diseases and ASD. We compiled a list with 195 SNPs reported as significant in genome-wide association studies (GWAS) of GI inflammatory diseases (inflammatory bowel disease, Crohn's disease, ulcerative colitis, and celiac disease), available either at the GWAS catalog (www.genome.gov/gwastudies) or curated manually. We used genotypic and phenotypic data available from the Autism Genetic Resource Exchange (AGRE) repository to evaluate GI loci that were associated with ASD in 1,510 trios and a subset of 269 trios where the affected child also has reported GI involvement (ASD-GI). The standard Transmission Disequilibrium Test (TDT) implemented in PLINK was performed using genotypic data from the Illumina Hap550 platform. The most significant P-value is in the Kruppel-like factor 12 (KLF12) region (rs11617463, PASD=6.95x10-04, OR [95%CI] =0.71 [0.58-0.87]), in a locus that has shown association to both Crohn's and celiac disease. An effect at the EPH receptor A7 (EPHA7) region, which has been associated with celiac disease, was observed in all ASD trios and the subset with GI symptoms (rs4446534, PASD=1.74x10-03, ORASD=0.84 [0.76-0.94], PASD-GI=5.75x10-03, ORASD-GI=0.74 [0.6-0.92]; rs4571541, PASD= 5.79x10-03, ORASD=0.85 [0.76-0.95], PASD-GI=9.51x10-03, ORASD-GI= 0.73 [0.57-0.93]). When we analyzed the subset of children with GI complications, the most significant locus was that of 6-phosphofructo-2-kinase/fructose-2, 6-biphosphatase 3 (PFKFB3, rs1064891, PASD-GI=4.99x10-04, OR=0.67 [0.53-0.84]), which has been associated with celiac disease and is near interleukin 2 receptor alpha (IL2RA). This study demonstrates that several loci associated with inflammatory GI diseases are also associated with ASD.

2534/W

A migraine susceptibility locus segregating in rolandic epilepsy families maps to 17q21.33-q22. *L. Addis[1], T. Chiang[2], W. Li[2], L.J. Strug[2], D.K. Pal[1].* 1) Clinical Neurosciences, Institute of Psychiatry, King's College London, London, United Kingdom; 2) Hospital for Sick Children, Toronto, ON, Canada.

Migraine without aura is co-morbid in Rolandic Epilepsy (RE) cases, and familial aggregation studies indicate a 3-fold elevated risk of migraine in their seizure-free siblings. These results suggest a shared susceptibility that is not directly mediated by seizures. Although migraine is believed to be a complex genetic disorder in the general population, the genetic model for migraine in a homogenous sample of RE families may be less complex. On this assumption we carried out a genome-wide linkage screen to map a migraine susceptibility locus in 23, 2-4 generation RE families. The families were singly ascertained in the USA as part of an investigation into the genetic causes of RE. These families contained at least one member who also met the International Classification of Headache Disorders (ICHD-2) criteria for migraine. Using Genehunter, the maximum HLOD obtained was 4.4 between markers D17S788-D17S787 under a recessive model of inheritance, 99% penetrance. At this locus, 17 of the families provided positive LOD scores. Singlepoint linkage analysis provided a LOD of 2.1 at the same markers and under the same inheritance model. Analysis of differential male and female recombination fractions did not provide evidence for imprinting. The 9MB, 2LOD interval on chromosome 17 contains a relatively small number of genes, which we are currently investigating to elucidate functional variants. Loci on chromosomes 1q and 2q also provided linkage evidence, with LODs above 2.3 for both single- and multipoint analysis. These loci will also be further investigated given the observed heterogeneity at the 17q locus. None of these loci have been previously reported in the literature as migraine susceptibility loci, and thus it is possible that the 17q locus and others are specific to migraine without aura susceptibility in RE. Siblings of RE cases are at a 50% risk of carrying the EEG abnormality associated with RE. Thus the presence of RE in a family may represent a marker of a heritable form of cortical excitability, which along with additional genetic factors, can result in either RE, migraine, or both. Understanding the genetic etiology of migraine in RE can also provide important insight into migraine in the general population.

2535/W

Using Ingenuity Pathway Analysis to study gene relationships under linkage peaks of interest in Autism Spectrum Disorders. *A. Hare[1], M. Azaro[1], V. Vieland[2], J. Flax[1], L. Brzustowicz[1].* 1) Gen, Rutgers Univ, Piscataway, NJ; 2) The Research Institute at Nationwide Children's Hospital, Columbus, OH.

Using behavioral and genetic information from the Autism Genetic Resource Exchange (AGRE) dataset we developed behavioral phenotypes and investigated linkage and association for individuals with and without Autism Spectrum Disorder (ASD) who exhibit expressive language behaviors consistent with a motor speech disorder. Speech and language variables from Autism Diagnostic Interview-Revised (ADI-R) were used to develop two motor speech phenotypes (NVMSD:ALL and NVMSD:C). Using Affymetrix 5.0 data, the Posterior Probability of Linkage (PPL) framework was used to assess the strength of evidence for or against trait-marker linkage and linkage disequilibrium (LD) across the genome. We identified several linkage peaks based on two related expressive language phenotypes consistent with a potential motor speech disorder: Chromosomes 1q24.2, 3q25.31, 4q22.3, 5p12, 5q33.1, 17p12, 17q11.2 and 17q22 for NVMSD:ALL and 4p15.2 and 21q22.2 for NVMSD:C. While no compelling evidence of association was obtained under those peaks, we were able to identify several potential genes of interest using the core analysis of Ingenuity Pathway Analysis (IPA). IPA utilizes a Right-tailed Fisher's exact test to calculate a p-value determining the probability that each biological function and/or disease represented by the genes in our linkage regions is due to chance alone. Genes with functions related to Nervous System Development and Function, Neurological Disorders, Genetic Disorders, and Psychological Disorders were identified from the core analysis. We conducted control analyses in IPA in order to verify the reliability of the use of IPA in candidate gene identification. The results of the IPA control analyses were treated as a background subtraction tool for our linkage analyses in order to ensure that the final candidate gene list was unique to our phenotype. Each control analysis was comprised of a core analysis of 645 randomly selected genes designed to mimic the number of genes included in the core analysis of our linkage regions. Overall, 52 candidate genes were selected from a total of 645 genes for NVMSD:ALL and 51 candidate genes were selected from 388 genes for NVMSD:C. Given that autism spectrum disorders are complex with a wide range of behaviors and a large number of underlying genes, this motor speech phenotype may be one of several that should be considered when developing narrow, well-defined, phenotypes in the attempt to reduce genetic heterogeneity.

2536/W

Identification of an autosomal recessive stuttering locus on chromosome 3q13. *M. Raza[1], S. Riazuddin[2], D. Drayna[1].* 1) SSBCD, LMG, NIDCD/NATIONAL INSTITUTE OF HEALTH, 5 RESEARCH COURT, ROCKVILLE, MD; 2) ALLAMA IQBAL MEDICAL COLLEGE, LAHORE PAKISTAN.

Stuttering is a common speech disorder characterized by involuntary disruptions of verbal expression. Although its causes remain poorly understood, there is good evidence for genetic contributions to this disorder. Because of marginally significant linkage scores and difficulties reproducing linkage observations across studies, we sought to increase the power to detect linkage using highly consanguineous families. We studied families from the Pakistani populations in which inbreeding is common. We enrolled 6 consanguineous families from Pakistan, all containing multiple members who stutter. Family members were diagnosed using the Stuttering Severity Instrument-3, and we performed a genome wide linkage scan using the SNP genotyping with the Illumina 6K Chip. The program Pedcheck was used to test for proper inheritance within families. Two-point parametric linkage analysis was performed using SuperLink v1.4 from the EasyLinkage v5.08 package. The disease allele frequency was set at 0.01 and Caucasian allele frequencies provided by Illumina were used for the analysis. Due to the high degree of consanguinity in this family and in the Pakistani population, autosomal recessive inheritance was assumed. Significant evidence of linkage, with a LOD score of 3.23 under a recessive mode of inheritance, was found at SNP rs7631540 on chromosome 3q13.31 (at 115.31 Mb) in one family, designated PKST77. No markers showed significant linkage under either additive or dominant models of inheritance in this family. The surrounding interval was genotyped with additional microsatellite and SNP markers. LOD scores of 3.13, 4.23 and 3.74 were obtained with markers rs1317244 (113.53 Mb), D3S1310 (116.26 Mb) and D3S1303 (119.64 Mb), respectively. Haplotype construction narrowed the linkage interval to 3.24 Mb (extending from SNPs rs13062867 to rs714697). Based on the UCSC genome browser, some 46 known and predicted genes are found in this region of chromosome 3q. Within this interval, the exons of the candidate gene DRD3 (Dopamine Receptor D3) were sequenced in affected as well as unaffected individuals from PKST77, however no novel variation was observed. Our results demonstrate strong evidence of a novel stuttering linkage locus based on studies in a consanguineous stuttering family PKST77. In addition to the previously reported stuttering genes on chromosomes 12q and 16p (Kang, et al., 2010), an additional stuttering locus resides on chromosome 3q.

2537/W

Homozygosity Haplotype analysis of high denisty SNP genotype data link an autosomal dominant atypical movement disorder to chromosome 19p13.3-q13.12. *L. Yu[1], Y. Liu[2], M. Kan[2], A. Nicolas[3], L. Gao[4], L. He[2,4], R. Margolis[1].* 1) Dept. of Psychiatry & Behavioral Sci, Johns Hopkins University, Baltimore, MD; 2) Institutes of Biomedical Sciences, Fudan University, Shanghai, China; 3) Department of Neurology, University of Alabama at Birmingham School of Medicine,Birmingham AL; 4) Bio-X Center, Shanghai Jiaotong University, Shanghai, China.

The considerable information content of high-density single nucleotide polymorphism (SNP) genotyping makes this approach potentially quite valuable for linkage analysis. However, high-density SNP genotype data present an analytic challenge. A recently developed non-parametric method termed Homozygosity Haplotype (HH) analysis allows for a genome-wide search of the shared autosomal segments among patients using high-density SNP genotype data. We have applied this method to individuals from a family with an atypical movement disorder, segragating in an autosomal dominant pattern. The phenotype consists primarily of a late onset slowly progressive gait ataxia complicated in some individuals by abnormal tone, dystonia, and/or episodic attacks of ataxia. We performed a genome wide scan using the Illumina human1M-duo beadChip. HH analysis revealed a 33.4 Mb genomic region on chromosome 19 suggestive for linkage (-log10P is between 2.9 and 5.7). We independently validated the region on Chr19p13.3-q13.12 using microsatellite markers, with a maximum two-point LOD score of 3.64 at D19S406. In conclusion, both parametric and non-parametric analyses support linkage of the disorder to Chr19p13.3-q13.12. The defined genomic interval will facilitate identification of the causative mutation.

2538/W

Nicotine Dependence: Linkage and Association Analyses Suggest a Role for ST8SIA4. *P.A. Madden[1], M. Pergadia[1], A.C. Heath[1], N.G. Martin[2], J. Kaprio[3,4,5], G.P. Montgomery[2], Nicotine Addiction Genetics Consortium.* 1) Psychiatry, Washington University, St. Louis, MO, USA; 2) Queensland Institute of Medical Research, Brisbane, Australia; 3) Department of Public Health, University of Helsinki, Helsinki, Finland; 4) Institute for Health and Welfare (THL), Helsinki, Finland; 5) Institute for Molecular Medicine Finland FIMM, University of Helsinki, Helsinki, Finland.

The Nicotine Addiction Genetics Project (NAG) is an international collaborative study between investigators from Australia, Finland and the USA to identify factors that affect risk for tobacco use and dependence. Presented will be linkage and gene-based genetic association findings for nicotine dependence (ND), defined using a quantitative measure derived from the factor analysis of survey items based on DSM-IV Nicotine Dependence and the Fagerstrom Test for Nicotine Dependence assessed during the heaviest period of cigarette smoking in Finnish and/or Australian subjects. Because no evidence suggests that dependence on nicotine is a quantitative trait, analyses using a quantitative measure should maximize our power to identify chromosomal regions that harbor risk loci for this public health problem. Linkage findings were obtained from the separate and combined analysis of 10cM microsatellite marker genome scan data on DNA obtained from 2300 Australian and Finnish subjects from 440 families ascertained for heavy lifetime cigarette use. Results indicate a linkage signal for ND on chromosomes 5 (LOD=3.9, empirical p=0.03) among Australian smokers. Subsequently, a gene-based test (VEGAS), that adjusts for both linkage disequilibrium and gene size, was applied to SNP data limited to the region under the chromsome 5 linkage peak. The top ranked gene was ST8SIA4 (p=7.0E-06; which exceeds the Bonferroni corrected threshold: p < 2.5E-04). ST8SIA4 is a polysialyltransferase which is involved in the polysialylation of the neural cell adhesion NCAM molecule, the expression of which has been reported in findings from animal research to be affected by the presence of nicotine. Our linkage findings were observed in the Australian, but not the Finnish linkage sample, suggesting that these findings might be most relevant in families with more severe levels of heavy smoking and ND.

2539/W

Using family-based association study to refine the candidate region for bipolar affective disorder on chromosome 3p22. *R. Secolin[1], C.E.M. Banzato[2], M.C.M. Oliveira[2], M.F.R. Bittar[1], P. Dalgalarrondo[2], I. Lopes-Cendes[1].* 1) Dept Med Gen, Univ Campinas - UNICAMP, Campinas, SP, SP., Brazil; 2) Dept Medical Psychology and Psychiatry, Univ Campinas - UNICAMP, Campinas, SP, SP., Brazil.

Background: BPAD is a common psychiatric illness, with a prevalence of 0.8-2.6 % in the general population. Clinical features include episodes of mania or hypomania, interspersed with periods of depression. Genetic factors are known to contribute to the etiology of BPAD. We have previously identified a candidate locus on chromosome (ch) 3p22 using a group of nuclear families segregating BPAD and a family based association strategy. **Objectives:** To refine the ch 3p22 candidate region for BPAD using additional SNPs in our group of families. **Methods:** We evaluated 97 nuclear families, with a total of 411 individuals, including 96 patients who fulfilled clinical criteria for BPAD according to DSM-IV classification. We genotyped 94 SNPs within the 3p22 candidate locus using the SNPlex® system (Applied Biosystems). Statistical power was verified using TDT POWER CALCULATOR. Genotyped data was processed by JINGLEFIX program, which also estimated minor allele frequency (MAF). Mendelian inconsistencies were evaluated by PEDCHECK software. We estimated Hardy-Weinberg equilibrium and linkage disequilibrium using HAPLOVIEW software. Family based association analysis was performed by the transmission disequilibrium test (TDT) using TDTAE software. Statistical results were adjusted for multiple comparisons using the Bonferroni correction. **Results:** Our sample showed statistical power higher than 80% to detect association. Four SNPs presented MAF < 0.05 (rs3772105, rs35107818, rs11928905 and rs35479964) and two were in Hardy-Weinberg disequilibrium (pvalue < 0.001; rs2290528, rs2685112), these were excluded from the final analysis. We found significant association signal for SNP rs166508, even after Bonferroni correction ($p_{corrected}$ = 0.0187). **Conclusions:** We demonstrated that SNP rs166508 is associated with BPAD in our family sample. This SNP is located within intron 15 of the integrin alpha 9 (*ITGA9*) gene. *ITGA9* encodes a subunit of the alpha 9 integrin, an integral membrane glycoprotein which is a receptor for a nerve growth factor, neurotrophin 3 and brain-derived neurotrophic factor. It remains to be determined whether *ITGA9* plays a role in the mechanism underlying BPAD. FAPESP, São Paulo, Brazil.

2540/W

Genome-Wide Copy Number Analysis of Schizophrenia Patients. *D. Rudd, M. Axelsen, E. Epping, T. Wassink.* Univ Iowa, Iowa City, IA.

Schizophrenia is characterized by positive and negative symptoms that include hallucinations, delusions, disorganized thought as well as flat affect and decreased speech. Previous studies have identified both common and rare copy number variants that confer risk to developing schizophrenia. Therefore, we analyzed our novel sample set of schizophrenia patients for genome-wide copy number variation in order to identify novel candidate genes. We used Affymetrix Genotyping Console 3.0 to analyze signal intensity data from Affymetrix Genome-Wide Human SNP Array 6.0 microarrays. To date we have analyzed 58 schizophrenia probands and 23 psychiatrically unaffected controls. CNVs discussed in this abstract were confirmed by qPCR and were not present in controls. First, we identified a 973kb deletion in one proband within *CNTN4* that disrupts the 5' untranslated region as well as the first 4 of exons of the long isoform. *CNTN4* is a GPI-anchored neuronal membrane protein that belongs to the super-family of neuronal cell adhesion molecules involved in axonal growth and guidance, and is involved in the fasciculation of the central nervous system. In addition, *Cntn4* knock out mice exhibit behavioral and neurological phenotypes. In humans, disruption of *CNTN4* is associated with an increased risk to develop an autism spectrum disorder (ASD) and is believed to contribute to the impaired social functioning observed in 3p deletion syndrome. Published data in two previous studies done by the same author reported no copy number variation in *CNTN4* out of 812 total controls. In two more unrelated probands we identified 435kb and 627kb deletions on chromosome 15q11.2 spanning the genes *NIPA1, NIPA2, CYFIP1* and *GCP5*. These four genes are highly conserved and located between breakpoint 1 and breakpoint 2 of the Prader-Willi/Angelman Syndrome (PW/AS) region. A previous report indicates that patients harboring an inherited or *de novo* heterozygous deletion in this region who additionally do not meet diagnostic criteria for PW/AS have severe psychiatric phenotypes such as attention deficit hyperactivity disorder, ASDs, and obsessive-compulsive behavior compared with 350 healthy, unrelated controls. Preliminary results in our genome-wide analysis of copy number variation in schizophrenia have identified promising candidates for disease etiology. Future directions include analysis and validation of CNVs in the remainder of the sample set of approximately 500 probands.

2541/W

Association of protein-coding variants in the microcephalin gene with cognition and symptoms of schizophrenia. *E.A. Epping[1], B.C. Ho[1], N.C. Andreasen[1], M. Axelsen[1], S. Mugge[1], T.H. Wassink[1,2].* 1) Department of Psychiatry, University of Iowa Carver College of Medicine, Iowa City, IA; 2) Interdisciplinary Program in Genetics, University of Iowa, Iowa City, IA.

Purpose: Schizophrenia is considered a highly heritable disorder disrupting normal brain development, but the etiologic factors responsible remain to be fully understood. The microcephalin protein encoded by the gene *MCPH1* is highly expressed early in brain development and regulates neuronal progenitor cell division. Deleterious mutations in *MCPH1* have been shown to result in primary microcephaly, and common polymorphisms have been implicated in evolution of human cortical size and cognitive functioning. In this work, it is hypothesized that protein-coding variants in *MCPH1* are associated with variation in brain morphology, cognition, and symptoms of schizophrenia. **Methods:** A sample of 313 individuals with schizophrenia or schizoaffective disorder and 175 healthy control subjects were genotyped at *MCPH1* protein coding SNPs rs930557 (His/Asp), rs1057090 (Ala/Val), and rs1057091 (Pro/Ser). The majority of these subjects have had structural brain MRIs, testing of cognitive function (27 tests grouped into 5 domains), and in patients, assessment of symptoms using scales for positive or negative symptoms of schizophrenia. Analyses of covariance using appropriate covariates for brain volumes or cognitive domains were performed to determine genotype effects. A genotype by diagnostic group interaction term was also included in the general linear models. Symptom measures were compared using t-tests. **Results**: Patients homozygous for the Pro variant at rs1057091 had significantly lower severity of hallucinations ($p < 0.02$) and delusions ($p < 0.05$) compared to Ser carriers. A significant genotype effect was also observed for the verbal memory domain with this variant ($p = 0.0001$), with poorer performance found in Pro homozygotes. A significant genotype effect was also observed for the rs1057090 SNP, with worse verbal memory performance in Ala homozygotes ($p < 0.01$). While there were no significant genotype effects on cortical brain volumes, Pro and Ala homozygotes had smaller hippocampus volumes, consistent with worse performance on tests of verbal memory. No significant effects were observed for the rs930557 SNP. **Summary:** Protein-coding variants in *MCPH1* were found to be associated with differences in verbal memory, hippocampus volumes, and positive symptoms of schizophrenia. The effect on verbal memory was found in both patient and control populations for two SNPs indicating these may contribute to variation in general cognitive functioning.

2542/W

Association of HLA class I markers with Multiple Sclerosis in the Italian and UK population: evidence of two independent protective effects. *L. Bergamaschi[1], M. Ban[2], N. Barizzone[1], L. Corrado[1], M. Leone[3], M.E. Fasano[4], F.R. Guerini[5], P. Naldi[3], C. Agliardi[5], E. Dametto[4], M. Salvetti[6], A. Visconti[6], D. Galimberti[7], E. Scarpini[7], P. Cavalla[8], R. Bergamaschi[9], D. Caputo[10], S. Cordera[11], D. Vecchio[3], P. Momigliano-Richiardi[1], S. D'Alfonso[1].* 1) Department of Medical Sciences, University of Eastern Piedmont, Novara, Italy , Novara, Italy; 2) University of Cambridge, Department of Clinical Neuroscience, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 0QQ, UK; 3) Department of Neurology, Ospedale Maggiore, and IRCAD, Novara, Italy; 4) Transplantation Immunology, San Giovanni Battista Hospital, Turin, Italy; 5) Laboratory of Molecular Medicine and Biotechnology, Don C. Gnocchi Foundation IRCCS, S. Maria Nascente, Milan, Italy; 6) Neurology and Center for Experimental Neurological Therapy (CENTERS), Università La Sapienza, Rome, Italy; 7) Department. of Neurological Sciences, University of Milan, Dino Ferrari Center, Fondazione Ospedale Maggiore Policlinico, Milan , Italy; 8) Department of Neurology, Ospedale San Giovanni Battista, Turin, Italy; 9) Neurological Institute C. Mondino, IRCCS, Pavia, Italy; 10) Multiple Sclerosis Unit, Don C. Gnocchi Foundation, IRCCS, S. Maria Nascente Milan, Italy; 11) Neurology Ospedale Umberto Parini, Aosta, Italy.

Background: We recently contributed to demonstrate for Multiple Sclerosis (MS) a second association signal in the class I HLA region independent of the well-established effect of the HLA-DR15 allele. In an Italian cohort we confirmed the association of HLA-A*02 and observed that the contemporary presence of HLA-Cw*05 significantly increased (3-fold) the protective effect of HLA A*02, independently of DR15. A*02-Cw*05 is carried by two HLA extended haplotypes characterized by the B*4402 and B*1801 alleles, respectively. Objectives and Methods: To better understand whether the strong protection of the A*02-Cw*05 combination was due to a haplotypic effect or a direct role of these two markers we extended the analysis in our Italian cohort (1445 MS cases and 973 controls) to the HLA-B*44 and -B*18 alleles and verified the association of the different combinations of tested HLA class I markers in an independent UK cohort (721 MS cases, 408 controls and 480 family trios). Results: The analysis of the Italian samples showed a strong protective effect of the A*02-Cw*05 combination carrying B*44 (OR=0.27 p=3.3x10-05), independently of DR15. This was confirmed in UK family trios (OR=0.33 p=5.5x10-4) and in a combined cohort of UK family trios and case/controls (OR=0.53 p=0.044). In both populations this protective effect was significantly stronger than that mediated by A*02 alone. At difference from A*02, the other HLA class I tested markers (Cw*05, B*44 and B*18) did not show individually any significant protection. Conclusions: This study identified two independent protective effects which are tagged by the A*02-Cw*05-B*44 haplotype and the A*02 allele, respectively.

2543/W

Racial disparity in regulatory genetics of SLC6A3, a dopamine transporter implicated in multiple neuropsychiatric disorders. *Z. Lin, Y. Zhou.* Dept Psychiatry, MRC 114, Mclean Hosp/Harvard Med Sch, Belmont, MA.

Chromosomal DNA sequence polymorphisms may contribute to individuality, confer risk for diseases and most commonly, are used as genetic markers in association study. Current genome-wide association studies or candidate gene approaches use same genetic markers to underpin risk factors for complex diseases in various world populations and often result in inconsistent findings. To better understand ethnicity-specific genetics, we deep-sequenced the regulatory regions of SLC6A3, a gene that encodes the dopamine transporter and is implicated in many brain disorders with racial disparity, in 30 African (AA) and 30 Caucasian (CA) Americans. AA and CA carry 50.9% and 33.3% ethnicity-specific common polymorphisms including all of the most common ones in the SLC6A3 promoter. More than a half of the polymorphisms are not in the dbSNP yet. In each sample, 95% of the chromosomes carry unique regulatory haplotypes and none of the haplotypes are shared between the two ethnicities. Finally, a quarter of the haplotypes evolved in an ethnicity-specific manner and the rest co-evolved independently of ethnicity. These findings suggest that we are still at the beginning stage of understanding human genetics and that deep-sequencing will help dissect genetic variations underlying individuality, ethnicity and complex disease.

2544/W

Analysis of SNCA-LRRK2 genes interaction in Parkinson's disease and controls. *A. Parsian[1], J.H. Zhao[2], B. Patra[3].* 1) Div Neuroscience & Behavior, NIAAA/NIH, Bethesda, MD; 2) MRC Epidemiology Unit, Cambridge, UK; 3) Department of Pathology, Thomas Jefferson University, Philadelphia, PA.

Our earlier reports of eight single nucleotide polymorphisms (SNPs) in the SNCA and LRRK2 regions have indicated that they are at most have modest increase of susceptibility. In the current investigation, we focused on their interactions. The sample included 227 familial and 355 sporadic cases of Parkinson's diseases and 236 unrelated controls. As SNP-wise analysis shows no evidence of association we selected haplotype showing increased risk according to haplotype specific analysis in SNCA as covariate to those in LRRK2 in an interaction analysis both using R/haplo.stats, and vice versa. As in our earlier report, haplotype 112 of 770-int4-Rep1 in SNCA has frequency of 0.04817 (haplo.em) and shows moderate significance (haplo.score score statistic 2.53, asymptotic and simulated p values = 0.01; haplo.glm t-statistic=2.37 and p = 0.0182). In contrast, haplotypes 211212 and 212211 for rs10506151-rs10784486-rs1365763-rs1388598-rs1491938-rs1491941 in LRRK2 have score statistics -1.79 and -1.42 and t-statistic -1.79 (p = 0.0744) and -1.76 (p = 0.0794). The strongest evidence of statistical significance is the presence of SNCA haplotype 112 and haplotypes 222111 and 222221 in LRRK2 (both with t-statistic>5, p<0.0001), or presence of haplotypes 211212 and 212211 in LRRK2 with haplotype 122 in SNCA (t-statistic=1.77, p=0.078). Given the recent successes of genome-wide association studies (GWASs) which largely indicate that SNPs established accounted for a small amount of phenotypic variations, it is hopeful that study of gene-gene interactions can help to identify further genotypic contributions to the "missing heritabilities". Our results are in line with earlier reports that specific haplotypes in these two genes might contribute to increased risks of Parkinson's disease.

2545/W

Lack of association between G-protein coupled receptor kinase 5 gene and Parkinson's disease. *P. Tarantino[1], E.V. De Marco[1], F.E. Rocca[1], F. Annesi[1], D. Civitelli[1], G. Provenzano[1], V. Scornaienchi[1], V. Greco[1], C. Colica[2], G. Nicoletti[3], A. Quattrone[3,4], G. Annesi[1].* 1) Inst Neurological Sci, National Research Council, Mangone, Cosenza, Italy; 2) Institute of Neurological Sciences, National Research Council, Catanzaro, Italy; 3) Institute of Neurology, University Magna Graecia, Catanzaro, Italy; 4) Neuroimaging Research Unit, National Research Council, Catanzaro, Italy.

The major component of Lewy Bodies (LB), the pathological hallmark of Parkinson's disease (PD), is α-synuclein, most prominently phosphorylated at serine 129. G-protein coupled receptor kinase 5 (GRK5) has been reported to phosphorylate α-synuclein in vitro, enhancing the α-synuclein toxicity to dopaminergic neurons in Drosophila model. Moreover, GRK5 was found in LBs from brain of PD patients. A genetic association study performed in the Japanese population revealed haplotypic association of the GRK5 gene with susceptibility to sporadic PD. We aimed at investigating whether four single-nucleotide polymorphisms (SNPs) within the GRK5 gene could represent risk factors for sporadic PD in Southern Italy. We studied 446 patients with PD and 450 controls coming from Southern Italy. Genomic DNA was extracted from peripheral venous blood using a standard phenol-chloroform method. All the subjects were genotyped for the SNPs rs871196, rs2420616, rs7069375, rs4752293 located in the GRK5 gene and selected from those previously used by other researchers. Genotyping was carried out with TaqMan assays on an ABI7900 real-time PCR instrument. A haplotype analysis was also performed.All markers were in Hardy-Weinberg equilibrium in both patients and controls. The distribution between the two groups did not show significant differences at allelic, genotypic and haplotypic level. This result was not modified either by using different models (dominant and recessive) or stratifying by sex.There is a considerable debate as to the precise role of members of the GRKs family. Besides their main role of selectively phosphorylating agonist-occupied G-protein-coupled receptors (GPCRs) for the desensitization, GRKs also phosphorylate non-GPCR substrates, such as α-synuclein. Finally, others studies report that only endogenous GRK3 or GRK6 phosphorylated α-synuclein at Ser 129 in HEK293 cells. However, they suggested that GRK2 or GRK5, when overexpressed, could expand beyond their physiological localization in the cell allowing GRK a non-physiological access to α-synuclein and, consequently, its phosphorylation. Our data do not suggest any implication of the GRK5 gene in susceptibility to PD in Southern Italy.Nevertheless, it is also possible that this gene plays a minor role in the pathogenesis of sporadic PD.Further studies in other populations could help to elucidate this debate.

2546/W

Variants on chromosome 15q25 are associated with age at initiation of daily smoking. *M. Kapoor[1], J. Wang[1], S. Bertelsen[1], A. Agrawal[1], J. Budde[1], B. Porjesz[2], H. Edenberg[3], K. Bucholz[1], J. Kramer[4], S. Kuperman[5], A. Brooks[6], J. Tischfield[6], L. Bierut[1], A. Goate[1].* 1) Department of Psychiatry, Washington University, St Louis, MO; 2) Department of Psychiatry, State University of New York, Brooklyn, NY; 3) Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN; 4) Department of Psychiatry, University of Iowa College of Medicine, Iowa City, IA; 5) Division of Child Psychiatry, University of Iowa Hospitals, Iowa City, IA; 6) Department of Genetics, Rutgers University, Piscataway, NJ.

Epidemiological research suggests that age at smoking initiation is related to consequent aspects of smoking behavior, such as cigarette consumption, nicotine dependence and smoking cessation. Studies have shown that the younger an individual starts smoking, the higher the subsequent cigarette consumption and risk of nicotine dependence, and the lower the likelihood of quitting. Several genome-wide association and candidate gene studies have linked chromosome 15q24-q25.1 (a region including the CHRNA5-CHRNA3-CHRNB4 gene cluster) with substance dependence and smoking-related illnesses such as lung cancer and chronic obstructive pulmonary disease. To further examine the impact of this gene cluster region on the development of substance use disorders, we tested whether variants within and flanking the CHRNA5/A3/B4 gene cluster affect age at onset of daily smoking. A total of 34 SNPS were analyzed in a European American only cross sectional sample of adolescents and young adults from the families recruited for the Collaborative Study of the Genetics of Alcoholism (COGA). A within-family quantitative transmission disequilibrium test (QTDT) was used to test for association between age at initiation of smoking and genetic markers on chromosome 15q25. Significant associations (p<0.05) were found between the age at smoking initiation and multiple SNPs in this region. There was very strong evidence of association between rs16969968 and age of onset of daily smoking. Additional SNPs in this region were also found to be moderately associated with the age of onset of regular drinking. Our results suggest that a gene or genes in this region may be involved in increased risk of nicotine dependence by influencing the age of onset of daily smoking. There may also be common genetic determinants, which govern the regular use of alcohol, tobacco and other substances of addiction.

2547/W

Analysis of Autism Spectrum Disorder Families Reveals an Enrichment of Immune Loci. *C.D. Langefeld[1], P.S. Ramos[1], S.J. Walker[2].* 1) Dept Biostatistical Sciences, Wake Forest Univ , Winston-Salem, NC; 2) Institute for Regenerative Medicine, Wake Forest Univ, Winston-Salem, NC.

Autism Spectrum Disorders (ASDs) encompass a heterogeneous group of clinical descriptors whose core features include deficits in cognition, communication, and social acuity, coupled with stereotypical behaviors. The etiology of ASDs, with very few exceptions, is unknown although a growing number of clinical and basic research studies have implicated immunological abnormalities as being associated with and/or responsible, at least in part, for the cognitive and behavioral deficits seen in ASD children. In order to assess a potential enrichment of immune loci in autistic children, we used genotypic data available at the Autism Genetic Resource Exchange (AGRE) repository to perform a family-based association analysis between variants in immune loci and ASD. We used a bioinformatics database (Ingenuity Pathway Analysis) to compile a list with 2,012 genes with known immune function. We tested 27,563 SNPs genotyped on the Illumina Hap550 platform in the AGRE collection of 1,510 trios, and performed the standard Transmission Disequilibrium Test (TDT) as implemented in PLINK. We have adjusted the reported P-values by running up to 1 million permutations. We observed a significant enrichment of results with P<0.001 (53 vs. 28 expected SNPs, P=1.0x10-05), suggesting the presence of true positive associations. Two of the most significant loci map to chromosome X: a significant variant near moesin (MSN, rs5918959, P=1.00x10-06, OR [95%CI] =0.24 [0.14-0.41]), and several variants in the CD99 molecule-like 2 (CD99L2, rs11796490, P=1.20x10-05, OR=0.68 [0.58-0.80]; rs11797645, P=1.58x10-03, OR=0.75 [0.63-0.89]; rs11797944, P=1.41x10-03, OR=0.75 [0.64-0.89]). Several SNPs in the jumonji AT rich interactive domain 2 (JARID2) gene, which has been previously implicated in schizophrenia, have also shown significant association with ASD (rs13193457, P=1.80x10-05, OR=0.61 [0.49-0.75]; rs794776, P=6.48x10-04, OR=0.64 [0.51-0.80]; rs6459404, P=7.15x10-04, OR=1.21 [1.09-1.34]; rs6921502, P=8.43x10-04, OR=1.20 [1.09-1.33]). A variant in the thyroid peroxidase gene (TPO), which has been associated with hypothyroidism, is also among the most significant (rs1514687, P= 1.50x10-05, OR=1.46 [1.24-1.72]). This study demonstrates that there is an enrichment of immune-function genes in autistic children, and that several novel genes with immune functions are associated with ASD.

2548/W

Confirmation of association between Multiple Sclerosis and CYP27B1. *E. Sundqvist[1], M. Bäärnhielm[2], L. Alfredsson[2], J. Hillert[1], T. Olsson[1], I. Kockum[1].* 1) Dept. Clinical Neuroscience, Karolinska Institutet, Stockholm, Stockholm, Sweden; 2) Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden.

Multiple Sclerosis, MS (OMIM #126200) is a complex inflammatory disease, characterised by lesions in the central nervous system. Both genes and environmental factors influence disease susceptibility. There is an apparent geographical pattern in MS prevalence, with increasing prevalence with increasing latitude, and the level of solar and UV radiation have been implicated in causing this difference in incidence. There is also a month-of birth effect in MS susceptibility, which could be linked to differences in climate, including sun exposure, during the year. Vitamin D levels are dependent on the level of UV radiation, and vitamin D deficiency has been implicated in MS and autoimmune disease such as type 1 diabetes, where patients have lower levels of 25-OHD3 in blood than controls. Previtamin D3 is produced in the skin, and in the liver turned into 25-OHD3. In the kidney, skin and immune cells 25-OHD3 is turned into the bioactive 1,25(OH)2D3 by the enzyme coded by CYP27B1 on chromosome 12q13.1-3. 1,25(OH)2D3 binds to the Vitamin D receptor, expressed in T cells and antigen presenting cells. 1,25(OH)2D3 has a suppressive role on the adaptive immune system, decreasing T cell and dendritic cell maturation, proliferation and differentiation, shifting the balance between Th1 and Th2 cells in favor of Th2 cells, and increasing the suppressive function of regulatory T cells. Rs703842 in the 12q13-14 region was associated to MS in a recent study by the ANZgene consortium, Nature Genetics, 41(7), 2009. We show associations to three SNPs in this region in our Swedish material (2158 cases, 1759 controls) rs4646536, rs10877012, rs10877015 (p=0.01, 0.01 and 3.5 x 10-3, respectively). We imputed rs703842 and performed a joint analysis with the ANZgene results, reaching a significant association of rs703842 with disease (p = 5.1 x 10-11 odds ratio 0.83 (0.79 - 0.88 95% confidence interval). Because of its close association with 25-OHD3 our results lend further support to the role of vitamin D in MS pathology. Lower exposure to sunlight and low expression of CYP27B1 mRNA will likely influence the amount of available active vitamin D3 in the body. In individuals with other genetic risk factor(s) and possibly exposed to other environmental factor(s), this could, through the effects of vitamin D3 on the immune system, lead to the development of MS or other autoimmune disease.

2549/W

Genetic association study of the monoamine oxidase A gene polymorphism (MAO-A VNTR) with gambling behaviour. *R.P.L. Tong[1], R.P. Souza[1,2], N. Freeman[1], M. Tampakeras[1], S. Shaikh[1], D.M. Casey[3], D.C. Hodgins[3], G.J. Smith[4], R.J. Williams[5], D.P. Schopflocher[6], R.T. Wood[7], N. el-Guebaly[8], D.S.S. Lobo[1,2,9], J.L. Kennedy[1,2].* 1) Neurogenetics Section, Neuroscience Department, Centre for Addiction and Mental Health, Toronto, Ontario, Canada; 2) Department of Psychiatry, University of Toronto, Toronto, Ontario, Canada; 3) Psychology Department, University of Calgary, Calgary, Alberta, Canada; 4) Faculty of Extension, University of Alberta, Calgary, Alberta, Canada; 5) School of Health Sciences, University of Lethbridge, Lethbridge, Alberta, Canada; 6) Faculty of Nursing, University of Alberta, Calgary, Alberta, Canada; 7) Department of Sociology, University of Lethbridge, Lethbridge, Alberta, Canada; 8) Division of Addiction, Department of Psychiatry, university of Calgary, Calgary, Alberta, Canada; 9) Problem Gambling Service, Addictions Program, Centre for Addiction and Mental Health, Toronto, Ontario, Canada.

Problem gambling (PG) is an impulse-control disorder with a life-time prevalence of 5%; in adults and its heritability has been estimated at 50-60%; in twin studies. Serotonin (5-HT) and dopamine (DA) dysregulation has been associated with PG. Thus, functional polymorphisms that affect the activity of 5-HT and DA may predispose individuals to PG. The monoamine oxidase (MAO) enzyme catabolizes 5-HT and DA in brain regions that regulate impulse control. A functional variable number tandem repeat (VNTR) polymorphism in the promoter region of the X-chromosome MAO-A gene (MAO-A VNTR) has demonstrated allele-specific enzyme expression where the 4-repeat allele has been associated with higher expression and lower impulsivity while the opposite is true for the 3-repeat allele. Here, we investigate the effect of the MAO-A VNTR polymorphism on gambling behavior. A sample of 1122 subjects (50.1% males, mean age = 39.95 ± 7.38) were stratified by ethnicity (Caucasian, African, and Asian). Each subject was assessed by the Canadian Problem Gambling Index (CPGI) or the South Oaks Gambling Screen (SOGS) and assigned to the PG or non-PG group. All participants were genotyped for the MAO-A VNTR functional polymorphism. An allelic analysis was performed on the overall sample as well as an analysis of males and females separately to analyze the association between the MAO-A VNTR and the gambling behaviour. The MAO-A VNTR polymorphism alleles were not significantly associated with gambling behaviour in the overall sample ($p>0.05$). Also, the allelic analysis of the ethnically stratified samples found that there was no significant association between the MAO-A VNTR alleles and gambling group across the different ethnic groups (all ethnicity groups: $p>0.05$). The results from our study suggest that the MAO-A VNTR polymorphism may not play a role in gambling behaviour. Further study should investigate whether other functional polymorphisms affecting 5-HT and DA activity are associated with gambling behaviour. Also, efforts should be taken to replicate the results of this study as genetic findings may have therapeutic implications and improve the treatment of PG.

2550/W

Gene x gene interaction analysis in susceptibility to bipolar spectrum disorder. *J.A. Donald[1], J.M. Fullerton[2], P.B. Mitchell[3], P.R. Schofield[2].* 1) Department of Biological Sciences, Macquarie University, Sydney, Australia; 2) Prince of Wales Medical Research Institute, Sydney, Australia; 3) School of Psychiatry, University of New South Wales, Sydney, Australia.

This study aims to identify interactions between genes of small effect that may contribute to susceptibility to bipolar spectrum disorder, a highly heritable psychiatric condition, for which few genes of large effect have been identified. A nonparametric linkage (NPL) analysis of 65 Australian extended pedigrees was conducted. Chromosome-by-chromosome correlation analysis of family-specific NPL scores was conducted to detect evidence of genetic interaction, followed by interaction-specific multipoint NPL and permutation analysis. Finally, a single nucleotide analysis of each interaction region was conducted to fine map putative gene x gene interactions using the publicly available GAIN and WTCCC genome-wide association (GWAS) datasets (2933 cases, 2534 controls), with follow-up analysis of SNP data from our Australian cohort (216 cases, 164 controls). We demonstrated four robust interchromosomal interaction clusters exceeding Bonferroni correction at α =.05 (uncorrected p < 5.38e-07) on 11q23-25-2p15-12, 4q32-35-1p36, 12q23-24-4p16-15, and 20q13-9q21-22. Using the combined GWAS dataset, we identified a suggestive SNP-based interaction on 2p14-11q23 after multiple testing correction (uncorrected p=5.761e-10, corrected p=0.06794). This interaction failed to replicate in our small Australian cohort. The 4q32-35-1p36 interaction was further investigated in the Australian cohort, examining 11 putative functional candidate genes in 1p36 with 4q35 SNPs in the FAT and MTNR1A genes, which were previously associated with bipolar disorder. We identified a nominal SNP x SNP interaction cluster (7 of the top 17 p values), spanning the promoter of the melatonin receptor MTNR1A and exons 7-22 of CAMTA1, a transcription activator known to potentiate deactivation of rhodopsin, a G-protein coupled light receptor. Expression array analysis of these genes shows a disease specific correlation in brain expression (p=0.009). This study highlights the difficulties in identifying robust genetic interactions underlying bipolar disorder susceptibility. However these results suggest an interaction between two genes that may result in increased sensitivity to circadian cues.

2551/W

Common variants in the regulative regions of GRIA1 and GRIA3 receptor genes are associated with migraine susceptibility. *D. Formicola[1], A. Aloia[1], S. Sampaolo[2], O. Farina[2], D. Diodato[2], L.R. Griffiths[3], F. Gianfrancesco[1], G. Di Iorio[2], T. Esposito[1].* 1) Inst Genetics & Biophysics, Italian Natl Research Council, Naples, Naples, Italy; 2) Headache Service - Department of Neurological Sciences, Second University of Naples, Italy; 3) Genomics Research Centre, School of Medical Science, Griffith University, Gold Coast, Queensland, Australia.

Glutamate is the principal excitatory neurotransmitter in the central nervous system which acts by the activation of either ionotropic (AMPA, NMDA and kainate receptors) or G-protein coupled metabotropic receptors. Glutamate is widely accepted to play a major role in the path physiology of migraine as implicated by data from animal and human studies. Genes involved in synthesis, metabolism and regulation of both glutamate and its receptors could be, therefore, considered as potential candidates for causing/predisposing to migraine when mutated. The association of polymorphic variants of GRIA1-GRIA4 genes which encode for the four subunits (GluR1-GluR4) of the alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptor for glutamate was tested in 244 migraineurs with and without aura (MA and MO) and 260 healthy controls. Two variants in the regulative regions of GRIA1 (rs2195450) and GRIA3 (rs3761555) genes resulted strongly associated with MA (P=0.00002 and P=0.0001, respectively), but not associated with MO, thus suggesting their role in cortical spreading depression. Whereas, the rs548294 variant in GRIA1 gene showed association with MO and MA phenotypes (Mig. all p=0.00009; MO p=0.0003; MA p=0.004), supporting the hypothesis that MA and MO phenotypes could be genetically related. Possible additive interactions of the two associated SNPs in GRIA1 (rs548294) and GRIA3 (rs3761555) genes were evaluated in those subjects who had both SNPs characterization. In Migraine patients, the p-value for the sole risk allele of the GRIA1 gene (TT/TT, CT/TT genotypes) was 0.03, OR 1.68 (CI = 1.02-2.77); the p-value for the sole risk allele of the GRIA3 gene (CC/TC, CC/CC genotypes) was 0.005, 2.28 (CI = 1.27-4.11) and the p-value for the combined two risk alleles (TT/TC, TT/CC, CT/TC, CT/CC genotypes) was 0.00001, OR 3.35 (CI = 1.96-5.74). Therefore, in migraine patients, when both mutant alleles are present, the risk to develop migraine is merely the sum of the risk effect carried by each single mutant allele. Preliminary functional studies showed that these variations modify binding sites for transcription factors suggesting a role in the regulation of the expression of GRIA1 and GRIA3 genes in different conditions. These results represent the first genetics evidence of a link between glutamate receptors and migraine and confirm that genetic variants in GRIA1 and GRIA3 genes are important in migraine aetiology.

2552/W

HLA class I genes involved in the triggering of multiple sclerosis. *J. Link[1], I. Kockum[1], A.R. Lorentzen[2], B.A. Lie[2], E.G. Celius[2], M. Schaffer[1], B. Brynedal[1], H.F. Harbo[2], J. Hillert[1].* 1) Karolinska Institutet, Stockholm, Sweden; 2) Oslo University Hospital, Oslo, Norway.

Aim: Our aim was to elucidate the role of alleles of human leukocyte antigen (HLA)-A, -B, -C and -DRB1 in the Scandinavian population in multiple sclerosis (MS) and in particular to study the previous suggested signals within the HLA.

Background: Several studies have reported associations with the class I genes in MS. Both HLA-A*02, HLA-C*05 and HLA-B*44 have been implicated to be responsible for this association. We have previously reported the possibility of two independent associations from class I in the Scandinavian population, HLA-A*02 and HLA-C*08.

Material and Methods: The study population consisted of 1230 patients and 1060 controls from Sweden together with 550 patients and 600 controls from Norway generating a Scandinavian population of 1780 patients and 1660 controls. The subjects were low resolution genotyped for HLA-A, -B, -C and -DRB1 giving the study an advantage over studies in which HLA genotypes were inferred from single nucleotide polymorphism data.

Results: Results from allele association with regard to already known risk factors in HLA will be presented as well as analyses of previously suggested signals within HLA class I. An investigation of interactions between genes will be performed and the dependence on HLA-DRB1*15 of novel MS associations will be assessed.

2553/W

Systematic Identification of new loci for non-specific autosomal recessive mental retardation. *R. Abou Jamra[1,3], A. Ekici[1], A. Abboud[2], B. AlHallak[2], S. Ibrahim[2], S. Muhammad[2], M. Fakher[2], A. Khateeb[2], J. Schumacher[3], M. Nöthen[3], A. Reis[1].* 1) Institute of Human Genetics, University of Erlangen-Nuremberg, Erlangen, Bavaria, Germany; 2) Paediatrician in private clinics, Syria; 3) Institute of Human Genetics, University of Bonn, Bonn, NRW, Germany.

Autosomal recessive forms of non-specific mental retardation (ARMR) make up a substantial part of mental retardation cases, but are poorly understood to date. Positional cloning in consanguineous families with multiple affected children currently represents the best strategy to identify causative genes. We interviewed and examined 68 consanguineous families of Syrian descent, with 1 - 5 affected children each. Genotyping of the first 35 families using SNP chips (Illumina or Affymetrix) allowed genome wide homozygosity mapping. We identified a single candidate region in each of 10 families: 3p26-p26 (3Mb), 4q26-4q28 (13Mb), 5q21-q21 (7Mb), 6p12-q12 (17Mb), 6q12-q15 (22Mb), 12q13-q15 (19Mb), 14q11-q-12 (9Mb), 15q23-q26 (23Mb), 16p12-q12 (24Mb), and 18p11-p11 (1.2Mb). In the remainder of the families more than one homozygous region was identified. These unique regions do not overlap. By comparing our results with published data we found that the candidate region in family MR019 (16p12-q12) overlaps with the recently described locus MRT10, and that of family MR061 (14q11-q12) with MRT9. Ongoing is the genotyping of further 33 families and whole exome sequencing of families with single linked loci. Our results support the previous observation that ARMR is extremely heterogeneous. It appears that only systematic analysis of large numbers of consanguineous families and identification of several mutations in each gene will allow identification of causative genes and ultimately contribute to understanding the neuronal network underlying normal and abnormal brain functioning. Supported by grants from the DFG and BMBF within the German Mental Retardation Network (MRNET).

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Crosstalk between histone modifications and DNA methylation in patients with intellectual disability due to JARID1C mutations. *B. Chung[1,2], D. Grafodatskaya[2], D.T. Butcher[2], S.W. Scherer[3], F.E. Abidi[4], C.E. Schwartz[4], R. Weksberg[1,2].* 1) Clinical & Metabolic Gen, Hosp Sick Children, Toronto, ON, Canada; 2) Genetics and Genome Biology, Hosp Sick Children, Toronto, ON, Canada; 3) The Centre for Applied Genomics, Hosp Sick Children, Toronto, ON, Canada; 4) Greenwood Genetic Center, South Carolina, USA.

BACKGROUND: Genes that function in epigenetic regulation play an important role in normal neurodevelopment. However, the molecular mechanisms by which multiple hierarchical epigenetic marks drive normal development are not well understood and the critical genomic targets are largely unknown. Therefore, disorders caused by mutations in genes that apply or remove epigenetic marks at specific genomic targets provide a unique opportunity to study the pathophysiology of epigenetic dysregulation in human disease. The X-linked gene, JARID1C, encodes a H3K4 demethylase. Mutations in this gene cause intellectual disability (ID). We hypothesized that JARID1C mutations would dysregulate DNA methylation at specific genomic targets. METHOD: A genome-wide approach was used to analyze sodium bisulfite modified genomic DNA from white blood cells of patients with known JARID1C mutations (n=13). The Illumina Methylation 27 Microarray with probes for 27,578 CpG sites covering >14,000 genes was used. DNA methylation profiles of patients were compared to sex- and age- matched controls. Differentially methylated CpG sites were identified using the Mann-Whitney test (absolute methylation difference >17% and p-value cut-off <0.05) with correction for multiple testing. RESULTS: Differential methylation analysis identified 17 genes with loss of CpG methylation. For 5 genes demonstrating the most significant loss of methylation in patients, the array findings were validated by pyrosequencing. Bioinformatic analyses showed that the DNA methylation alterations co-localized with the expected types of histone modifications in the target genes. CHIP-qPCR and expression array analyses, using lymphoblastoid cell lines from the same patients, are in progress. CONCLUSION: Our study has identified a specific pattern of DNA methylation alterations in patients with JARID1C mutations. These data demonstrate the functional specificity of these epigenetic regulators and also the cross-talk that occurs between histone modifications and DNA methylation in chromatin-mediated transcriptional regulation.

2555/W

Frequent disruption of SYNGAP1 in non-syndromic intellectual disability. *F.F. Hamdan[1,6], H. Daoud[2,6], A. Piton[2,6], J. Gauthier[2,6], S. Dobrzeniecka[2,6], J-C. Lacaille[3], L. Mottron[4,6], M. Beauchamp[1,5], G.A. Rouleau[2,6], J.L. Michaud[1,6], Synapse to Disease (S2D).* 1) Sainte-Justine Hospital Research Center, Montreal, QC, Canada; 2) Centre Hospitalier de l'Université de Montréal Research Center, and the Department of Medicine, University of Montreal, Montreal, QC, Canada; 3) Le Groupe de Recherche sur le Système Nerveux Central, Department of Physiology, Université de Montréal, Montréal, Canada; 4) Centre de Recherche Fernand-Séguin, Hôpital Rivière-des-Prairies, Montréal, Canada; 5) Departement of psychology, University of Montréal, Qc., Canada; 6) Centre of Excellence in Neuromics of Université de Montréal and Synapse to Disease (S2D) group, Montréal, Canada.

Background. Little is known about the genes associated with autosomal dominant non-syndromic intellectual disability (NSID). We recently identified de novo truncating mutations in *SYNGAP1*, which encodes a Ras/Rap GTPase activating protein that is critical for cognition and synapse function, in 3 out of 94 (3%) sporadic NSID cases that were screened and none in controls [Hamdan etal., 2009 *NEJM* 360(6):46-52)], suggesting that *SYNGAP1* disruption may represent a common cause of autosomal dominant NSID. In order to further explore this possibility, we sequenced in this study all *SYNGAP1* exons and intronic boundaries in 50 additional sporadic cases of NSID and 380 control individuals. **Results**. We identified a splicing mutation (c.2294+1G>A) and a 1-bp deletion causing a frameshift (c.2677delC, p.Q893RfsX183) in *SYNGAP1* in 2 patients with NSID whereas no splicing or truncating mutations were found in controls. RT-PCR and sequencing showed that the c.2294+1G>A mutation causes skipping of exon-13, resulting in a frameshift and a premature stop codon. Both mutations are de novo and are predicted to truncate SYNGAP1 upstream of important functional domains. No truncating or deleterious mutations were identified in controls. **Conclusions.** These results further confirm that de novo truncating mutations in SYNGAP1 represent a common cause of NSID, explaining ~ 3% of the sporadic cases.

2556/W

Mitochondrial Polymorphisms Impact Mitochondrial Function and Outcomes Attained after Severe Traumatic Brain Injury. *Y. Conley, D. Okonkwo, S. Deslouches, S. Alexander, D. Ren.* University of Pittsburgh, Pittsburgh, PA.

Aim: Determine the impact of mitochondrial genome variation on mitochondrial function and outcomes attained after a severe traumatic brain injury (TBI). Methods: Subjects had a severe (GCS£8), non-penetrating head injury with cerebrospinal fluid (CSF) sampling, 16-70, not brain dead, and no history of cardiovascular-related diseases. Outcomes included Glasgow Outcome Scale (GOS), Disability Rating Scale (DRS), and Neurobehavioral Rating Scale (NRS) at 3/6/12 and 24 months. ATP, measured using luminometry, and lactate/pyruvate, measured using HPLC/UV detection, were measured from CSF every 24 hours for the first five days. Genotyping used a multistage design. SNPs spanning the mitochondrial genome, using the mitochip, were assessed for n=136. Then SNPs significantly associated with outcomes, neurochemical levels or both; were assessed using TaqMan® or RFLP for n=336. Multivariate and trajectory analyses were used. Results: T195C, T4216 and A10398G were significantly associated with lactate/pyruvate levels (p=0.0134, p=0.0218, p=0.0167) but only in females. T195C was associated with ATP trajectory group (p=0.0098) in the total sample. T195C was associated with GOS at 3 months (p=0.0098), but only in females, while A10398 was associated with DRS at 6 and 12 months in the total population. Conclusions: Mitochondrial genetic variation impacts outcomes attained after a severe TBI as well as mitochondrial function during the acute period, providing a potential explanation for variability in outcomes in this population. A window of opportunity exists during the acute phase following injury when intervention may impact long term outcomes. This project helps elucidate this acute period, which could lead to interventions to improve patient outcomes.

2557/W

Investigating Mitochondrial Variation in Autism. *J.L. McCauley[1], A. Hadjixenofontos[1], M.A. Schmidt[1], I. Konidari[1], W.F. Hulme[1], P.L. Whitehead[1], H.H. Wright[2], R.K. Abramson[2], R.K. Menon[3], S.M. Williams[4], D.J. Hedges[1], M.L. Cuccaro[1], J.R. Gilbert[1], E.R. Martin[1], J.L. Haines[4], M.A. Pericak-Vance[1].* 1) Hussman Institute for Human Genomics, University of Miami, Miami, FL; 2) University of South Carolina School of Medicine, Columbus, SC; 3) Dept. of Epidemiology and Dept. of OB & Gyn, Rollins School of Public Health, Emory University, Atlanta, GA; 4) Center for Human Genetics Research, Vanderbilt University, Nashville, TN.

Despite the increasing speculation that oxidative stress and abnormal energy metabolism may play a role in Autism Spectrum Disorders (ASD), and the observation that patients with mitochondrial defects have symptoms consistent with ASD, there are no comprehensive published studies examining the role of mitochondrial variation in autism. We have employed a multiphase approach to the examination of mitochondrial DNA (mtDNA) variation in autism. In our first phase, we examined 128 mitochondrial single-nucleotide polymorphisms (SNPs) common to both the Illumina 1M and Illumina 1M Duo BeadChip arrays in a dataset of Caucasian cases and controls (964 probands, 923 father controls, and 723 pediatric controls). In phase two we resequenced the entire mtDNA in a subset of these Caucasian samples, ~400 proband-father pairs (379 probands, 372 father controls), using the Affymetrix Human Mitochondrial Resequencing Array 2.0. In phase three we genotyped our entire autism cohort and pediatric controls on additional SNPs which define the major European haplogroups, using the Sequenom MassARRAY iPLEX and TaqMan genotyping platforms. Case-control tests of association for both SNPs and haplogroups were performed ultilizing Fisher's exact, χ^2, and logistic regression procedures. Examination of heteroplasmy within our resequencing dataset is ongoing. In phase one, we found no significant association between autism and any of the 128 SNPs analyzed from the Illumina GWAS panel. Our resequencing efforts identified eighteen SNPs within the entire mtDNA that demonstrated nominally significant (p£0.05)association with autism. However these SNPs failed to replicate in our extended dataset of autism cases and pediatric controls. In our third phase we analyzed 987 Caucasian autism cases and 1145 Caucasian controls (including fathers and pediatric controls). We found no evidence to suggest a significant association between any of the major European haplogroups (H,I,J,K,T,U,V,W, and X) and autism in our Caucasian dataset. These data indicate there is no strong evidence for the role of mtDNA variation in Caucasian ASDs. We cannot however rule out the potential for a more subtle or interactive effect.

2558/W

Invastigation of 22 mitochondrial tRNA genes in Persian Alzheimer's disease patients. *S. Sheibani nia[1,4], M. Houshmand[2,4], F. Assarzadegan[3].* 1) Science and Research Branch, Islamic Azad university, Tehran, Iran; 2) National InstituTe of Genetic Engineering and Biotechnology,Tehran, Iran; 3) Shahid beheshti university, Tehran,Iran; 4) special medical lab, Tehran,Iran.

Alzheimer's disease (AD) shows itself as a gradual loss of episodic memory (for instance, forgetting that a conversation took place the day before). A lot of evidence suggests that neurodegenerative diseases, including AD, stem from the abnormal accumulation of harmful proteins in the nervous system . In AD,these include Aβ peptides, the lipid-carrier protein apolipoprotein E (apoE), the microtubuleassociated protein tau, and the presynaptic protein α-synuclein, which is also involved in growth factors. A form of apolipoprotein E, apoE4, contributes to the abnormal accumulation of Aβ and tau, but probably also damages mitochondria and the cellular cytoskeleton. small number of patients (probably fewer than 1%) have early-onset AD because they have inherited autosomal dominant mutations in genes whose protein products APP, presenilin 1 (PS1) or PS2 are involved in the production of Aβ peptides. Mitochondria play important role in apoptosis. Epidemiological studies suggest an increased risk of late-onset AD in offspring of mothers with AD compared to those of fathers with AD, consistent with maternal transmission patterns in AD. Moreover, advanced maternal age at birth may constitute an independent risk in the offspring. Mutations in mtDNA were suspected to be causally related since mtDNA is maternally inherited in a non-Mendelian way. The human mitochondrial genome is composed of a circular double-stranded DNA of 16 569 bp, which encodes 37 genes. These include 22 tRNAs, 2 rRNAs and 13 subunits of respiratory enzyme complexes. Since mtDNA partly encodes four out of five complexes in the oxidative phosphorylation pathway, mutations in mtDNA may result in defective energy homeostasis. A mutation identified as increased in AD patients affects a mitochondrial tRNA gene. Possible consequences for mitochondrial protein synthesis, particularly for complex I have been suggested. An increased sensitivity of cells bearing a variety of mtDNA mutations to Calcium-dependent oxidant stress was shown. Both qualitative and quantitative defects of mtDNA have been implicated in the pathogenesis of AD. 22 tRNA genes in mitochondrial genome were investigated by PCR-sequenced method for 24 AD Iranian Patients. Common deletions (~5 kb) were also determined by real-time PCR. Our results showed new mutations in tRNA genes and also percentage of mtDNA deletion was varied from patient to patient.

2559/W

A high resolution Genome-Wide Association of Prepulse Inhibition for 37 mouse inbred strains. *N. Alliey, C. Liu.* Department of Psychiatry and Behavioral Neuroscience, University of Chicago, Chicago, IL. USA.

Prepulse Inhibition (PPI) of the startle response is a largely studied endophenotype related to psychosis and mental disorders that is studied similarly in humans and mice. Based on data mining, we retrieved PPI data from 37 different inbred mouse strains from the publicly available Mouse Phenome database, and ran a Genome-Wide association analysis using 2 different platforms, with 132K and 4M SNPs respectively, using the EMMA Server at UCLA. Statistically significant association with PPI is found in a region of Chromosome 1 containing the gene Scg2, only region that survived Bonferroni multiple test correction. Scg2 is expressed in brain, involved in the packaging or sorting of peptide hormones and neuropeptides into secretory vesicles, and is highly conserved among mammals. Pathways and comparative analyses to human are also performed. Our finding of this Scg2 is consistent with a previous report using low resolution genetic map, but only a single region produced strong association using high density mapping. Scg2 is proposed as candidate gene for the studied trait.

2560/W

TAA repeat variation in the GRIK2 gene does not influence age at onset in Huntington's disease. *J.H. Lee[1], J.M. LEE[1], M. Ramos[1], T. Gillis[1], L. Mysore[1], A.E. Hendricks[2], R.M. Myers[2], V.C. Wheeler[1], M.E. MacDonald[1], J.F. Gusella[1].* 1) Molecular Neurogenetics Unit, Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA 02114, USA; 2) Department of Neurology, Boston University School of Medicine, Boston, MA 02118, USA.

In Huntington's disease (HD), genetic modifiers in addition to the HD CAG repeat lengths play a significant role in determining age at onset (AO). GRIK2, a mediator of excitatory neurotransmission in the brain, has been suggested to be a modifier gene, but the results are still controversial. To confirm GRIK2 modifier effect in HD, we collected all available samples over twenty-five hundreds from different cohorts and tested this using different analysis methods (linear regression analysis using both global regression line and own regression line, extreme analysis). No significant associations of the GRIK2 TAA repeat polymorphism for HD modifier were seen in all analysis from the large sample size. However, the subgroup analysis using brain samples shows a trend towards association but did not reach statistical significance. These results indicate that GRIK2 does not have significant HD modifier effect and previously shown positive association may be due to unrepresentative small sample size.

2561/W

Case-control association study of PARK2 exon rearrangements in Parkinson disease using an array comparative genomic hybridization analysis. *J. Mitsui[1], Y. Takahashi[1], T. Matsukawa[1], J. Goto[1], Y. Saito[2], S. Murayama[3], S. Tsuji[1].* 1) Dept of Neurology, Univ of Tokyo, Tokyo, Japan; 2) Dept of Pathology, National Center Hospital of Neurology and Psychiatry, Tokyo, Japan; 3) Dept of Neuropathology, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan.

[Background] PARK2 is the responsible gene for autosomal recessive juvenile parkinsonism (AR-JP), which are estimated to account for as much as 30-40% of early-onset Parkinson disease (PD). It has been reported that most of the mutations in PARK2 are gross exon rearrangements. Since single heterozygous mutations in PARK2 in sporadic cases may also increase susceptibility to PD, the knowledge of the frequency and spectrum of PARK2 variants in both PD cases and controls is needed. [Patients and Methods] We screened 145 PD cases (mean age at onset, 50.1 years old) and 148 controls for PARK2 exon deletions and duplications using an array comparative genomic hybridization analysis. All the participants were Japanese, and the PD cases were not selected based on an age at onset or a family history of PD. In 145 PD cases, 27 were early-onset (age at onset younger than 40) and 118 were late-onset (age at onset older than 40). Forty-three cases (29.7%) had a family history of PD. [Results] In 27 early-onset PD cases, 8 (29.6%) had homozygous or compound heterozygous rearrangements that were compatible with AR-JP. In 118 late-onset PD cases, 2 (1.7%) had homozygous or compound heterozygous rearrangements that were compatible with AR-JP (ages at onset were 43 and 47 years old, respectively), and 1 (0.8%) had a heterozygous rearrangement (age at onset was 75 years old). None of the 148 controls had any PARK2 rearrangements. The one carrier patient was a 76-year-old man when examined. At 75 years of age, he showed irritability and suffered from hallucination. Three months after onset, he developed bradykinesia and rigidity with gait disturbance. It was revealed that his sister also developed parkinsonism and dementia at 80 years of age, and that she had the same heterozygous rearrangement. [Conclusions] Among early-onset PD, the frequency of patients with AR-JP was similar to what has previously been reported. Although further large-scale studies will be needed to determine the implication of the heterozygous rearrangement in PARK2 in late-onset PD, the frequency of a heterozygous rearrangement was low in the present study, which suggests that it is likely irrelevant in the majority of late-onset PD.

2562/W

***LINGO1* is not associated with Parkinson's disease in German patients.**

S. Thier[1], S. Klebe[1], D. Lorenz[1], M. Nothnagel[2], S. Schreiber[3], C. Klein[4], J. Hagenah[4], M. Kasten[4], D. Berg[5], K. Srulijes[5], T. Gasser[5], G. Deuschl[1], G. Kuhlenbäumer[6]. 1) Neurology, University Hospital Schleswig-Holstein, Campus Kie, Kiel, Germany; 2) Institute of Medical Informatics and Statistics, Christian-Albrechts University, Kiel, Germany; 3) Institute of Clinical Molecular Biology, Christian-Albrechts University Kiel, Germany; 4) Section of Clinical and Molecular Neurogenetics at the Department of Neurology, University of Lübeck, Germany; 5) Hertie-Institute for Clinical Brain Research, Tübingen, Germany; 6) Institute of Experimental Medicine, Christian-Albrechts University, Kiel, Germany.

Background: Essential tremor (ET) and Parkinson's disease (PD) are the most common movement disorders and show clinical, genetic and pathophysiological overlap. Single nucleotide polymorphisms (SNPs) in the leucine-rich repeat (LRR) and immunoglobulin (Ig) domain-containing, Nogo receptor-interacting protein gene (*LINGO1*) are associated with ET. *LINGO1* is overexpressed in the substantia nigra (SN) of PD patients and inhibition of LINGO1 can protect dopamineric neurons against degeneration in a rodent model of PD. **Objectives:** Three *LINGO1* SNPs (rs8030859, rs9652490, and rs11856808), which were recently associated with ET, were tested for association with PD in three German case-control samples. **Methods:** Three large independent German case-control samples with 1798 cases and 1482 controls were genotyped for the three *LINGO1* SNPs associated with ET. Association was assessed using allele and genotype based tests in each of the three samples separately, in the combined sample, and in subsets of patients with early-onset PD (<50 years) and of patients with a positive family history of PD. **Results:** Neither of the three samples alone nor the combined sample showed evidence for association between *LINGO1* SNPs and PD. The subsets with early-onset PD or a positive family history did also not reveal evidence for association. **Conclusion:** SNPs in the *LINGO1* gene associated with ET could not be shown to be associated with PD in our three independent large German samples. Despite clinical and pathophysiological overlap a molecular-genetic link between the two common neurological disorders PD and ET is still missing.

2563/W

The Paradox of Williams Syndrome: An imaging genetics study of non-social anxiety and hypersociability.

J.U. Blackford[1], E.M. Dykens[2,3], T.A. Thornton-Wells[2,3,4]. 1) Psychiatry, Vanderbilt University, Nashville, TN; 2) Kennedy Center for Research on Human Development, Vanderbilt University, Nashville, TN; 3) Psychology & Human Development, Vanderbilt University, Nashville, TN; 4) Center for Human Genetics Research, Molecular Physiology & Biophysics, Vanderbilt University, Nashville, TN.

Williams syndrome (WS) is a neurodevelopmental disorder caused by a microdeletion of about 25 genes on chromosome 7q11. Anxiety and fears are robustly associated in the general population with inhibited temperament. However, persons with WS are engaging and socially disinhibited while still having significant nonsocial fears. We employed two novel control groups—persons with inhibited (IT) or uninhibited temperament (UT)—to provide a match for the two key features of this dissociation. Ten subjects (4 females) per group were recruited. A functional MRI study was conducted using a block design in which subjects passively viewed social and non-social images of three different emotional valences (fear, neutral, or happy). We performed a region of interest analysis comparing amygdala response to the nonsocial and social images for each emotion. Continuous measures of brain activation were extracted for genetic analysis. Candidate genes were chosen from genetic pathways related to mood, sociability and appetite, including GTF2IRD1, located in the WS deletion region. 38 SNPs and 6 CNVs in these genes were assayed using TaqMan technology and passed quality control. There were significant group differences in amygdala response to the nonsocial and social stimuli for the fearful stimuli, with lower amygdala activation to viewing social versus nonsocial fear stimuli in the WS and UT groups, which might underlie heightened sociability, or decreased social inhibition. In contrast, the IT group had similar amygdala activation to social versus nonsocial stimuli, reflecting a more general, indiscriminate fear response, or hyper-vigilance. Logistic regression using an additive allelic model showed a trend for association between amygdala activation to non-social fear stimuli and 2 SNPs---rs1042713 in 5-HTT and rs1629816 in GHRL. Association was dependent on group status, with IT and WS groups showing similar genotype-dependent responses to non-social fearful stimuli. Larger sample sizes will be needed to confirm these moderate effects (estimated Cohen's f=0.09).

2564/W

Novel mutation in *VAMP1* gene causes hereditary spastic ataxia in 3 Newfoundland families mapping at the *SAX-1* locus.

C.V. Bourassa[1], I.A. Meijer[1], N.D. Merner[1], K.K. Grewal[2], M.G. Stefanelli[2], E.J. Ives[2], P.A. Dion[1], G.A. Rouleau[1,3]. 1) The Centre of Excellence in Neuromics, CHUM Research Center and the Department of Medicine, Montreal, Quebec, Canada; 2) Discipline of Medical Genetics and Division of Neurology, Faculty of Medicine, Health Sciences Center, Memorial University, Saint John's, Newfoundland; 3) Sainte-Justine Hospital, Montreal, Canada.

Hereditary spastic ataxia (HSA) is a large group of neurodegenerative disorders characterized by lower-limb spasticity and generalized ataxia. The neuronal tracts involved are pyramidal and cerebellar, which is why the phenotype is different from spinocerebellar ataxia (SCA) and hereditary spastic paraplegia (HSP). Three large families from Newfoundland were found to have an autosomal dominant form of this disease, and they were all linked to the *SAX-1* locus on chromosome 12p13 in 2002; which was the first locus mapped for autosomal dominant HSA. Since then, the causative gene has been looked for actively and we recently observed a variant in the synaptobrevin 1 gene (*SYB1*); also known as vesicule-associated membrane protein 1 (*VAMP1*). VAMP1 is one of the three soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) proteins involved in neuronal exocytosis in response to calcium; these proteins are thought to drive the fusions of opposing membranes. A direct sequencing of a few key family members from all 3 pedigrees has revealed a heterozygous base pair change that is predicted to affect the splicing of the VAMP1A and VAMP1B isofoms, one being cytoplasmic and the other mitochondrial. *VAMP1* has another annotated isoform, deemed to be cytoplasmic, and the missense variant is predicted to be damaging to this protein, according to SIFT and SNAP; the other softwares could not align the sequence properly due to the fact that other species lack this specific isoform. The validation of the mutation involved sequencing of all three families through which we found the variant to be segregating in the family members with the *SAX-1* haplotype. Moreover, the family members that do not have the haplotype do not have the variant and sequencing of random population controls allows us to see if this variant is common in the Newfoundland population. Also, sequencing of cDNA from two patients and one control sample, a French-Canadian person, allows us to test for the effect of the splicing. Further validation of this *VAMP1* variant will consist on protein expression using lymphoblastoid cell lines derived from key HSA patients and cerebellum of human control samples.

2565/W

Strong evidence that multiple genes involved in glutamate neurotransmission interact to modulate risk of schizophrenia.

L.M. Brzustowicz[1], Y. Huang[2], S.C. Seok[2], J.E. Hayter[1], J.S. Messenger[1], R.A. Zimmerman[1], A.S. Bassett[3], V.J. Vieland[2]. 1) Department of Genetics, Rutgers University, Piscataway, NJ; 2) Battelle Center for Mathematical Medicine, The Research Institute at Nationwide Children's Hospital, Columbus, OH; 3) Department of Psychiatry, University of Toronto, and Centre for Addiction and Mental Health, Toronto, ON, Canada.

We have previously demonstrated linkage between 1q23 and schizophrenia and linkage disequilibrium (LD) with markers within NOS1AP, a gene involved in glutamate neurotransmission, in a set of Canadian families of European descent. We have reported significantly increased expression in schizophrenia of NOS1AP in postmortem brain samples. We have shown that rs12742393, a SNP within NOS1AP, is in strong LD with schizophrenia, and that the disease associated allele can significantly increase expression from the NOS1AP promoter in a cell culture system. To search for possible epistatic interactions between NOS1AP and other risk genes we have reanalyzed our microsatellite genome scan data from the Canadian families, conducting a two-locus linkage analysis with rs12742393 set as a fixed locus. A baseline, single locus linkage scan produced four peaks (1p, 2p,13q,17q) with Posterior Probabilities of Linkage >20% in addition to the peak at NOS1AP (PPL=97%). Excluding the region around NOS1AP, the two-locus analysis produced eight peaks with a PPL>20%. SNPs from under these linkage peaks available from Affymetrix 6.0 arrays were then tested for evidence of association to schizophrenia in these families individually and in a two locus analysis allowing for epistatis with NOS1AP. Six peaks (1p, 2p, 2q, 3p, 7p, 13q) contained clusters of multiple SNPs that produced Posterior Probabilities of Linkage Disequilibrium Given Linkage (PPLD|L) >20%. SNPs within the genomic extent of GRM7 and GPR39, genes also involved in glutamate neurotransmission, produced evidence of LD in epistasis with NOS1AP. We next sought to extend these results to another sample and used subjects from the schizophrenia collections in dbGAP. Despite no significant evidence of association of either gene individually with schizophrenia in this sample, we obtained significant evidence of an epistatic interaction between NOS1AP and GRM7 that modulates schizophrenia risk. Our results support the possibility that epistatic interactions can obscure linkage and association signals and could contribute to inconsistent "replication" across data sets. Using a 2-locus method, we have demonstrated strong evidence of gene x gene interaction between NOS1AP and additional schizophrenia susceptibility loci involved in glutamate neurotransmission.

2566/W

Regulators of CHRNA5 gene expression and their association with nicotine dependence: A systems biology approach. *R.F. Clark[1], L.J. Bierut[2], A.M. Goate[2], J.A. Stitzel[3], E.O. Johnson[1].* 1) RTI International, Research Triangle Park, NC; 2) Dept of Psychiatry, Washington University School of Medicine, St Louis, MO; 3) Institute for Behavioral Genetics, University of Colorado, Boulder, CO.

Differences in gene expression in the brain influence behavior and disease susceptibility. However, the contribution of single genes to complex behavioral phenotypes is usually small. Therefore, such phenotypes need to be described in terms of networks of interacting genes. The established association between the α5 cholinergic nicotinic receptor subunit gene (*CHRNA5*) and nicotine dependence (ND) presents an attractive point from which to identify a network of co-expressed genes that may influence nicotine addiction. In this study we used *in silico* systems biology tools to identify networks of genes that are co-regulated with Chrna5 in mice and to evaluate the association of nominated regulatory candidate genes with ND in humans.

We used expression-QTL mapping to identify the sources of variation in gene expression in the mouse hippocampus and to enable global mapping of factors regulating such transcriptional responses. We investigated natural genetic variation in gene expression levels of >39,000 transcripts in related mouse strains in two RI panels of C57BL/6J and DBA/2J to gain insights into molecular networks that might alter the transcription of Chrna5 mRNA. SNPs in positive candidate genes were then tested for association with ND in humans.

We found that the expression of >25 proximal genes on chromosome 9 (including Chrna5) in mice are coordinately expressed and cis-regulated. We additionally found that Chrna5 has two statistically significant trans-QTLs on chromosomes 13 and 17. Genes whose expression correlates most similar to those of Chrna5 in both RI panels include three genes proximal to Chrna5 on chromosome 9 and two genes located within the trans-eQTL loci for Chrna5. SNPs within the human homologues of these two genes were associated with ND in humans with odds ratios of ~1.2 and p-values <0.05.

These analyses suggest that we have found two good candidate genes for regulating hippocampal expression of Chrna5 in mice (and *CHRNA5* in humans). Both genes are highly expressed in the hippocampus, as well as in several other brain regions. Furthermore, these two genes are novel candidates for involvement in nicotine addiction pathways. These findings emphasize the utility of a systems biology approach to analyze complex data to model interacting biological processes.

2567/W

Association analysis of AVPR1A in Finnish autism families. *K. Kantojärvi[1], P. Onkamo[2], J. Oikkonen[1,2], L. Ukkola-Vuoti[1], R. Vanhala[3], I. Järvelä[3,4].* 1) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 2) Department of Biological and Environmental Sciences, University of Helsinki; 3) Department of Child Neurology, Helsinki University, Helsinki, Finland; 4) Laboratory of Molecular Genetics, Helsinki University Central Hospital, Helsinki, 00029, Finland.

Autism spectrum disorders are characterized by defects in language development and social communication as well as by routines and rituals. In humans as well as other mammals the hormone arginine vasopressin (AVP) has a prominent role in affecting many social, emotional and behavioral traits, including pair bonding and altruism (Donaldson and Young 2008). The AVP receptor 1A, that is coded by the AVPR receptor 1A gene, mediates the influences of the AVP hormone in the brain (Fink et al., 2007). We have previously shown association of the AVPR1A haplotypes with auditory structuring ability in music (KMT) (Ukkola et al., 2009). Three independent studies have reported an association of AVPR1A and autism (Kim et al.,2002; Wassink et al., 2004; Yirmiya et al., 2006). Based on comparative phylogenetic analyses the site of regulation of AVPR1A expression is currently unknown (Rosso et al., 2008). We have analyzed the association of AVPR1A in Finnish families with autism and compared the allele and haplotype associations with those obtained in musical families (Ukkola et al., 2009). A total of 170 Finnish families with 626 members, each with at least one autistic proband, participated in the study. The diagnosis was based on ICD-10 and DSM-IV. Highly variable microsatellites RS1 and RS3 residing in the promoter region and the AVR microsatellite in the intron of the AVPR1A gene were genotyped. The allele 4 at marker RS3 showed nominally significant association with autism (p=0.042) using FBAT. In previous studies RS3 have been associated in autism with differing alleles (Kim et al. 2002; Wassing et al. 2004, Yirmia et al. 2006). The global haplotype analysis of all three markers RS3-RS1-AVR showed overall association in autism (UNPHASED p-value 0.0490). Previously, the same 3-marker area has shown positive association in autism (p-value 0.004) (Yirmiya et al. 2006). Furthermore, a specific AVPR1 haplotype, containing allele 4 from RS3 and allele 4 from AVR, suggested a positive association in autism (p-value 0.041). Interestingly, this haplotype has also been associated with musical aptitude (p=0.005) (Ukkola et al. 2009). Similar AVPR1A haplotypes showing association with two very different phenotypes, autism and musical aptitude, might suggest that the regulatory region of AVPR1A will reside outside the promoter region. Currently, more families are being genotyped and thus, refined results will be presented in the meeting.

2568/W

Search for mutations in the DMXL1 gene on chromosome 5 associated to a Prader-Willi like phenotype. *J.L Mandel[1,2,3], C. Seifert[2], C. Gasnier[2], N. Haumesser[1], J. Böhm[1], J. Laporte[1], J. Muller[1,2], N. Calmels[2].* 1) IGBMC (CNRS, INSERM, Universite de Strasbourg), Illkirch Cedex, France; 2) Laboratoire de Diagnostic Genetique, NHC, CHU Strasbourg; 3) College de France, Paris.

The DMXL1 gene has been proposed recently to be mutated in patients with a Prader-Willi like phenotype (Gokhale et al., abstracts at ASHG 2008 and ASHG 2009), with the finding of novel heterozygous missense variants in 14/114 patients. It was orally reported at ASHG that most of these variants occurred in exons 20-24 of this gene, encoding a large (3027aa) WD repeat protein. We have sequenced either directly (the large exon 24) or after HRM screen (exons 20-23) these exons that encode 20% of the protein, in more than 300 patients refered to our molecular diagnostic lab for a broadly defined Prader-Willi like phenotype, and in whom no chromosome 15 anomalies were observed. We found 7 novel missense variants, 5 of which observed in a single patient each. The two recurrent missenses appear non pathogenic, as they have been found in controls (M1589V), or (I2000V) in one patient later found to carry a 16p11.2 deletion. Among the 5 variants observed only once, 3 were predicted as possibly or probably pathogenic using Polyphen. We expect to be able to analyse the parents to investigate whether the variants occurred *de novo* and will report on the phenotype of the patients. Our results suggest a rarer frequency of possibly pathogenic variants than reported by Gokhale et al.

2569/W

A Common *SNCA* Allele Modifies Risk for Parkinson's Disease, Independent of REP1, and is Associated with Increased Plasma α-Synuclein. *I.F. Mata[1,2], M. Shi[3], P. Agarwal[4], K.A. Chung[5,6], K.L. Edwards[7], S.A. Factor[8], D.R. Galasko[9], C. Ginghina[3], A. Griffith[4], D.S. Higgins[10], D.M. Kay[11], H. Kim[1,2], J.B. Leverenz[1,2,12], J.F. Quinn[5,6], J.W. Roberts[13], A. Samii[1,2], K.W. Snapinn[7], D.W. Tsuang[1,12], D. Yearout[1,2], J. Zhang[3], H. Payami[11], C.P. Zabetian[1,2].* 1) Dept Neuroscience, VA Puget Sound Healthcare Sys, Seattle, WA; 2) Department of Neurology, University of Washington School of Medicine, Seattle, WA; 3) Department of Pathology, University of Washington School of Medicine, Seattle, WA; 4) Booth Gardner Parkinson's Care Center, Evergreen Hospital Medical Center, Kirkland, WA; 5) Department of Neurology, Oregon Health and Science University, Portland, OR; 6) Portland Veterans Affairs Medical Center, Portland, OR; 7) Department of Epidemiology, University of Washington, Seattle, WA; 8) Department of Neurology, Emory University School of Medicine, Atlanta, GA; 9) Department of Neurosciences, University of California, San Diego, La Jolla, CA; 10) Neurolgy Service, Samuel Stratton VA Medical Center, Albany, NY; 11) Genomics Institute, Wadsworth Center, New York State Department of Health, Albany, NY; 12) Department of Psychiatry and Behavioral Sciences, University of Washington School of Medicine, Seattle, WA; 13) Virginia Mason Medical Center, Seattle, WA.

Background: Rare missense mutations and multiplications in *SNCA*, which encodes α-synuclein, result in autosomal dominant Parkinson's disease (PD). Our group and others have also shown that common *SNCA* variants, including the "REP1" promoter repeat polymorphism and several noncoding SNPs, modify susceptibility for non-Mendelian PD. Families with *SNCA* triplications display elevated expression of α-synuclein in a variety of tissues, including brain and blood, but whether a similar relationship exists for common risk alleles is unknown. We sought to further explore the association between common *SNCA* variation and PD, determine whether evidence of allelic heterogeneity exists, and examine the correlation between PD-associated variants and plasma α-synuclein levels. Methods: We performed a two-tiered analysis of 1,956 PD patients and 2,112 controls from the NeuroGenetics Research Consortium. In Tier 1 (n=692 cases and 692 controls), we genotyped a comprehensive set of 13 tagSNPs, and two additional SNPs selected from the literature. SNPs associated with PD under an additive model (α=0.05) in Tier 1 were then validated in Tier 2 (n=1,277 cases and 1,422 controls). Previously published REP1 genotypes were also included. Plasma α-synuclein was assayed in 86 cases and 78 controls using a highly sensitive Luminex assay. Results: Five SNPs were associated with PD under an additive model in Tier 1 (α=0.05). Of these, four were successfully replicated in Tier 2. In the combined sample, the most significant marker was rs356219 (OR, 1.41; CI, 1.28-1.55; p = 1.6 x 10-12) located ~ 9 kb downstream from the gene. The effect of this marker was similar in early onset, late onset, familial, and sporadic disease. A regression model containing rs356219 alone best fit the data. The linkage disequilibrium correlation coefficient between this SNP and REP1 was low (r^2=0.09). The risk-associated C allele of rs356219 was also correlated with higher transformed plasma α-synuclein levels in cases under an adjusted additive model (p= 0.005). Conclusions: Our data suggest that one or more unidentified functional *SNCA* variants tagged by rs356219 modify risk for PD, and that the effect is larger than, and independent of, REP1. We have also demonstrated for the first time that a common PD-associated *SNCA* allele correlates with increased levels of α-synuclein protein in vivo, in a dose-dependent manner.

2570/W

Genome-wide Association Analysis of Age-At-Onset in Late-Onset Alzheimer Disease Implicates Genetic Variation on Chromosome 9. *Y.S. Park[1], A.C. Naj[1], G.W. Beecham[1], M.A. Slifer[1], E.H. Powell[1], P.J. Gallins[1], I. Kondari[1], P.L. Whitehead[1], G. Cai[2], V. Haroutunian[2], J.D. Buxbaum[2], H.E. Gwirtsman[3], J.R. Gilbert[1], J.L. Haines[4], M.A. Pericak-Vance[1].* 1) John P. Hussman Institute for Human Genomics, Dr. John T Macdonald Foundation Department of Human Genetics, University of Miami Miller School of Medicine, Miami, FL; 2) Department of Psychiatry, Mount Sinai School of Medicine, New York, NY; 3) VA Medical Center, Nashville, TN; 4) Vanderbilt Center for Human Genetics Research, Vanderbilt University, Nashville, TN.

While Alzheimer disease (AD) is diagnosed in fewer than 1% of those age 65 years and younger, it affects as much as 40% of the population after age 90. AD is highly heritable (h2~70%), with much of the genetic effects uncharacterized. We examined age-at-onset (AAO) among Late-onset AD (LOAD) cases from a genome-wide association study (GWAS) to identify novel loci for LOAD, testing the variants most strongly associated with AAO for association with LOAD risk. We evaluated the association of 483,399 single nucleotide polymorphisms (SNPs) with AAO in a discovery dataset of 821 cases, with replication in a genotyped and imputed dataset of 1,243 cases from four additional case-control GWAS datasets (three publicly available). Our analyses used linear regression under an additive model with correction for population substructure in PLINK (v1.07). Associations with P£10^{-5} were followed up by (a) evaluating association in the replication case set and jointly in combined discovery/replication case sets, and (b) examining association with LOAD risk in our GWAS case-control dataset. We also examined SNP associations within strata of cases with 0, 1, or 2 copies of the APOE ε4 allele. We confirmed the association of APOE with AAO (rs2075650, P=3.387×10^{-10}; rs8106922, P=2.40×10^{-7}). We also observed a number of strong associations with AAO for SNPs on chromosome 12 in a region of linkage for LOAD (rs1010096, P=3.37×10^{-7}; rs1010095, P=1.58×10^{-6}) but these did not replicate. Only the top SNPs near APOE, rs2075650 and rs8106922, showed highly statistically significant associations with both AAO and LOAD. Stratifying by number of APOE ε4 alleles, two intronic SNPs within the Dopamine beta-hydroxylase precursor (DBH) gene demonstrated associations with lower AAO (rs1108581, P=1.76×10^{-7}; rs3025388, P=5.55×10^{-6}) among ε4/ε4 cases. DBH encodes dopamine beta-hydroxylase, an enzyme converting dopamine to noradrenalin, which is involved in catecholamine biosynthesis and norepinephrine biosynthesis pathways. Furthermore, DBH deficiency has been hypothesized to affect memory consolidation of emotional events in mice. Examining AAO in 821 cases from a GWAS of LOAD, we confirmed associations of APOE variants and observed strong associations of SNPs in a new locus of interest, DBH, with AAO among APOE ε4/ε4 cases, meriting further investigation.

2571/W

Mitochondrial DNA tRNACys mutation in a family with Frontotemporal Dementia and Parkinson's disease. *G. Provenzano[1], M. D'Amelio[4], P. Tarantino[1], V. Greco[1], V. Scornaienchi[1], S. Realmuto[4], F. Annesi[1], F.E. Rocca[1], E.V. De Marco[1], D. Civitelli[1], C. Cupidi[4], I. Manna[1], G. Savettieri[4], A. Quattrone[2,3], G. Annesi[1].* 1) Institute of Neurological Sciences, National Research Council, Cosenza, Italy; 2) Institute of Neurology, University Magna Graecia, Catanzaro, Italy; 3) Neuroimaging Research Unit, National Research Council, Catanzaro, Italy; 4) Department Experimental BioMedicine and Clinical Neurosciences, University of Palermo, Palermo, Italy.

Objective Mitochondrial dysfunction has been implicated in the pathogenesis of some neurodegenerative diseases, including Parkinson's disease (PD) and Frontotemporal dementia (FTD), because of the essential role of mitochondria in energy metabolism and apoptosis. FTD is the second most common type of primary degenerative dementia. Some patients present an overlap between PD and FTD both in neuropathological and clinical aspects. This may suggest a similar physiopathology and an involvement of mitochondrial DNA in FTD, as it has been associated to PD. Patients and Methods In order to explore whether mitochondrial mutations contribute to the susceptibility of these diseases, we analysed an italian family with parkinsonism and FTD. Genomic DNA was isolated from whole blood and the entire mitochondrial gene was amplified by PCR and sequenced. We also screened the proband of the family for PINK1, DJ-1, LRRK2, PGRN and TAU genes and we excluded mutations in these gene. Results From the sequencing of the entire mitochondrial genome, we identified a G5783A homoplasmic mutation, already reported in literature. This mutation was identified in the proband (FTD + PD), in his brother (FTD), in his maternal uncle (PD), in his mother, in his sister but not in his father. Restriction enzyme digestion revealed absence of the mutation in 50 controls. Conclusion This mutation occurs at the T arm of tRNAcys, resulting in the disruption of the stem structure, which may reduce the stability of the tRNA. In conclusion, we provided further evidence of the involvement of mitochondrial DNA variation in PD and FTD.

2572/W

***IL1B* and *PTPRM* genes are associated with mesial temporal lobe epilepsy.** *R.O. Santos[1,4], M.S. Silva[1,4], R. Secolin[1,4], C.L. Yasuda[2,4], T.R. Velasco[3,4], A.C. Sakamoto[3,4], F. Cendes[2,4], I. Lopes-Cendes[1,4], C.V. Maurer-Morelli[1,4].* 1) Department of Medical Genetics, Faculty of Medical Sciences, University of Campinas, Campinas-SP, BRAZIL; 2) Department of Neurology, Faculty of Medical Sciences, University of Campinas, Campinas-SP, BRAZIL; 3) Department of Neurology, Psychiatry and Clinical Psychology, University of São Paulo, Ribeirão Preto-SP, BRAZIL; 4) CINAPCE - Interinstitutional Cooperation for Brain Research.

BACKGROUND: Epilepsies are a group of chronic neurological disease characterized by recurrent seizures. Mesial temporal lobe epilepsy (MTLE) is one of the most common and intractable forms of epilepsy showing a complex mode of inheritance. Previous studies have associated polymorphisms in pro-inflammatory cytokine interleukin 1-beta (*IL1B*) gene and increased predisposition to MTLE associated; however these findings are still controversial. In addition, we have found that the protein tyrosine phosphatase, receptor type, M gene (*PTPRM*) is up-regulated in brain tissue from patients with MTLE. *PTPRM* gene product regulates a variety of cellular processes including cell growth, differentiation and mitotic cycle. OBJECTIVE: To investigate if *IL1B* and *PTPRM* genes are associated with the phenotype in mesial temporal lobe epilepsy (MTLE). DESIGN/METHODS: DNA samples were obtained from 204 unrelated patients with MTLE, as well as 204 unrelated controls, with no history of epilepsy. We selected five SNPs within *IL1B* and 110 SNPs within *PTPRM* from HapMap database. SNPs were genotyped using the SNPlex™ genotyping system (Applied Biosystems). Minor allele frequency (MAF>0.05), linkage disequilibrium (r^2>0.8) and Hardy-Weinberg equilibrium (HWE $_{pvalue}$>0.05) were estimated using the HAPLOVIEW software. Statistical analysis was performed by a logistic regression model with Bonferroni correction for multiple comparisons. RESULTS: We found association between SNP rs3730364 in the *IL1B* gene and MTLE (p=$1.4\text{x}10^{-14}$, OR=0.11; 95%CI: 0.06 - 0.21). Furthermore, we found 11 SNPs in with *PTPRM* gene which were significantly associated with MTLE (rs727037, rs638251, rs671369, rs1443616, rs1016188, rs583909, rs565798, rs9807775, rs8087904, rs3786368, rs727027 and rs634438) (p=$1.2\text{x}10^{-11}$, OR= 0.12; 95%CI: 0.07-0.24 for rs727027). CONCLUSIONS/RELEVANCE: Our association study shows that there is a relationship between one SNP in the *IL1B* gene as well as several SNPs in the *PTPRM* gene and MTLE. However, none of these SNPs appear to be functional variants. Although much progress has been made in the characterization of genes for the monogenic and rare forms of epilepsy the common epilepsy syndromes, usually showing complex inheritance remain a major challenge for gene identification, our study hopes to shed some light into this area. Supported by FAPESP.

2573/W

Deletions of the *DCC* gene cause congenital mirror movements. *B. Tarshish[1], A. Moreno-de-Luca[1], L. Wang[2], H. Mao[2], K.R. Schmidt[1], K. Rudd[1], L.M. Knight[1], C.L. Martin[1], D.H. Ledbetter[1], M.R. Rossi[1].* 1) Department of Human Genetics, Emory University School of Medicine, Atlanta, GA; 2) Department of Radiology, Emory University School of Medicine, Atlanta, GA.

Mirror movements (MM) are contralateral involuntary movements that mirror voluntary ones. MM are occasionally present in healthy children, but persistence beyond age 10 is considered abnormal. Mutations in the *DCC* (deleted in colon cancer) gene were recently identified as the cause of congenital MM in two pedigrees with multiple affected family members (Srour et al. 2010). Here, we report an 11-week-old female referred to genetics for agenesis of the corpus callosum, ventriculomegaly, and colpocephaly with thinning of the periatrial white matter. She was generally non-dysmorphic, but was noted to have a marked frontal hair upsweep, suggestive of abnormal brain development. We performed array comparative genomic hybridization analysis and identified a 655 kb interstitial deletion of chromosome 18q21.2 that includes the 3' end (exons 23-29) of the *DCC* gene (chr18:49229956-49884580; hg18 genome assembly). Subsequent FISH analysis on the proband's father revealed that he has the same deletion. Interestingly, he has a history of MM since early childhood that is not associated with other neurologic abnormalities. To further characterize this condition, we performed a brain MRI exam on the father using high-resolution T1 and T2-weighted structural MRI, diffusion tensor imaging and tractography, and functional MRI. MRI findings suggested an abnormal corpus callosum with a thin genu and thick splenium, the presence of a fifth ventricle, and mild atrophy in the superior portion of the cerebellum. In conclusion, our findings confirm that haploinsufficiency of the *DCC* gene causes MM, possibly due to a defect in axonal midline guidance and inter-hemisphere connection. Furthermore, these results expand the phenotype associated with *DCC* mutations to include structural brain abnormalities, especially of the corpus callosum. We therefore recommend mutation analysis of *DCC* in individuals with similar phenotypes.

2574/W

Resequencing of a ~60Kb genomic region at Chr1q21 centered on the GBA locus in Ashkenazi Jewish Parkinson's Disease cases and controls. *M. Verbitsky, J. Lee, K. Marder, L.N. Clark.* Taub Institute, Columbia University, New York, NY.

Mutations in the Glucocerebrosidase gene (GBA) located at Chr1q21 are the cause of Gaucher Disease (GD). We and others have shown that mutations in GBA are also a risk factor for Parkinson's disease (PD). More than 200 mutations have been described in the GBA gene in patients with GD including point mutations, small indels and recombinant mutations that involve a nearby pseudogene. The frequency of specific alleles varies in different populations; for example, the mutations N370S, L444P, c.84insGG and IVS2+1 G>A are the most frequent mutations in the Ashkenazi Jewish (AJ) population. Fewer point mutations have been reported in AJ PD patients (the most common mutations reported are N370S and c.84insGG) and CNVs at the GBA locus have not been described. Given the clinical and genetic heterogeneity at the GBA locus we hypothesize that additional genetic changes at the GBA locus may contribute to PD. To identify additional genetic variation, including new mutations and genetic modifiers at the GBA locus we performed deep resequencing of a ~60Kb genomic DNA region at 1q21 centered on the GBA locus in a total of 47 AJ subjects. The AJ subjects consisted of 3 groups: PD cases carrying GBA mutations (N = 22), PD cases without known GBA mutations (N = 13) and matched controls (N = 12). In addition to GBA the region analyzed included the following genes: GBAP, MTX1, MTX1P, Clorf3, CLK2 and COTE1. Libraries were prepared from equimolar pools of thirteen 4-6 kb amplicons for each sample and sequencing was performed on a Roche 454 GS titanium FLX system. Data from the sequencing run on the Roche 454 GS titanium FLX was processed using 454's standard data analysis software and aligned to the reference sequence. (Chr1:155,177,506-155,243,197, GRCh37/hg19). SAMtools was used to call targeted bases, with valid-adjacent base calls that deviate from the reference treated as potential variations (SNP/indel) and assigned a coverage-dependent Phred-scaled mutation probability. CNV-seq was used to predict CNVs in the dataset. A preliminary analysis identified an average of 38 high confidence variants per subject. We have compiled a comprehensive catalog of genetic variation at the Chr1q21 region containing GBA. Our results may shed light on the modifying effects that genetic variation in regulatory regions and genes in the vicinity of GBA have on the genetic risk for PD. This study was funded by the National Institute of Health NS050487 and NS060113 (Clark).

2575/W

Distinct genetic effects lead to different forms of panic disorder. *R. Subaran.* Biostatistics, Columbia Univ, New York, NY.

Panic Disorder is common and complex disorder characterized by recurrent and debilitating bouts of uncontrollable fear. While clearly influenced by genetic contributions, the strong heritability seen in panic disorder has been contrasted by paucity in discoveries of genes exerting a strong effect. Various genetic models, including the existence of locus heterogeneity, can explain this missing heritability. Locus heterogeneity, the presence of more than one causative disease gene in a population, drastically reduces power to detect a genetic effect for any given locus. One approach to overcome this is to examine large numbers of cases and controls. However, this approach has often yielded only modest effect sizes for common diseases making it difficult to justify the resources needed to undertake such studies. Another means of addressing potential heterogeneity is to apply strict phenotypic constraints in hopes of separating different genetic contributions through differential phenotypic assignments. Using the latter approach, we previously showed that a syndrome, in which panic disorder is accompanied by comorbid phenotypes including the rare bladder disorder interstitial cystitis (IC), is a genetically distinct form of panic disorder syndrome linked to a region of chromosome 13q. We also showed that in a separate sample, panic disorder is associated with polymorphisms in the serotonin transporter-encoding SLC6A4 gene. In our current study, we have revisited this association, this time stratifying all panic disorder cases by the presence or absence of accompanying phenotypes, including IC. We found that panic disorder cases without IC sufficiently explain the association at SLC6A4, while panic disorder cases with IC show no significant association. This suggests that panic disorder with IC has distinct genetic underpinnings from panic disorder without IC. Currently, we are examining in our stratified sample, a panel of markers consisting of a subset of SNPs reported to be associated with panic disorder in the literature. Our preliminary results are again consistent with a model of underlying genetic differences in phenotypically separated strata of panic disorder cases. Taken together our results suggest that careful stratification of individuals in cases-control analysis can be a powerful tool in resolving the common problem of locus heterogeneity in traits such as panic disorder.

2576/W

Early cannabis exposure: genetic contributions and epistatic interactions relevant to addiction risk. *M.M. Jacobs[1], X. Wang[2], E. Keller[3], D. Dow-Edwards[4], Y.L. Hurd[1].* 1) Departments of Psychiatry, Neuroscience and Pharmacology, Mount Sinai School of Medicine, New York, NY, USA; 2) Department of Clinical Neuroscience, Karolinska Institute, Stockholm, Sweden; 3) Department of Forensic Medicine, Semmelweis University, Budapest, Hungary; 4) Department of Pharmacology, State University of New York, Downstate Medical Center, New York, NY, USA.

Vulnerability to drug addiction is thought to be caused by a combination of genetic and developmental environmental factors. Human studies have linked prenatal marijuana (Cannabis sativa) exposure with increased susceptibility to drug abuse later in life. But, little is known regarding the neurobiology underlying this increased risk and there is minimal insight regarding the contributions of genetic risk to drug addiction vulnerability associated with prenatal exposure. To address these questions, we examined brain tissue from human fetuses (18-22 weeks) whose mothers used cannabis during pregnancy (95 controls and 45 cannabis exposed) for single nucleotide polymorphisms (SNPs) of genes within the endocannabinoid, dopaminergic, opioid and glutamatergic systems that are direct and indirect targets of THC and known to be important in addiction risk. SNPs within the fatty acid amide hydrolase (FAAH) gene, the dopamine receptor 1 (DRD1) gene and the cannabinoid receptor 1 (CNR1) gene were significantly associated with fetal cannabis exposure ($P<0.05$). Given the functional overlap between the neural systems of interest, we also asked if interactions between SNPs might produce larger genetic effects than individual SNPs alone. The most significant two-locus epistatic interactions were found between rs13356332 and rs4323213 of homer homolog 1 (HOMER1) and rs2075572 of opioid receptor, mu 1 (OPRM1) ($P=0.0002$ and 0.009, respectively). None of these markers were significant at the single locus level, suggesting that the combination of these SNPs increase addiction risk more than either alone. We have begun to delineate the specific gene expression profile linked to these epistatic interactions in the fetal brain. Our data demonstrate that the HOMER1 SNPs invert the normal pattern of OPRM1 mRNA expression in discrete brain regions, suggesting a possible neurobiological mechanism by which these genetic alterations may act. We also observe the same HOMER1 x OPRM1 epistatic interactions and brain mRNA expression level changes in adult heroin abusers, lending further evidence that these SNP interactions may be responsible for some genetic risk in this unique fetal sample at high risk for drug abuse. These findings demonstrate specific genetic dysregulation of neural systems involved in addiction in individuals at increased risk for later drug abuse and begin to shed light on the interaction of genetic and environmental risk for drug abuse.

2577/W

Interrogating the complex genetic effects of chromosome 16p13.13 in multiple sclerosis susceptibility. *D. Mortlock[1], R. Zuvich[1], W. Bush[1], J. McCauley[2], A. Beecham[2], P. De Jager[3,4], A. Ivinson[5], A. Compston[6], D. Hafler[4,7], S. Hauser[8,9], S. Sawcer[6], M. Pericak-Vance[2], L. Barcellos[10], J. Haines[1], International Multiple Sclerosis Genetics Consortium.* 1) Ctr Human Gen Res, Vanderbilt Univ Sch Med, Nashville, TN; 2) John P. Hussman Institute for Human Genomics, University of Miami, Miller School of Medicine, Miami, FL; 3) Program in NeuroPsychiatric Genomics, Center for Neurologic Diseases, Department of Neurology, Brigham & Women's Hospital and Harvard Medical School, Boston, MA; 4) Program in Medical & Population Genetics, Broad Institute of Harvard University and Massachusetts Institute of Technology, Cambridge, MA; 5) Harvard NeuroDiscovery Center, Harvard Medical School, Boston, MA; 6) University of Cambridge, Department of Clinical Neurosciences, Addenbrooke's Hospital, BOX 165, Hills Road, Cambridge, CB2 2QQ, UK; 7) Department of Neurology, Yale School of Medicine, New Haven, CT; 8) Department of Neurology, School of Medicine, University of California, San Francisco, CA; 9) 9 Institute for Human Genetics, School of Medicine, University of California, San Francisco, CA; 10) Division of Epidemiology, School of Public Health, University of California, Berkeley, CA.

Multiple sclerosis (MS) is characterized as a neurodegenerative, autoimmune disease of the central nervous system. Various family studies have shown that MS has a strong genetic component. With the advent of collaborative efforts in genotyping and analysis, at least thirteen non-MHC genes have been associated with MS in the past three years. With MS as for other diseases, independent candidate gene or pathway approaches have often indicated independent genetic signals arising from physically overlapping genomic regions, clouding interpretation of which specific genes are functionally involved. A ~600 KB stretch of chromosome 16 contains several MS-associated genes that were the focus of three recent publications: a candidate gene study of CIITA, an extensive follow-up analysis of CLEC16A, PRM1, PRM2, and PRM3 from a GWAS performed by the International Multiple Sclerosis Genetics Consortium (IMSGC), and a candidate pathway approach examining genes involved in the IL7RA-IL7 pathway including SOCS1. We subsequently genotyped 149 SNPs across this region (bp: 10,870,067-11,462,368). Sixty-three of the SNPs (42%) in this region had significant results (p-values<0.05); while, 43 SNPs (29%) had very significant results (p-values<0.001). Additionally, some of the SNPs near CIITA and SOCS1 had differentially significant results when the dataset was stratified by a surrogate SNP (rs3135388) for the HLA-DR*1501 allele (59/149 SNPs from HLA+ (presence of at least one risk allele) group, 36/149 SNPs from HLA- (absence of a risk allele) group, and 70/149 unique SNPs). By investigating the linkage disequilibrium in the region and using interaction analyses within logistic regression analysis, we have determined that this region may harbor three independent disease loci. Thus, we hypothesize that there may be evolutionary pressures underlying this unique genomic configuration in the etiology of MS. For the genes in this region, whole-genome expression data was examined for the possibility of correlated expression in lymphoblastoid cell lines. Two of the three independent disease loci (CLEC16A and SOCS1) show significant correlation. This could be explained by co-regulation, but functional studies will be necessary to understand the importance of this chromosome 16 region in MS pathogenesis.

2578/W

Two stage case-control association study of chromosome 6p SNPs in schizophrenia: extension of recent genome-wide association studies. *M. Bamne[1], J. Wood[1], H. Mansour[1], F. Dickerson[2], R. Yolken[3], B. Devlin[1,4], V. Nimgaonkar[1,4].* 1) Dept Psychiatry, Univ Pittsburgh, Pittsburgh, PA; 2) Sheppard and Enoch Pratt Hospital, Baltimore, MD, USA; 3) Stanley Laboratory of Developmental Neurovirology, Department of Pediatrics, Johns Hopkins School of Medicine, Blalock 1104,600 N Wolfe St., Baltimore, Maryland, USA; 4) Department of Human Genetics, University of Pittsburgh, Graduate School of Public Health, Pittsburgh, Pennsylvania 15213, USA.

Objective: To evaluate the role of variations in the HLA region in schizophrenia (SZ) genesis. The study was motivated by recent schizophrenia genome-wide association studies (GWAS), as well as our earlier published studies of MHC class I chain-related genes B (MICB). The GWAS observed genome-wide significant associations for single nucleotide polymorphisms (SNPs) across the HLA region. Our earlier studies suggested interactions between host genetic variation in the MICB gene located in HLA region and exposure to neurotropic herpes viruses in SZ risk. **Method**: In phase I, we comprehensively genotyped 61 'tag' SNPs from a ~ 100 KB region around MICB [31546573 -31657535 (NCBI genome build 36.1)] among Caucasian SZ cases (n= 517), and screened adult controls (n=314). In Phase II, we integrated SNPs from Phase I showing significant associations with selected SNPs from recent GWAS. The SNPs were genotyped among independent African-American SZ cases (n=600) and controls (n=404). **Results**: In Phase I, SNP-based tests yielded significant associations (p-value £ 0.05, uncorrected) for 11 SNPs. Phase II results also showed 8 SNPs with significant association (p-value £ 0.05). Of these, five SNPs were derived from the GWAS (rs12214031- BTN3A2_3UTR, p= 0.006; rs6932590- BTN3A2, p= 0.007; rs12199613- BTN3A2, p= 0.02413; rs9393709, p= 0.02471; rs926300, p= 0.05587). In addition, Phase II analyses continued to implicate two SNPs in the vicinity of MICB (rs6940467, p= 0.0343, OR 1.25 & rs6915833, p=0.079, OR 1.93) that were also associated in the Phase I samples. **Conclusion**: Variations in the HLA region may be associated with schizophrenia risk. Additional studies integrating exposure to neurotropic infectious agents are warranted.

2579/W

Mental Retardation Associated With Retinitis Pigmentosa in the Xp11.3 Deletion Syndrome: ZNF674 in the Dock. Guilty or Innocent? *J. Kaplan[1], N. Delphin[1], D. Bonneau[2], X. Zanlonghi[3], S. Gerber[1], JP. Bonnefont[1], A. Munnich[1], JL. Dufier[4], O. Roche[4], JM. Rozet[1].* 1) Department of Genetics, Paris Descartes University, INSERM Unit 781, Hôpital Necker-Enfants Malades, 75015 Paris, France; 2) Biochemistry - Genetics, INSERM U694 - Angers University and CHU, Angers, France; 3) Ophthology, Clinique Sourdille, Nantes, France; 4) Ophthalmology, Ophthalmology - Paris Descartes University - CHU Necker, Paris, France.

Purpose: X-linked retinitis pigmentosa (XLRP) are among the most severe retinal dystrophies owing to their early onset (1stdecade) and their rapid progession (blindness in the 3rd-4th decades). Two genes account for almost all XLRP cases: RPGR (Xp21.1-11.4; ca.70% of XLRP) and RP2 (Xp11.23; ca.30% of XLRP). In the 90s, a 1.2 Mb deletion in the Xp11.3 region was reported in two independent families segregating XLRP with mental retardation (MR). While the retinal dystrophy was ascribed to the deletion of the RP2 gene, the mental retardation was suggested to be accounted for by the loss of the ZNF674 gene which mutations were independently reported to account for isolated XLMR. Here, we report two unrelated families with XLRP with no mental retardation harbouring a large Xp11.23 deletion. The purpose of this study was to characterize the deletion boundaries and to determine whether the absence of mental retardation could be accounted for by the absence of ZNF674 deletion. Methods: Two large unrelated families were ascertained gathering respectively 7 and 2 males affected with severe forms of XLRP. Indirect studies with highly polymorphic markers of the RP3 and RP2 loci were undertaken to direct the molecular study towards one gene or the other. The Xp11.23 deletion identified in both families was characterized by PCR using STS markers of the region.Results and Conclusions: Indirect studies at the RP3 and RP2 loci in both families suggested the existence of a large deletion encompassing RP2 and polymorphic markers flanking the 5 'region of the gene. PCR-based studies of STS markers in the Xp11.23 region demonstrated that in one of the family the deletion included PHF16 (first two exons) RP2, SLC9A7, CHST7, and ZNF674 while in the second the deletion encompassed the first three exons of RP2, SLC9A7, CHST7, and ZNF674. The breakpoints of both deletions are under characterization. Owing to the loss of ZNF674, affected males patients were interviewed and examined to state on their mental development. None of them presented with any mental disabilities raising the question to know whether the loss of ZNF674 accounted for the mental retardation in families co-segreating the feature with XLRP.

2580/W

MOTOR AND MENTAL DYSFUNCTION IN WOMEN WITH FXTAS: two families with mother-daughter transmission. *L. Rodriguez-Revenga[1,2], J. Pagonabarraga[3,4], B. Gómez-Anson[4,5], O. López-Mourelo[6,7], I. Madrigal[1,2], M. Milà[1,2,8].* 1) Biochem & Molec Gen, Hosp Clinic, Barcelona, Spain; 2) CIBER de Enfermedades Raras (CIBERER), Barcelona, Spain; 3) Neurology Service. Hospital Hospital Sant Pau, Barcelona, Spain; 4) CIBER de Enfermedades Neurodegenerativas (CIBERNED), Barcelona, Spain; 5) Neuroradiology Unit, Radiology Department. Hospital Sant Pau, Barcelona, Spain; 6) Fundació de Recerca Hospital Sant Pau, Barcelona, Spain; 7) PIC, IFAE, Universitat Autonoma Barcelona, Spain; 8) IDIBAPS (Institut d'Investigacions Biomèdiques August Pi i Sunyer). Barcelona, Spain.

Fragile X-associated tremor/ataxia syndrome is a late-onset neuropsychiatric degenerative disorder that occurs predominantly in male *FMR1* premutation carriers (55-200 CGG repeats). Clinical symptoms appear in patients in their 50s or later, and they usually begin with an action tremor. Afterwards, different findings including ataxia, loss of sensation in the distal lower extremities and autonomic dysfunction may occur and gradually progress. Cognitive deficits are also observed, including memory problems and executive function deficits, with a gradual progression to dementia in some individuals. Some patients also have Parkinsonian symptoms and experience neuropsychological disturbances, including anxiety, reclusive behavior, and irritability or mood lability. Magnetic resonance imaging demonstrates cerebellar and brain atrophy, as well as white matter hyperintensities. Additionally, hyperintensities in the middle cerebellar peduncles on T2 have been described as a characteristic finding. Although FXTAS was firstly identified among older male premutation carriers, to date, it has also been described among premutated women. Even though, it has been suggested to occur less frequently, having a milder phenotype and onset at an older age. As more female cases of FXTAS are being described, a wider clinical spectrum is being reported. Nevertheless, dementia has only been described in two patients. Herein, the molecular, neurological, neuropsychiatric, psychological, cognitive, and neuroradiological features of two fragile X syndrome families with a mother-daughter FXTAS transmission are described and, interestingly, dementia is present in both mothers. ACKNOWLEDGMENTS We thank Dr. Willemsen (Rotterdam, ND) for kind antibody donation and the families for their cooperation. This work was supported by FIS 07-0770, and FIS 09-00413. The CIBER de Enfermedades Raras is an initiative of the ISCIII.

2581/W

Improving Access to Clinical and Genetic Data on Mental Disorders. *J.L. Ambite, S. Sharma, C.N. Hsu, Y. Arens.* Information Sciences Institute, University of Southern California, Marina del Rey, CA.

The NIMH Center for Collaborative Genetic Studies of Mental Disorders (CGSMD) - nimhgenetics.org - maintains a large collection of phenotypic and genotypic data on studies of autism, bipolar disorder, schizophrenia, depression, Alzheimer's disease, anorexia nervosa, attention-deficit hyperactivity disorder and obsessive-compulsive disorder, with biological samples on the corresponding affected and control individuals and families stored at the Rutgers University Cell and DNA Repository (RUCDR). The collection comprises over 64,000 subjects, over 7,000 distinct phenotypic variables, and over 2,700,000 genetic markers. These data was previously disseminated through the nimhgenetics.org website to NIMH-authorized researchers as file-based distributions (in file formats such as csv, SAS, plink, etc) for each disorder. While these distributions made this wealth of data broadly available to the community, the file-based format required a researcher to spend significant effort processing and collating data from many files in a variety of formats to select individuals of particular characteristics for further analysis. To improve the efficiency of data analysis, we developed the CGMSD Data Warehouse and Explorer. The CGSMD Data Warehouse is a relational database that includes all the data previously available as file-based distributions, but further harmonized and presented through a uniform schema that allows the researcher to query within and across diseases in a more convenient way. The warehouse not only provides more efficient and flexible access, but it also enables better curation, since many quality control checks can be implemented as database queries. Although expert users may benefit from direct access to the database, we expect that most researchers would prefer a simpler, more intuitive access to the data. Thus we developed the CGSMD Data Explorer that provides a web-based access-controlled intuitive query interface to the warehouse. A researcher can easily identify individuals that satisfy given demographic, pedigree, genetic and phenotypic characteristics (e.g., all the African-American autistic children, with samples at RUCDR, with particular value ranges on the Autism Diagnostic Interview, and their alleles for SNPs in the BDNF gene). We expect that the CGSMD Data Warehouse and Explorer will empower scientists to efficiently test novel scientific hypotheses that will lead to a better understanding of genetic architecture of mental disorders.

2582/W

Do glucocerebrosidase mutations affect the neurobiology of PD? : Dopamine synthesis and regional brain activity in *GBA*-associated parkinsonism. *O. Goker-Alpan[1, 3], J. Masdeu[2], A. Ianni[2], G. Lopez[1], D. Eisenberg[2], P. Kohn[2], C. Groden[1], M. Chalfin[2], K.F. Berman[2], E. Sidransky[1].* 1) Section on Molecular Neurogenetics, I MGB/NHGRI, NIH, Bethesda, MD; 2) Section on Integrative Neuroimaging, Clinical Brain Disorders Branch, NIMH, NIH, Bethesda, MD; 3) Center for Clinical Trials, Springfield VA.

Mutations in the gene encoding glucocerebrosidase (GBA), the enzyme deficient in Gaucher disease (GD), are a common genetic risk factor for parkinsonism. Subjects with Parkinson disease (PD) are five times more likely to carry *GBA* mutations. Although the clinical phenotype of patients carrying *GBA* mutations may resemble sporadic PD, the age-of-onset of parkinsonian manifestations is earlier, and cognitive impairment is more prevalent. We investigated whether *GBA* mutations alter the neurobiology of PD, studying *in vivo* brain dopamine synthesis as a marker of neurodegeneration, and resting regional Cerebral Blood Flow (rCBF) as an index of brain activity in 103 subjects (38F/65M). Positron emission tomography (PET) was performed using ^{18}F-Fluorodopa (FDOPA) and $H_2{}^{15}O$ to evaluate regional brain dopamine synthesis and resting rCBF respectively. Eight subjects had sporadic PD [mean age 60±6 years]; 8 had GD and parkinsonism [54±10 years]; 10 had GD but no parkinsonism [50±13 years]; and 7 were Gaucher carriers, but did not exhibit parkinsonism [50±18 years]. Subjects in the last two groups had a family history of PD. All PD subjects had similar UPDRS scores and disease duration. As the age-of-onset was earlier among *GBA* mutation carriers, each study group was compared to its own age and sex matched healthy control group, made up by twice the number of study subjects. Data were assessed with both region of interest and voxel-based methods. Striatal dopamine synthesis was markedly decreased in PD subjects regardless of *GBA* mutation status, with a greater loss in the caudal striatum (51% loss in putamen Ki), and relative sparing of the caudate (29% loss). Striatal dopamine was decreased ($p < 0.05$) in the putamen of somesubjects with GD and a family history of parkinsonism, without overt signs of parkinsonism. rCBF was decreased only in the patients with GD and parkinsonism, in a pattern resembling that in diffuse Lewy body disease. This, the first *in vivo* study evaluating the pattern of neurodegeneration in both *GBA* hetero- and homozygotes, demonstrates that although the pattern of dopamine loss in *GBA*-associated parkinsonism is similar to sporadic PD, the resting brain activity is decreased in areas affected in diffuse Lewy body disease, potentially explaining the increased cognitive impairment. The absence of a gene dosage effect may support the gain-of function-hypothesis as a mechanism for the development *GBA*-associated parkinsonian pathology.

2583/W

Disease severity and parkinsonism in rapid-onset dystonia-parkinsonism, DYT12. *B.M. Snively[1], L.J. Ozelius[2], D.F. Hill[1], L.S. Light[1], M.A. Stacy[3], A. Brashear[1].* 1) Wake Forest University School of Medicine, Winston-Salem, NC; 2) Mount Sinai School of Medicine, New York, NY; 3) Duke University and Medical Center, Durham, NC.

Rapid-Onset Dystonia-Parkinsonism (RDP), DYT12, is characterized by rapid onset and stabilization of symptoms without recovery. RDP is caused by mutation of the α3 subunit of the Na+/K+-ATPase gene (ATP1A3) and is dominantly inherited with reduced penetrance. Onset of RDP has been associated with triggers including physical and life stress, and alcohol use. To date, factors specifically associated with RDP severity have not been systematically studied. We collected movement disorder rating scale data by neurologic examination of 35 carriers of ATP1A3 mutations, including E277K, T613M, S712P, A714T, I758S, D801Y, and D923N. Each mutation was represented by one family or individual, except T613M, which was represented by two families and two unrelated individuals. Thirty mutation carriers manifested RDP, with RDP severity scores from 1 (mild limb dystonia) to 4 (severe bulbar and limb involvement with inability to walk; mean= 3.2, SD=1.0). Parkinsonism was scored 1 to 4 (absent, possible, probable, definite) by two reviewers and the two scores were averaged (mean=3.3, SD=0.7). Ages at onset of movement disorder symptoms ranged from 2 to 61 years (mean=24, SD=14). RDP severity score, Burke-Fahn-Marsden movement disorder rating scale (BFM), and Unified Parkinson's Disease Rating Scale motor score (UPDRS) did not differ by proband status, gender, and mutation origin (paternal, maternal, de novo or unknown). Parkinsonism scores were lower in probands (mean difference=0.36 versus non-probands, p=0.025) and did not differ by gender and mutation origin. Differences by ATP1A3 mutation were not evaluated due to limited numbers of independent families and individuals. RDP severity was inversely related to age at onset: Spearman correlations (SC) with onset age were -0.21 (p=0.27), -0.43 (0.019), and -0.30 (0.11) for RDP severity, BFM, and UPDRS, respectively. Age at onset and parkinsonism were unrelated (SC=0.071, p=0.71; SC=-0.17 adjusted for age at exam, p=0.38). Familial aggregation was low to moderate for age at onset (Intraclass correlation, ICC=0.0), RDP severity (0.10), BFM (0.11), and UPDRS (0.28), and was greatest for parkinsonism (ICC=0.42). In conclusion, we observed an inverse relationship between RDP age at onset and severity as measured by the BFM scale in individuals with various ATP1A3 mutations. Familial aggregation of the parkinsonism component of RDP may be due to ATP1A3 or genetic modifiers. This work is supported by NINDS grant R01NS058949 (AB).

2584/W

Expanding the phenotype of Autosomal Dominant Leukodystrophy associated with LMNB1 duplication. *C. Toro[1], SG. Ziegler[2], C. Groden[1], CD. Blair[3], K. Cao[3], H. Carlson-Donohoe[2], DR. Simeonov[2], FS. Collins[3], WA. Gahl[1,2].* 1) Undiagnosed Diseases Program, NHGRI, National Institutes of Health, Bethesda, MD; 2) Medical Genetics Branch, NHGRI, National Institutes of Health, Bethesda, MD; 3) Genome Technology Branch, NHGRI, National Institutes of Health, Bethesda, MD.

Autosomal Dominant Leukodystrophy (ADLD) is a rare disease due to duplications of LMNB1, encoding lamin B1. Patients typically present in the third or fourth decade of life, with progressive dysautonomia, spasticity and cerebellar dysfunction. Patients with ADLD are often misdiagnosed early on in their disease with multiple sclerosis. Dominant inheritance, distinct MRI features of widespread subcortical demyelination with sparing of subcortical U fibers, involvement of corticospinal tracts and middle cerebellar peduncles, and absence of inflammatory CSF changes, support the diagnosis. Histopathological abnormalities available in a few cases are restricted to glial cells. Over-expression of LMNB1 has been suggested as the mechanism leading to ADLD. We expand the phenotype of ADLD by describing an otherwise previously healthy 47 year-old man with a 5-year history of dysautonomia, spasticity, ataxia and lower extremity pain. An Illumina Omni-Quad Chip identified a ~360 Kb duplication encompassing the LMNB1 gene on chromosome 5q23.2, confirmed by multiplex PCR. The absence of family history suggested a *de-novo* duplication. PAS positive bodies in eccrine sweat glands were present in a forearm skin biopsy. Sural nerve biopsy had no axon or Schwann cell abnormalities. Immunofluorescence staining for nuclear envelop proteins revealed abnormal nuclear morphology. The patient's cultured fibroblasts had increased nuclear blebbing at passage 7 (30% compared to 11% in controls); cellular senescence was also enhanced, as measured by senescence associated β-galactosidase assay. Melanocytes grew slowly, with ballooned cytoplasm and misshapen nuclei (76% blebbed vs 47% in controls; 11% binucleated vs 0% in controls). However, qRT_PCR and Western blotting revealed that lamin B1 mRNA and protein were not over-produced in our patient's cultured fibroblasts. The role of lamin B1 as an integral structural component of the nuclear envelope explains the morphological changes expressed in cultured cell lines from our patient. In addition, lamin B1 exerts transcription regulatory roles through its dynamic interactions with chromatin. A disturbance in these regulatory processes might be central to the emergence of demyelination in AOLD. Our patient's cells provide a model system to study the LMNB1-dependent events, including those required for myelin maintenance in the adult human brain. The cells also provide a venue in which to investigate therapeutic interventions.

2585/W

Clinical and Behavioural phenotype associated with 12q24.3 subtelomeric duplication. *A.D. Rasalam[1], J.C. Dean[2].* 1) Learning Disability Team, Royal Aberdeen Childrens Hosp, Aberdeen, United Kingdom; 2) Department of Clinical Genetics, Ashgrove House, Foresterhill, Aberdeen, United Kingdom.

In Aberdeen, Scotland, children with developmental delay undergo genetic testing for identification of an underlying cause for their difficulties. This includes standard karyotyping, Fragile X testing and MLPA for subtelomeric duplications and deletions and also for microdeletions and duplications commonly associated with a learning disability. Among the children who have had an underlying cause identified, there are 3 families with a subtelomeric duplication of 12q24.3 including ZNF10. Two of the index cases are boys and the other is a girl. The duplication was paternally inherited in two of the families. All the children have developmental delay and speech and language difficulties. Features of inattention, hyperactivity and impulsiveness are more prominent in the boys. Their symptoms fulfil diagnostic criteria for Attention Deficit Hyperactivity Disorder (ADHD). We have achieved good control of the symptoms using Atomoxetine and Methylphenidate in one of the children and are in the process of initiating treatment in some of the others. All the children are in receipt of special educational support and treatment from Speech and Language Therapy. We have assessed one of the fathers who also carries the duplication and he had features that are consistent with ADHD as a child and he has continued to have difficulties into adulthood. Studies into ADHD have previously found susceptibility loci on Chromosome 12q. Cappellacci et al, American Journal of Medical Genetics Part A 140A:1203-1207 (2006) published a case report of pure 12q22q24.33 duplication with features of developmental delay and ADHD responsive to Fluoxetine treatment. Our cases are described to contribute to the clinical phenotype associated with 12q subtelomeric duplication.

2586/W

Mutations in Polynucleotide Kinase 3' Phosphatase Cause the Novel Neurological Disease, Microcephaly with Seizures. *E.C. Gilmore[1,2], J. Shen[3], C.A. Marshall[3], M. Haddadin[4], J.J. Reynolds[5], W. Eyaid[6], A. Grix[7], R.S. Hill[3], M. Topcu[8], K.W. Caldecott[5], A.J. Barkovich[9], C.A. Walsh[1,3,10].* 1) Division of Genetics, Children's Hospital Boston, Harvard Medical School, Boston, MA; 2) Division of Child Neurology, Department of Neurology, Massachusetts General Hospital, Harvard Medical School, Boston, MA; 3) Howard Hughes Medical Institute, Department of Neurology, Beth Israel Deaconess Medical Center and Program in Neuroscience, Harvard Medical School, Boston, MA; 4) Department of Pathology, Al-Bashir Hospital, Ministry of Health, Amman, Jordan; 5) Genome Damage and Stability Centre, University of Sussex, Falmer, Brighton, UK; 6) Genome Damage and Stability Centre, University of Sussex, Falmer, Brighton, BN1 9RQ, UK 6Genetics & Endocrinology, Department of Pediatrics Mail Code 1510, King Fahad National Guard Hospital, King Abdul Aziz Medical City, Saudi Arabia; 7) Department of Medical Genetics, Kaiser-Permanente Point West Medical Offices, Sacramento, CA; 8) Hacettepe University, Ihsan Dogramaci Children's Hospital, Section of Pediatric Neurology, Ankara, Turkey; 9) Department of Radiology, University of California at San Francisco, San Francisco, CA; 10) Broad Institute of Massachusetts Institute of Technology and Harvard, Cambridge, MA.

We have identified a novel autosomal recessive disease that we have called microcephaly with seizures. Patients with this disease are generally born with microcephaly that becomes very severe with time and intractable seizures starting in infancy. Interestingly, the patients have structurally normal MRIs except for the small brain size, indicating no neuronal migration abnormality. We have identified the cause of the disease as mutations in the DNA repair gene, polynucleotide kinase 3' phosphatase (PNKP). Since identifying the genetic cause of the disease, we have found an additional family with a likely less detrimental mutation with significantly less clinical severity. This indicates the clinical spectrum of this disease is wide and the burden of this disease remains to be determined. EBV transformed lymphocytes derived from the patients have abnormalities in multiple DNA repair pathways. However, the patients do not appear to be at a greater risk for cancer or immunodeficiency, in contrast other DNA repair mutations that lead to microcephaly. In addition, the presence of seizure is unique among non-degenerative neurological diseases associated with DNA repair. We believe novel pattern of neurological symptoms reflect the various pathways that require PNKP and give insight into the requirements of DNA repair in the brain.

2587/W

Fragile X premutation rCGG repeats alter the nuclear export of specific mRNAs. *A. Qurashi, P. Jin.* Dept Human Molec Genetics, Emory Univ Sch Med, Atlanta, GA.

Fragile X-associated tremor/ataxia syndrome (FXTAS) is a neurodegenerative disorder in fragile X premutation carriers with FMR1 alleles containing 55-200 CGG repeats. Using a Drosophila model of FXTAS, we previously demonstrated that transcribed premutation repeats alone are sufficient to cause neurodegeneration, suggesting that rCGG repeat-binding proteins (RBPs) may be sequestered from their normal function by rCGG binding. Recently we identified Pur alpha and hnRNP A2/B1 as RBPs, which could modulate the rCGG-mediated neuronal toxicity. To further investigate the role of Pur alphain rCGG-mediated neurodegeneration, we have taken a proteomic approach to identify the proteins that interact with Pur alpha. Over 100 proteins, including several known interactors such as Fmrp, were found to directly interact with Pur alphain vitro. To evaluate the potential contribution of Pur alpha-interacting proteins to rCGG-mediated toxicity, we further tested their genetic interactions with rCGG repeats using the FXTAS fly model, and identified several interactors of Pur alpha that indeed could genetically modulate the toxicity caused by rCGG repeats. Of particular interest among them is Rm62, the Drosophila ortholog of the p68 RNA helicase. Rm62, physically interacting with Pur alpha, could influence rCGG-mediated neurodegeneration negatively. Biochemically, fragile X rCGG repeats could decrease the soluble protein level of Rm62, leading to the accumulation of Hsp70 transcript, a previously identified target of Rm62, in the nucleus. Further microarray analyses revealed the nuclear accumulation of additional mRNAs involved in stress and immune responses in fragile X premutation flies. These findings suggest an unexpected nuclear export deficit of specific mRNAs caused by fragile X premutation rCGG repeats, and indicate that the altered nuclear export of these mRNAs could contribute to the pathogenesis of FXTAS.

2588/W

Quantitative mapping of deleted mitochondrial DNA and gene expression analysis of the the brain region with highly accumulated deleted mitochondrial DNA in transgenic mice exhibiting bipolar disorder-like phenotypes. *A. Takata[1,2], T. Kasahara[1], S. Kanba[2], T. Kato[1].* 1) 1Laboratory for Molecular Dynamics of Mental Disorders, RIKEN Brain Science Institute, Saitama, Japan; 2) Department of Neuropsychiatry, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.

Bipolar disorder (BD) is a psychiatric disease characterized by recurrent episodes of mania and depression and is one of the ten leading causes of disability worldwide. To elucidate the pathological mechanism of BD, numerous neuroimaging and post mortem brain studies have conducted and implicated involvement of several brain regions. However the responsible neural system, such as substantia nigra dopaminergic neurons in Parkinson's disease, has not been identified. Based on the mitochondrial hypothesis of BD, we previously created the transgenic mice (Tg mice) expressing defective mitochondrial DNA (mtDNA) polymerase in forebrain neurons and they exhibited BD-like phenotypes. In Tg mice, these phenotypes were caused by functional alternations due to accumulation of aberrant mtDNA. Therefore, we hypothesized that the most damaged region in Tg mice brain could be revealed by the quantitative analysis of aberrant mtDNA. The results would also provide some insights into identification of the neural system responsible for BD. In this study, we conducted semi-comprehensive quantitative mapping of deleted mtDNA (Δ-mtDNA) using laser microdissection and quantitative PCR technique. As a result, we found large amounts of Δ-mtDNA in some mood disorder-related regions including mouse equivalent of subgenual cortex and nucleus accumbens. The most prominent accumulation of Δ-mtDNA was detected in paraventricular thalamic nucleus (PVT), which plays a role in stress response and circadian behavior and has connections with many mood disorder-related regions such as subgenual cortex, insular cortex, nucleus accumbens, amygdala, suprachiasmatic nucleus and midbrain monoaminergic neurons. To investigate the consequence of Δ-mtDNA accumulation in PVT of Tg mice, we analyzed global gene expression profile of PVT. Gene Ontology analysis suggested alternation of genes related to regulation of neurological system, protein complex assembly and cell death. Involvement of PVT dysfunction and alternation of these biological processes in bipolar-disorder like phenotypes in Tg mice and the pathophysiology of human bipolar disorder should be tested in further experiments.

2589/W

Gly269Ser mutation in compound heterozygosity with Leu127Arg is associated with adult onset of Tay-Sachs: a case report from south Italy. *P. Spadafora[1], M. Liguori[1], M. Caracciolo[1], G. Tagarelli[1], A. Bagalà[1], I.C. Cirò Candiano[1], G. Spinelli[1], A. Gambardella[1,2], A. Quattrone[2].* 1) Inst Neurological Sci, National Research Council, Mangone (CS), Mangone, Italy; 2) Department of Medical Sciences, University "Magna Graecia", Catanzaro, Italy.

Tay-Sachs disease (TSD) is an autosomal recessive neurological disorder characterized by significant deficiency of the lysosomal enzyme β-Hexosaminidase A (HexA) that results in cerebral GM2 ganglioside accumulation. Mutations in HEXA gene are responsible for deficiency or reduced activity of β-Hexosaminidase A that correlate directly with disease severity. HEXA gene is located on chromosome 15q23-q24; it contains 14 exons and encodes for the α-subunit of β-Hexosaminidase A. We describe an Italian family in which the proband showed psychiatric symptoms, ataxia and other cerebellar signs, seizures and proximal weakness of arms and legs. Proband's parents, originated from the same isolated village in southern Italy, were consanguineous. Materials and methods. HexA activity was assayed in homogenates of leucocytes using MUG as substrate. All exons and flanking intronic boundaries of HEXA gene were analysed by direct sequencing on ABI 3130XL. Results. In the proband, in vitro determination of HexA activity showed a 10% residual activity. Molecular analysis of HEXA gene revealed Gly269Ser mutation in compound heterozygosity with Leu127Arg, which is one of the infantile Tay-Sachs alleles. Proband's father and mother were heterozygous for Gly269Ser and Leu127Arg alleles, respectively. Discussion and conclusions. We identified a Tay-Sachs patient from southern Italy by biochemical and molecular analysis. This subject was compound heterozygous for Gly269Ser and Leu127Arg HEXA mutations. Gly269Ser is one of the most common mutations found in late-onset TSD, either in Jewish and non-Jewish populations. This genetic variation leads to defective processing and association of α-chain with β-chain of β-Hexosaminidase A giving rise, however, to some residual activity. On the contrary, Leu127Arg mutation is responsible for infantile onset of TSD in Italy. As in our observation, several studies reported that patients with Gly269Ser showed milder and slowly progressive disease with more pronounced behavioral or psychiatric problems, and proximal weakness. The identification of two different mutations in HEXA gene from an isolated village of southern Italy, together with the finding that the proband's parents were second-degree cousins, suggested the presence of Ashkenazi Jews ancestors in this country. In fact, the incidence of TSD in Ashkenazi Jewish population is 1/3900 birth. Evidences derived from the genealogical tree seem to furher support this hypothesis.

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Clinical Genetic Clues to the Origins and Outcomes of Autism Spectrum Disorders. *E. Lopez[1,2,7], L. Kasmara[1,2,7], K. Calli[1,2,7], M.J. Hildebrand[1,2,7], P. Carrion[1,2,7], L. Swinton[1,2,7], C. Tyson[3,7], M.A. Hrynchak[3,7], J.J.A. Holden[4,5,7], E. Rajcan-Separovic[2,6,7], M.E.S. Lewis[1,2,4,7].* 1) Dept of Medical Genetics, University of British Columbia, Vancouver, BC, Canada; 2) Child and Family Research Institute, Vancouver, BC, Canada; 3) Cytogenetics Research Laboratory, Dept. of Pathology, Royal Columbian Hospital, New Westminster, BC, Canada; 4) Department of Physiology, Queens University, Kingston, ON, Canada; 5) Department of Psychiatry, Queens University, Kingston, ON, Canada; 6) Department of Pathology, University of British Columbia, Vancouver, BC, Canada; 7) The Autism Spectrum Interdisciplinary Research (ASPIRE) Program of ASD-CARC (www.AutismResearch.com).

Purpose: The Autism Spectrum Disorders (ASDs) are the most common childhood developmental disorder. The ASD population is heterogeneous and despite high prevalence and clear evidence that early interventions can optimize development, ASDs are often not recognized until age 3 years or older. In up to 40% of cases, ASD behaviours are recognized as a component of specific medical, genetic or chromosomal disorder. In most others, affected children can have seizures, co-existing psychiatric disorders, intellectual disability, and major or minor physical anomalies. These co-morbidities often occur as clusters of symptoms, suggesting syndromic relationships to each other and to ASDs. We aim to identify reliable biomarkers of ASD susceptibility, etiology and co-morbidity that may lead to earlier detection and improved management. **Methods:** We applied a series of standardized measurement tools to identify specific genetic, medical and teratogenic conditions known to co-exist with ASDs amongst a randomly selected cohort of >300 individuals with an ADOS-G and ADI-R confirmed ASD. All individuals received standardized clinical genetics consultation by a certified medical geneticist including review of family, medical and developmental history and dysmorphology exam. Standard clinical investigations included Fragile X gene testing, karyotype, subtelomeric and targeted FISH testing for common rearrangements of autism-associated loci at 2q37, 7q11, 15q11, 22q11 and 22q13. Data were analyzed to identify ASD clusters/subgroups that may differ in etiology, associated co-morbidities, outcome and genetic measures. **Results:** Comprehensive clinical genetic assessments revealed heightened frequency of co-existing intellectual disability (ID), as well as craniofacial, systemic, growth and neuroclinical anomalies (seizures, vision) within each different ASD-associated genetic, chromosomal, genomic syndromic and non-syndromic disorders. **Conclusions:** By identifying several cases sharing the same phenotypic pattern of symptoms, co-morbidities and/or clinical genetic/syndromic contributors to ASDs, we can begin to generate guidelines to facilitate optimal anticipatory management and functional outcomes for individuals and families living with ASDs. Our findings aim to set the standard for Clinical Genetic and Child Health Services critical to recognizing and managing brain and body features/co-morbidities of autism to improve individualized therapies and management.

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Decreased Wnt signaling pathway is associated with augmented APC in a mice Mk-801 model of schizophrenia. *DH. Cui[1], JF. Yu[1,2], D. Qi[3], MJ. Xing[1], KD. Jiang[1], YW. Peng[2].* 1) Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine, Shanghai, Shanghai, China; 2) Department of Anatomy, Histology and Embryology, Shanghai Medical College, Fudan University, Shanghai, China; 3) The Section of Cardiovascular Medicine, Department of Internal Medicine, Yale University School of Medicine, New Haven, USA.

Background: The wingless (Wnt) pathway is relatively new to the psychiatric literature, particularly in relation to schizophrenia. Some members of the Wnt signal transduction pathway were recently found to be altered in schizophrenia. Our recent study showed that the level of adenomatous polyposis coli (APC) mRNA, a negative regulator of the Wnt signaling, in peripheral blood leucocytes of patients with schizophrenia was significantly increased. The current study investigated the role of Wnt signaling components in an animal model of schizophrenia, induced by the non-competitive NMDA antagonist (MK801). MK-801 was chosen to be studied since it produces a state of NMDA receptor hypofunction in rats that may be analogous to the one hypothesized to occur in schizophrenia. **Methods:** Male C57BL/6 mice received intraperitoneal injections of MK-801 or vehicle (controls) for 7 days. Behavioral tests were performed first. Then Prefrontal cortex (PFC) and ventral tegmental area (VTA)of mice were removed for measuring APC, glycogen synthase kinase 3 β (GSK-3β) and β-catenin mRNA and protein levels and activities using western blotting analysis and real-time PCR measurement. Pheochromocytoma (PC12) and SK-N-SH cells were also used to elucidate how APC affected the neural proliferation and differentiation by immunofluorescence staining technique with and without transfection of APC siRNA. **Results:** In experimental mice, MK-801 produced characteristic behavioural changes that modeled schizophrenia. MK-801 treatment induced a significantly increased phosphorylation and inactivation of glycogen synthase kinase 3 β (GSK-3β) in PFC and VTA, which was disassociated with augmented β-catenin phosphorylation, whereas APC mRNA and protein levels in the PFC and VTA were significantly increased, but was associated with increased β-catenin phosphorylation. Furthermore, APC protein showed to facilitates neural growth in vitro in cultured SK-N-SH and PC12 cells. **Conclusions:** These data suggest that treatment with an NMDA receptor antagonist MK-801 can produce a hypoactive Wnt pathway, which may be associated with increased APC protein levels. Furthermore, APC may facilitate neural growth independent of Wnt signaling. **Key words:** Canonical Wnt Signaling Pathway, adenomatous polyposis coli, Schizophrenia, PFC, VTA.

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Phosphorylation of serine-710 in DISC1 activates a molecular switch from progenitor proliferation to neuronal migration in the developing cortex. *N. Katsanis[1], K. Ishizuka[2,3], A. Kamiya[2,3], J. Robinson[1], E.C. Oh[1], N. Mitsuma[1], K. Furukori[2,3], S. Seshadri[2,3], B. Huang[2,3], A. Hayashi-Takagi[2,3], K. Kubo[4], K. Nakajima[4], A. Sawa[2,3].* 1) Dept Cell Biol, Duke Univ, Durham, NC; 2) Department of Psychiatry and Behavioral Sciences; 3) Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21287 USA; 4) Department of Anatomy, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan.

Regulatory mechanisms governing the sequence from progenitor cell proliferation to neuronal migration during corticogenesis are poorly understood. Disrupted-In-Schizophrenia-1 (DISC1), a major susceptibility factor for schizophrenia and related disorders, is highly expressed in both progenitor cells and postmitotic neurons in the developing cerebral cortex. Here we report that a specific phosphorylation of DISC1 at serine-710 (S710) acts as a molecular switch from maintaining proliferation of mitotic progenitor cells to activating migration of postmitotic neurons. Non-phosphorylated DISC1 at S710 regulates canonical Wnt signaling via an interaction with GSK3β, whereas phosphorylation of this residue triggers the recruitment of Bardet-Biedl-Syndrome (BBS) proteins to the dynein motor complex associated with the centrosome. Knockdown of DISC1 leads to deficits in both cell proliferation and neuronal migration; however a phospho-dead mutant A710-DISC1 can only rescue the former, while phospho-mimic mutant E710-DISC1 rescues specifically and exclusively the latter. These data highlight a dual role for DISC1 in corticogenesis and suggest that phosphorylation of this protein at S710 represents a key developmental switch.

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3D digital morphometry of craniofacial dysmorphology in the Simons Simplex Collection of Autism. *C.K. Deutsch[1,2], A.R. Shell[1], R.W. Francis[1], J.R. Cuomo[1], A.T. Hunt[1], L. Kowalski[3], C. Lord[3].* 1) Psychobiology Program, Eunice Kennedy Shriver Ctr UMMS, Waltham, MA; 2) Harvard Medical School, Boston, MA; 3) University of Michigan Autism and Communication Disorders Center, Ann Arbor, MI.

This project documents for the first time craniofacial anomalies in simplex autism utilizing new 3D morphometry methods. These methods provide reliable, quantitative measures of dysmorphology, and employ a new digital craniofacial normative database that permits conditioning on age, gender, and ethnicity. Both face and brain derive from common embryonic primordia and are shaped by shared forces; thus, a genetic or environmental insult that disrupts early development could manifest itself both as brain pathology and craniofacial dysmorphology. We have adopted an embryologically-derived approach that permits hypothesis testing about the underlying biology of dysmorphogenesis in autism. Here, we present findings by regions of primordia (Anlagen). These initial results focus on the first cohort of probands (N=70) seen within the quantitative dysmorphology section of the Simons Simplex Collection (SSC), imaged using 3D stereophotogrammetry at the University of Michigan Autism & Communication Disorders Center (C Lord, Director). All probands met criteria for autism based on ADI-R and ADOS criteria. We established the degree to which craniofacial anomalies are statistically overrepresented among SSC probands, using full-scale scores and combinations of individual anomalies chosen to reflect embryological factors. There was a marked statistical overrepresentation of full-scale summary scores (anomaly counts) among the probands with autism ($p < .0001$). Anomalies were frequent in several Anlagen derivatives, particularly within the frontonasal and mandibular regions (both $p < .0001$). Several laboratories, including ours, have found excessive cranial dimensions associated with autism. In the present study, we noted marked increases in the skull base width ($p < .0001$). Further, the magnitude of these measurements was significantly correlated with the full-scale scores ($p < .015$). Stratification on skull base width identified particularly dysmorphic cases, and conceivably may provide a tool for delineating more homogeneous subgroups of autism. *Acknowledgements:* The authors would like thank the Simons Foundation (SSC, SFARI) for their generous support, and NIH (R42 DE016442; FaceValue normative database). We are also grateful to the families within the SSC who participated in these studies.

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Genotype-controlled analysis of Serum Dopamine β-hydroxylase across Pregnancy in Women seeking care for Mental illness. *D.L. Perdomo[1, 2], W. Li[2], R.W. Bonsall[3], Y-L. Tang[2], D.J. Newport[3], B. Knight[3], Z.N. Stowe[3], J.F. Cubells[2, 3].* 1) Graduate Program in Genetics and Molecular Biology, Emory University, Atlanta, GA, 30322; 2) Department of Human Genetics, Emory University School of Medicine, Atlanta, GA , 30322; 3) Department of Psychiatry and Behavioral Sciences, Emory University, Atlanta, GA, 30322.

DBH encodes Dopamine Beta Hydroxylase (DβH), a vesicular enzyme that plays a crucial role in the catecholaminergic pathway by converting dopamine into norepinephrine. DβH activity levels are easily assayed in serum due to the release of DβH along with NE from noradrenergic neurons as well as from chromaffin granules of the adrenal medulla. Early efforts to use serum DβH as an index of sympathetic activity rapidly led to the finding that serum DβH activity is regulated largely by genetic inheritance. Subsequent work has demonstrated conclusively that *DBH* is the major quantitative trait locus regulating serum DβH [1]. Norepinephrine is an essential neurotransmitter in development: Dbh -/- mouse embryos die *in utero* and mothers of persons born with the rare Mendelian disorder of DβH deficiency report high rates of miscarriage [2]. Prior studies have demonstrated slight fluctuations in the serum DβH activity of women over the menstrual cycle or after administration of oral contraceptives [3,4]. However, those studies did not account for the large stable effect of *DBH* genotypes on serum DβH activity. Normal pregnancy associates with increased maternal sympathetic activity to meet the demands of increased cardiac output necessary to support the growth of the fetus. In the current study, we examine serum DβH activity across pregnancy in women seeking care for mental illness. We hypothesize that: (1) serum DβH activity will increase over the course of pregnancy, reflecting increased activity of the sympathetic nervous system; (2) maternal stress associated with depressive symptoms will elevate serum DβH activity beyond the physiological increase in uncomplicated pregnancy. Serum samples were drawn during the pre-conception period, the 1st, 2nd, and 3rd trimester, and the early (first 4 weeks) and late (8- 15 weeks) post postpartum period. To control for the effect of *DBH* genotype, we will examine the residual activities after accounting for rs1611115, a well-documented functional SNP at *DBH* that accounts or ~50% of the variance in serum DβH activity.

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Mutational Analysis of the DCX, LIS1 and FLNA genes in patients with neuronal migration disorder from Southern Italy. *V. Greco[1], E.V. De Marco[1], F.E. Rocca[1], F. Annesi[1], D. Civitelli[1], G. Provenzano[1], P. Tarantino[1], V. Scornaienchi[1], A. Labate[2], A. Gambardella[1,2], G. Annesi[1].* 1) Institute of Neurological Sciences, National Research Council, Cosenza, Italy; 2) Institute of Neurology, University Magna Graecia, Catanzaro, Italy.

Classical lissencephaly (LIS) and subcortical band heterotopia (SBH) or 'double cortex syndrome', are neuronal migration disorders associated with epilepsy and mental retardation. SBH is usually caused by mutations in the doublecortin (DCX) (Xq22.3-q23) gene, and much less frequently in the LIS1 (17p13.3) gene. Both genes encode proteins involved in microtubule homeostasis. DCX mutations predominantly cause SBH in heterozygous females and severe lissencephaly in males, although rare males with SBH and DCX mutations have been reported. Periventricular nodular heterotopia (PNH) is another human neuronal migration disorder in which a subset of neurons fails to migrate into the developing cerebral cortex and is often associated with focal epilepsy. Most sporadic as well as familial female patients carry a heterozygous FLNA mutation (Xq28) predicted to result in loss of function due to protein truncation. In this study, we have analyzed familial and sporadic patients with SBH or LIS from Southern Italy to investigate abnormalities of the DCX and LIS1 genes. Furthermore, we are attempting to evaluate the occurrence of mutations in FLNA gene in familial patients affected by PHN. We have performed by direct sequencing a mutational analysis of DCX and LIS1 genes in 5 patients (4 sporadic and 1 familial) with SBH or LIS. We are also carrying out the analysis of the FLNA gene in 2 familial patients with PNH from Southern Italy. Esonic rearrangements of DCX, LIS1 and FLNA were performed by multiplex ligatio-dependent probe amplification (MLPA). We have found 2 different nonsense mutations of the DCX gene in 2 unrelated female with SBH: a mutation previously described (R303X) in the exon V and a novel mutation (Tyr151X) in the exon 2. We did not detect mutations in LIS1 gene in the DCX-negative patients. We have excluded deletions and duplications in DCX, LIS1 and FLNA gene by MLPA in all the patients. The analysis of the FLNA gene in the patients affected by PNH, is still in progress. These preliminary data suggest an involvement of DCX gene in the pathogenesis of double cortex syndrome. However, since a small percentage of genetically undiagnosed cases of lissencephaly have been reported, it would be interesting to extend our study to other genes, such as ARX, TUBA1A, involved in cortical development.

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SPINOCEREBELLAR ATAXIA WITH HYPOGONADISM: AN INTRIGUING GROUP OF GENETIC DISORDERS. *C. Lourenco, C. Sobreira, A. Barreira, W. Marques Jr.* Neurology, Univ Sao Paulo, Ribeirao Preto, Sao Paulo, Brazil.

INTRODUCTION The association between cerebellar ataxia and hypogonadism was first described in four sibs by Holmes, and since has become known as Holmes type ataxia. At the time of his description, it was not possible to determine whether hypogonadism was hypogonadotropic or hypergonadotropic hypogonadism. Although the clinical pictures may share similar phenotype, the pathogenic mechanism involved in the two types of hypogonadism is different. Several syndromes with hypo/hypergonadotrophic hypogonadism and ataxia have been published, however there is a remarkable clinical heterogeneity among them. Here, we present the clinical data and molecular/biochemical studies of fifteen Brazilian patients with cerebellar ataxia and hypogonadism. MATERIAL AND METHODS All patients were evaluated in the neurogenetics clinics by geneticists, neurologists and endocrinologists. Brain MRI, ophtalmological exam, EMG/NCV, hormone and biochemical tests, screening for CDG, karyotype, muscle biopsy with chain respiratory enzyme assays and measurement of coenzyme Q10, molecular tests for Friedreich ataxia and for SCAs 1,2,3,6 and 7 were performed in the course of the investigation. RESULTS All patients had cerebellar ataxia, but the age of the onset was variable; it was worthy to note that ten patients had early onset ataxia. Consanguinity of parents was noted in two families; five patients had hypergonadotropichypogononadism. Mental retardation was seen in two u nrelated girls with hypergonadotropic hypogononadism. None of the patients had chromosomal anomalies. Molecular tests for Friedreich and SCAs were all negative. Optic atrophy and retinochoroidal degeneration were found in five patients; axonal neuropathy was present in four patients. Cerebellar atrophy with pons or prominent vermis involvement was a constant feature. In two patients, coenzyme Q10 deficiency was confirmed in muscle biopsy. CONCLUSIONS One family have features consistent with a rare neurological disorder, Boucher-Neuhauser syndrome; two other unrelated patients had coenzyme Q10 deficiency; CDG Ia was identified in one adult patient. The remaining patients had features that may fit in the Gordon-Holmes phenotype although we believe this entity should not be an homogenous disorder. Thus, the association between cerebellar ataxia and hypogonadism comprise hetereogenous entities whose clinical investigation can elighten the pathological basis of these fascinating neuroendocrinological syndromes.

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An Evidence-Based Approach to Treatable Metabolic Disorders Causing Intellectual Disability. *C.D. van Karnebeek[1], S. Adam[2], A.G. Leenders[3], J.F.M. Friedman[2], S. Stockler-Ipsiroglu[1].* 1) Div Biochemical Diseases, Dept Pediatrics, BC Children's Hospital, Vancouver, Canada; 2) UBC Dept Medical Genetics, BC Children's & Women's Health Centre, Vancouver, Canada; 3) Emma Children's Hospital, Academic Medical Center, Amsterdam, The Netherlands.

Background: Intellectual disability (ID), a debilitating condition with deficits in cognitive functioning (IQ<70) and adaptive skills, affects 2.5% of children and adults worldwide. Studies reporting the yield of complex diagnostic work-up in individuals with ID and developmental delay (DD) have focused primarily on the frequency of causal conditions. However, the primary aim of every clinician should be not to miss the small but increasing subset of treatable conditions, foremost inborn errors of metabolism (IEMs). **Aims:** To provide the clinician with an overview of the currently treatable IEMs causing ID/DD, and establish evidence-based guidelines for a structurd diagnostic approach. **Methods:** Two independent researchers used the Cochrane Collaboration methodology to identify and evaluate published information on potentially treatable IEMs that may cause ID/DD: formulation of definitions, search (strategy) in Pubmed (1965-2010) & '*The Online Metabolic and Molecular Bases of Inherited Disease*', selection & categorization of ' treatable IEMs', and establishing clinical guidelines. **Results:** Inherent to rare diseases, available evidence for effect of therapy is scarce and often of limited quality. A total of 65 treatable IEMs known to cause ID/DD were identified. An overview of the genetic, biochemical and clinical features of these conditions is provided. Therapeutic modalities (ranging from standard of care to experimental) include diet, co-factor/vitamin supplements, substrate inhibition, enzyme replacement, stem cell transplant. Outcomes (effect on: IQ, developmental test scores, behaviour, epilepsy, neuro-imaging) varied from improvement to halting or slowing neurocognitive regression. Side-effects are summarized. Using this evidence a stepwise diagnostic protocol was formulated: A 1st tier 'metabolic screening' detects 30% of 65 treatable IEMs: glucose, ammonia, lactate, plasma amino-acids, urine organic acids, acylcarnitine profile. The 2nd tier is based on clinical findings, frequency, invasiveness: a specific biochemical (metabolite, enzyme assay, profile) or gene analysis. For those conditions without reliable biomarkers, a High-Throughput Sequencing technique was designed, which potentially allows screening large ID/DD groups for multiple genes simultaneously (in pilot phase). **Conclusions:** This literature review generated the evidence needed to formulate guidelines to aid the clinician in recognition of treatable conditions causing ID/DD.

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Contribution of copy number variation in familial Parkinson disease. *A.L. Torres[1], S. Zuchner[1,2], C. Jauregui[1], E.R. Martin[1,2], W.K. Scott[1,2], J. Vance[1,2], L. Wang[1,2].* 1) Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL; 2) Dr. John T. Macdonald Foundation Department of Human Genetics, University of Miami Miller School of Medicine, Miami, FL.

PARK2, SNCA, DJ1, PINK1, ATP13A2 and LRRK2 are known familial Parkinson disease (PD). A comprehensive assessment of the contribution of copy number variations (CNVs) in these genes to PD risk is important for medical genetic testing strategies and molecular genetic understanding of disease mechanisms. We used high-density custom comparative genomic hybridization (CGH) arrays to evaluate CNVs in the introns, exons, and 5 kb flanking regions of the six PD genes in 182 PD cases and 67 unaffected controls. The average probe spacing was 150 bp. A DNA pool of 16 (8 males and 8 females) CEPHs was used as reference. CGH data were analyzed using Nexus 4.0 Copy Number software. No CNVs were found in DJ-1, ATP13A2, PINK1, and LRRK2. In both SNCA and PARK2, intronic CNVs were found at similar frequency in cases and controls. In SNCA, a duplication of the entire gene was found in one sporadic PD case with age-at-onset (AAO) of 36 years, which is consistent with the literature on rare duplication of SNCA. In PARK2, CNVs involving one or multiple exons were found in 19 individuals and 17 of them (16 cases and 1 control) were confirmed using a second method (Multiple Ligation Probe Amplification, Illumina Human610-SNP chip, and/ or quantitative PCR). Exonic CNVs in PARK2 were more frequent in cases than in controls (9.4% vs 1.5%, p= 0.05) and the difference was mainly driven by early-onset cases (AAO£40) (14.5% vs 1.5%, p=0.01). Since PARK2 is known as a recessive gene for juvenile Parkinsonism, we attempted to evaluate the contribution of PARK2 compound heterozygotes in PD with different AAO. HPLC and sequencing were used to search for additional missense variations in individuals with heterozygous PARK2 exonic CNVs. Family structure was used to determine the phase whenever necessary. We have finished complete evaluation of 11 individuals (6 early- and 5 late-onset cases). All evaluated early-onset cases and no late-onset case carry two alleles (a homozygote or a compound heterozygote) of PARK2 exonic variations (structural or sequence) (p= 0.002). In summary, exonic but not intronic CNVs in PARK2 were found to confer risk to PD, but CNVs were not a major contributing mechanism for the other PD genes examined. Additional studies are underway to evaluate compound heterozygote in more PD patients. Our study stresses the importance of determining compound heterozygote in PARK2 evaluations.

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Determining Prevalence Rates and Characterizing the Autism Spectrum Disorder Phenotype in the Old Order Amish. *J. Lee Robinson[1], L. Nations[1], S. Sacharow[1], T. McGregor[2], N. Suslowitz[2], R. Laux[2], J. Haines[2], M. Pericak-Vance[1], M. Cuccaro[1].* 1) John P. Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL; 2) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN.

The prevalence of Autism Spectrum Disorders (ASDs), most recently 1 in 110 children (CDC, 2009), makes it one of the most common neurodevelopmental disorders. It is etiologically complex and its genetic architecture has been difficult to determine. The study of genetically homogeneous populations, such as the Old Order Amish, may provide clues as to the genetic etiology of ASDs. However, little is known about prevalence rates of ASD in this population. It has been postulated that ASD is rarely found among the Amish. Thus, a study to determine the prevalence rate of ASD in the Amish is a crucial first step. Our study seeks to determine the rate of ASD in two Amish communities: Holmes County, Ohio and Elkhart-Lagrange County, Indiana. All Amish children between the ages of 3 to 21 in those counties (~8000) will be screened for the presence of an ASD. Trained clinicians ascertained door to door using a published Amish Directory. Families were approached and asked to participate in a brief interview regarding their children's development. Two primary screening instruments were used: the Social Communication Questionnaire (SCQ) and the DSM-IV-TR Checklist (created by authors). A dysmorphology screen and family history including questions specific to the ASD phenotype were also collected. Children screening positive on either screener received a comprehensive clinical evaluation by two licensed psychologists. This included the Autism Diagnostic Observational Schedule (ADOS) and Autism Diagnostic Interview (ADI), in which items were modified to exclude mention of modern technology and toys the Amish do not typically use and include toys and examples in alignment with Amish lifestyle. From September 2008 to April 2010, 2211 Amish children were screened in the two communities. A total of 26 children screened positive for ASD. Of those 26 children, 17 were evaluated (three were excluded for other reasons; six declined follow-up) and nine children were diagnosed with ASD using the ADI, ADOS, and/or clinical judgment. Interestingly, all nine children identified reside in Holmes County, Ohio. Four of the children had a prior ASD diagnosis. Preliminary data have identified the presence of ASD among the Amish at a rate of approximately 1 in 228 children (.4% of those screened) using standard ASD screening and diagnostic tools. Accurate determination of the ASD phenotype in the Amish is an initial step in the design of genetic studies of ASD in this population.

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Calpain: A facile diagnostic marker for identifying the true Carriers in Duchenne muscular dystrophy. *M. V. Rao[1,2], MPJS. Anandraj[1].* 1) Department of Biocemistry& Molecularbiology,Institute of Genetics and Hospital for genetic diseases,Osmania University,Begumpet,Hyderabad-500016,India.E:mail mohan777r@yahoo.com; 2) MoncoBiotechnologies, Plot.no 7-1-562, Near MRO Office,Ameerpet,Hyderabad-500016,INDIA mohan@moncobiotechnologies.com,.

DMD is the most frequent and best known of the childhood muscular dystrophies. The incidence rate is in the range of 13 to 33 per 100,000 yearly or about one in 3300 live male births in every part of the world. Approximately 30 percent of patients have a negative family history and are said to represent spontaneous mutations.The available methods for carrier detection such as PTT, S.Blot, STR, Q-PCR, SSCP, DHPLC and SCAIP analyses yield definitive results but they are labor intensive, expensive and are in the realm of special laboratory methods requiring special skills. Assessment of m-Calpain for true Carrier detection, if validated by parallel assessment of DNA mutations would standardize the diagnostic value of Calpain index. The platelet m-calpain levels were elevated 10 folds in DMD patients and true carriers (32-6ng/μg) when compared to age and sex matched controls(2.9-1.4ng/μg).

Using the18 exons of dystrophin by qantitaive M- PCR for 10 females at risk the gene dosage was quantified and it was identified that 6 showed low dosage and conformed the carrier state.Of 9 (5deletional and 4 nondeletional) families at risk for DMD were analyzed by radioactive PCR for 4 STRs (44,45,49and 50) polymorphism markers and it was found that 8 families were informative for all STR markers and in one family, all the 4 STR markers were non informative. Of 10 females at risk analyzed for these 4 STR polymorphisms it was found that 8(86%) were heterozygotes as compared to controls.

Of the same 10 females at risk analyzed calpain analysis by ELISA it was found that all 9(90%) were informative as compared to M-PCR and STR analysis it was found that the enhanced level of m-calpain as measured by ELISA and the presence of deletions or the mutant chromosome tracked by inherited STR polymorphisms while validating (ELISA was 100% reliable diagnostic method) the status of m-calpain as marker for diagnosis of disease/true carrier state, would form part of a strategy for definitive diagnosis of true carriers and prenatal diagnosis. Such a marker would also help in population screening.

2601/W

Genotype - phenotype correlations in Pontocerebellar Hypoplasia. *Y. Namavar[1], PG. Barth[2], PR. Kasher[1], F. van Ruissen[1], VRC. Eggens[1], WB. Dobyns[3], F. Baas[1], BT. Poll-The[2], PCH consortium.* 1) Department of Neurogenetics, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands; 2) Division of Pediatric Neurology, Emma's Childrens Hospital, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands; 3) The University of Chicago, Department of Human Genetics, 920 E. 58th Street, Chicago, IL 60637, USA.

Pontocerebellar hypoplasia (PCH) is a group of autosomal recessive neurodegenerative disorders with prenatal onset. The common characteristics are cerebellar hypoplasia with variable atrophy of the cerebellum and the ventral pons and progressive microcephaly. Neocortical atrophy and ventriculomegaly are present in variable degrees. So far, mutations in the tRNA splicing endonuclease subunit genes (TSEN54, TSEN2, TSEN34) and the mitochondrial tRNA arginyl synthetase gene (RARS2) were found to be associated with PCH types 2 and 4 and 6. We studied a large cohort of 169 PCH patients for mutations in these genes, of whom 106 patients tested positive for mutations in one of the TSEN genes or the RARS2 gene. We compared this group with 63 patients suspected of PCH who were negative on mutation analysis. We found a strong correlation between TSEN54 mutations and flat cerebellar hemispheres in which the vermis is relatively spared. We present evidence that the common homozygous mutation in TSEN54 can be predicted reliably from the PCH2 phenotype. Nonsense or splice site mutations in TSEN54 are associated with a more severe phenotype of more perinatal symptoms, ventilator-dependency and early death. Our data provide further evidence that the homogeneity of the phenotype, both from a clinical perspective and by neuroimaging, correlates strongly with the genotype and can facilitate early diagnosis and assist in molecular genetic testing. Furthermore, we show that PCH1 together with elevated CSF lactate may be caused by RARS2 mutations. Our study will enhance the clinical description of PCH and will assist with the neuroradiological and genetic diagnosis of PCH.

2602/W

Using genetic information in risk prediction for alcohol dependence in the Collaborative Study on the Genetics of Alcoholism GWAS sample. *J. Yan[1,2], F. Aliev[2], V.S. Williamson[2], B.T. Webb[2], A.M. Goate[3], J. Kramer[4], J.I. Nurnberger Jr[5], M.A. Schuckit[6], J.A. Tischfield[7], J.M. Quillin[1], D.M. Dick[1,2].* 1) Human and Molecular Genetics, Virginia Commonwealth University, Richmond, VA; 2) Virginia Institute for Psychiatric and Behavioral Genetics, Virginia Commonwealth University, Richmond, VA; 3) Department of Psychiatry, Washington University School of Medicine, St. Louis, MO; 4) Department of Psychiatry, University of Iowa College of Medicine, Iowa City, IA; 5) Department of Psychiatry, Indiana University School of Medicine, Indianapolis, IN; 6) Department of Psychiatry, University of California-San Diego, La Jolla, CA; 7) Department of Genetics, Rutgers University, Piscataway, NJ.

A number of studies investigating the clinical utility of genetic variants associated with complex disorders have illustrated the limitations and potential benefits of using genetic information in risk prediction for complex traits (Evans, D. M., Visscher, P. M., Wray, N. R., 2009, Human Molecular Genetics. 18(18), 3525-3531; Janssens, A.C., van Dujin, C.M., 2008, Human Molecular Genetics. 17(2), R166-R173.). The focus of this study was to assess the clinical validity of previously published genes associated with alcohol dependence (AD) in predicting risk for AD in an independent sample. The predictive ability of these genes was compared to family history. Using the Collaborative Study on the Genetics of Alcoholism (COGA) genome wide association study (GWAS) sample, we performed receiver operating characteristic (ROC) curve analysis to estimate the ability of a panel of SNPs to correctly classify cases and controls for DSM-IV AD. Specifically, sum scores of risk alleles were generated for a panel of 24 semi-independent SNPs, covering 15 genes, that had reported associations with alcohol dependence in the COGA family-based association sample. We identified a subset of individuals consisting of 627 cases and 454 controls from the COGA GWAS sample that were not part of the original family-based association sample and performed ROC analysis for the sum scores in this subset. These analyses did not result in significant discriminative ability for the sum scores; the area under the ROC curve (AUC) was 0.498 (95% CI = 0.463, 0.533, $p > 0.05$), suggesting that the SNPs are not predicting better than chance. The presence or absence of family history for AD was a better classifier of case control status in the COGA sample, with an AUC of 0.686 (95% CI = 0.654, 0.718, $p < 0.001$). This study shows that these SNPs currently have limited clinical utility and illustrates the need for further expansion of prediction panels for a complex disorder that encompasses both environmental and genetic risk factors of small effect such as AD.

2603/F

Concurrent mutations in AFG3L2 and paraplegin in patients with spinocerebellar degeneration. *S. Magri[1], V. Fracasso[1], M. Plumari[1], S. Caldarazzo[1], E. Sarto[1], C. Gellera[1], C. Mariotti[1], C. Pantaleoni[2], P. Plevani[3], F. Lazzaro[3], M. Muzi-Falconi[3], D. Di Bella[1], F. Taroni[1].* 1) Genet Neurodegen Metab Dis, IRCCS Ist Neurol Carlo Besta, Milan, milan, Italy; 2) Unit of Developmental Neurology, IRCCS Ist Neurol Carlo Besta, Milan, Italy; 3) Dept. Biomolecular Sciences and Biotechnology, University of Milan, Italy.

Autosomal dominant spinocerebellar ataxias (SCA) are a heterogeneous group of neurological disorders characterized by cerebellar dysfunction. We recently showed that *AFG3L2* mutations cause dominant ataxia SCA28 (Di Bella *et al*, *Nat Genet* 2010). AFG3L2 and its partner protein paraplegin, which causes recessive spastic paraplegia SPG7, are components of the *m*-AAA complex, involved in mitochondrial protein quality control. Since yeast functional studies showed that paraplegin coexpression can complement AFG3L2 mutations in some cases, we investigated the possible coinheritance of *AFG3L2* and *SPG7* mutations in patients with spinocerebellar syndromes. We identified 3 probands with heterozygous mutations in both the *AFG3L2* and the *SPG7* genes. Two ataxic patients carry an *AFG3L2* mutation affecting highly conserved amino acids located in the ATPase (E428D) or in the proteolytic (R702Q) domains of the protein along with paraplegin^{A510V}. Paraplegin^{A510V} occurs in a highly-conserved region of the ATPase domain and results in dramatically-reduced levels of paraplegin in homozygous patients. In one family, double heterozygosity for AFG3L2^{R702Q} and paraplegin^{A510V} resulted in a full-blown ataxic phenotype while AFG3L2^{R702Q} heterozygotes manifested only moderate cerebellar atrophy at MRI and paraplegin^{A510V} heterozygous carriers were completely unaffected. The third proband carry a *de novo* AFG3L2 mutation (R468C) in the highly conserved SRH region of the ATPase domain along with the inherited deletion of SPG7 exons 4-6. Notably, the R468C substitution abolishes the arginine finger in the AFG3L2 ATP-binding site which is necessary for ATP hydrolysis and crucial for intersubunit communication and/or catalysis. The clinical presentation of this patient is characterized by early-onset optic atrophy and a L-dopa-responsive spastic-ataxic syndrome with extrapyramidal signs. Muscle biopsy revealed an isolated defect of mitochondrial respiratory chain complex I. Functional analysis of AFG3L2 and SPG7 mutants in *m*-AAA-deficient yeast showed respiratory deficiency and defective substrate processing. In conclusion, our data indicate that a loss-of-function mutation in the AFG3L2 partner paraplegin may act as a disease modifier for heterozygous AFG3L2 mutations. Moreover, concurrent mutations in both components of the mitochondrial *m*-AAA complex may result in a complex phenotype, thus expanding the clinical spectrum of AFG3L2-associated mutations. [Telethon grant GGP09301 to FT].

2604/F

Mutations detected in the SHANK2 synaptic scaffolding gene in autism spectrum disorder and mental retardation affect dendritic spine morphology. *S. Berkel[1], C.R. Marshall[2], B. Weiss[1], U. Moog[3], G. Schratt[10], J. Howe[2], R. Roeth[1], V. Endris[1], W. Roberts[4], P. Szatmari[5], M. Bonin[6], A. Riess[6], H. Engels[7], R. Sprengel[8], S.W. Scherer[2,9], G.A. Rappold[1].* 1) Human Molecular Genetics, Institute of Human Genetics, Heidelberg, Germany; 2) The Centre for Applied Genomics, The Hosptal for Sick Children, University of Toronto, Toronto, Ontario, M5G 1L7, Canada; 3) Department of Human Genetics, Heidelberg University, Heidelberg, Germany; 4) Autism Research Unit, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, M5G 1Z8, Canada; 5) Department of Psychiatry and Behavioural Neurosciences, Offord Centre for Child Studies, Mc Master University, Hamilton, Ontario, L8N 3Z5, Canada; 6) Department of Medical Genetics, Institute of Human Genetics, Tuebingen University, Tuebingen, Germany; 7) Institute of Human Genetics, Rheinische Friedrich-Wilhelms-University Bonn, Bonn, Germany; 8) Max Planck Institute of Medical Research, Heidelberg University, Heidelberg, Germany; 9) Department of Molecular Genetics, University of Toronto, Ontario, M5S 1A8, Canada; 10) Interdisciplinary Center for Neurosciences, Heidelberg University, Heidelberg, Germany.

Autism spectrum disorder (ASD) and mental retardation (MR) represent clinically distinct neurodevelopmental disorders with a complex genetic etiology. Using microarrays we identified *de novo* copy number variations in the *SHANK2* synaptic scaffolding gene in two unrelated ASD and MR patients. DNA sequencing of *SHANK2* in ASD and MR revealed additional deleterious variants including a *de novo* nonsense C-terminal mutation and 7 rare inherited changes (six missense and one exonic microduplication). We demonstrated that three of these alterations reduced the ability of the SHANK2 protein to enlarge dendritic spines in primary hippocampal neurons. SHANK2 truncation leads to a complete loss-of-function while two more subtle mutations significantly impair spine enlargement indicating that haploinsufficiency of SHANK2 (like SHANK3) is etiologic in ASD. Our finding that SHANK2 is also independently involved in MR further links common genes between ASD and intellectual disability, and in this case dysregulation of dendritic spine formation is an underlying mechanism.

2605/F

Drosophila models of TDP-43 proteinopathies reveal that both cytoplasmic and nuclear accumulations of the protein are neurotoxic. *M. Lecourtois, L. Miguel, T. Frebourg, D. Campion.* Inserm U614, Institute for Medical Research, Rouen University, Normandy, France.

Physiologically, the TAR DNA-binding protein-43 (TDP-43) is a highly conserved protein, ubiquitously expressed and primarily located to the nucleus. TDP-43 protein has been implicated in several steps of gene expression regulation including transcription, RNA splicing, RNA transport, and translation. Recently, TDP-43 has been identified as a major constituent of nuclear and/or cytoplasmic ubiquitin-positive inclusions in patient with amyotrophic lateral sclerosis or frontotemporal lobar degeneration. Pathological proteins are abnormally hyperphosphorylated and partially cleaved to generate C-terminal fragments (TDP-43 CTF). Although the identification of alteration of the TARDBP gene, which encodes TDP-43 itself, in patients demonstrate that abnormal forms of TDP-43 can directly trigger neurodegeneration, the molecular mechanisms underlying TDP-43 toxicity are currently not understood. To address this question, we developed new Drosophila transgenic models expressing different variants of full-length human TDP-43 proteins presenting different subcellular localizations or the hTDP-43 CTF fragment. Using an inducible GAL4 system, we showed that both cytoplasmic and nuclear accumulations of TDP-43 are neurotoxic in flies. This toxicity occurs regardless of inclusions formation. Biochemical data showed that TDP-43 expression in flies recapitulates several key biochemical features of human TDP-43 proteinopathies, including abnormally phosphorylation on the disease-specific Ser409/Ser410 site and processing. Finally, to address the molecular mechanisms underlying TDP-43 toxicity in vivo, we tested focused genetic interactions and identified several Drosophila splicing regulators, as genetic modulators of cytoplasmic TDP-43 toxicity, highlighting that alteration of RNA processing could be a key mechanism in TDP-43 pathogenesis.

2606/F

Development of a mouse model of primary open angle glaucoma reveals a disease mechanism. *G.S. Zode[1], K. Bugge[1], C.C. Searby[1], E.M. Stone[1], M.H. Kuehn[2], V.C. Sheffield[1].* 1) Howard Hughes Medical Institute, Univ. of Iowa, IA 52242; 2) Department of Ophthalmology and Visual Sciences, University of Iowa Carver College of Medicine, Iowa, IA 52242.

Primary open angle glaucoma (POAG) is a common cause of blindness characterized by retinal ganglion cell (RGC) death and is associated with elevated intraocular pressure. Heterozygous myocilin (MYOC) mutations cause 2-4% of POAG cases, but the pathogenic mechanisms remain largely unknown. The objective of this study was to develop a mouse model of POAG and determine the pathogenic mechanisms involved. Transgenic mice (Tg) were generated which express the human Tyr437His MYOC mutation under the control of the CMV promoter. Mutant MYOC expression in the anterior segment of Tg mice was demonstrated by RT-PCR, immunohistochemistry and western blot analysis. Tg mice expressed higher levels of myocilin in the trabecular meshwork than wild type littermates. Starting at 3-months of age, Tg mice exhibited significantly higher intraocular pressure and demonstrated progressive loss of RGCs and axonal damage. In addition, Tg mice demonstrated significant lower response in pattern ERGs indicating functional loss of retinal ganglion cells. Interestingly, expression of mutant myocilin in the trabecular meshwork of Tg mice induces ER stress and activates the unfolded protein response (UPR). ER stress in the Tg mice switched from the UPR to the pro-apoptotic response as demonstrated by upregulation of chop and processing of pro-caspase-12. In addition, anterior chamber injection of tunicamycin or mutant myocilin virus significantly elevated IOP and activated UPR in control mice. Our observations indicate that Tg mice exhibit pathological changes similar to those in glaucoma patients validating this as a novel genetic animal model of glaucoma. Furthermore, this work reveals that expression of the Tyr437His mutation of human myocilin leads to ER stress and activation of UPR in the trabecular meshwork. These data indicates that sustained UPR compromises trabecular meshwork function leading to outflow deficiency, elevated intraocular pressure, and RGC death in glaucoma patients with myocilin mutations.

2607/F

Mechanisms underlying recessive mutations in Human Hyperekplexia. *S. Chung[1], A. Robinson[1], J. Vanbellinghen[2], MI. Rees[1,3].* 1) Institute of Life Science, School of Medicine, Swansea University, United Kingdom; 2) Laboratoire de Génétique Moléculaire, University of Liége, Liége, Belgium; 3) Wales Epilepsy Research Network, School of Medicine, Swansea University, United Kingdom.

Glycinergic neurotransmission is a major inhibitory influence in the CNS and defects are associated with paroxysmal neuromotor disorder, hyperekplexia which is characterized by exaggerated startle reflexes and hypertonia in response to sudden, unexpected auditory or tactile stimuli. Mutations in the genes encoding the glycine receptor (GlyR) α1 subunit (GLRA1) and the presynaptic glycine transporter GlyT2 (SLC6A5) are the most common cause of hyperekplexia. DNA sequencing of GLRA1 in 88 hyperekplexia patients revealed 19 variants in 30 index cases; 21 were inherited in recessive mode or part of compound heterozygosity. The expression levels and functional properties of these hyperekplexia mutants were analyzed using a high-content imaging system and patch-clamp electrophysiology. Hyperekplexia mutants with low level expression of functional GlyRs were further investigated for the surface expression. Biotinylation of cell-surface proteins revealed that the reduction in the number of functional channels observed in recessive mutants is due to the decreased cell-surface expression of GlyRs. Although the whole-cell expression of recessive mutants was comparable to that of WT GlyR α1, cell-surface expression levels were significantly decreased. This indicates that subcellular localization defects were the major mechanism underlying recessive mutations. For the dominant mutations, there was no alteration in the cell-surface expression indicating that the decreased level of dominant GlyRs is due to non-functional surface-targeted channels rather than a reduction in surface-expression. The discovery of hyperekplexia associated mutations and subsequent mechanisms underlying the mutations will provide invaluable opportunities to study the pathophysiology of the ancient startle response.

2608/F

An Integrated Examination of the Relationship of Genotype, Thyroid Function and Major Depression in Three Independent, Ethnically Diverse Longitudinal Samples. *R.A. Philibert[1], T.D. Gunter[2], S.R. Beach[3], C. Cutrona[4], D. Russell[4], G. Brody[3], N. Hollenbeck[1], M. Vijayendran[1], L. Elliot[1], D. Gugliano[1].* 1) Psychiatry, University of Iowa, Iowa City, IA; 2) Psychiatry and Neurology, St. Louis University, St. Louis, MO; 3) University of Georgia, Athen, GA; 4) Iowa State University, Ames, IA.

Thyroid function and major depression are complex disorders that are hypothesized to have overlapping diatheses. However, the exact nature of these overlapping diatheses remains completely undefined. In an effort to understand the pathophysiology of interlinked complex illnesses such as hypothyroidism and depression, we genotyped 1500 subjects from three longitudinal, ethnically diverse populations, for a series of 17 SNPs identified in prior genome wide association analyses of thyroid function. We demonstrate robust associations at least three of those SNPs from iodothyronine doiodinase, type 1 (DIO1) and phosphodiestarase 7B (PDE7B) with serum free thyroxine levels (FT4) and show strong evidence that these same SNPs also confer increased risk for major depression. The strength of the associations with both thyroxine levels and major depression appear to be gender and ethnicity specific. In summary, we confirm certain prior GWAS findings with respect to thyroid function in general population samples of both white and African-American ancestry and suggest that these SNPs may also contribute to alter vulnerability to major depression.

2609/F

Role of microRNAs in Fragile X-associated Tremor/Ataxia Syndrome. *H. Tan, P. Jin.* Department of Human Genetics, Emory University School of Medicine, Atlanta, GA 30322.

Fragile X-associated tremor/ataxia syndrome (FXTAS), a late age of onset neurodegenerative disorder, has been recognized in older males of fragile X premutation carriers, which is uncoupled from Fragile X syndrome. Using a Drosophila model of FXTAS, we previously demonstrated that transcribed premutation repeats alone are sufficient to cause neurodegeneration. MiRNAs are sequence specific regulators of posttranscriptional gene expression and are believed to regulate the expression of thousands of target mRNAs, with each mRNA targeted by multiple miRNAs. To determine the role of miRNAs in rCGG repeat-mediated neurodegeneration, we have analyzed the expression of all known miRNAs and identified select miRNAs, including miR-14 and miR-277, that are altered specifically in the brains derived from the Drosophila model of FXTAS. To evaluate the potential contribution of these miRNAs to rCGG-mediated toxicity, we further tested their genetic interactions with rCGG repeats using FXTAS fly model, and found that miR-277 could modulate rCGG repeat-mediated neurodegeneration. Using several miRNA target prediction algorithms, we have also identified several mRNA targets of miR-277 that could modulate rCGG repeat-mediated neurodegeneration. These results together suggest that selective miRNAs aberrantly expressed could modulate the pathogenesis of FXTAS by post-transcriptionally regulating the expression of specific mRNAs that are involved in FXTAS.

2610/F

Brain region-specific increased PKA activity is associated with alterations in behavioral responses to stress in Prkar1a-defective mice. *M.F. Keil[1, 3], G. Briassoulis[1], M. Nesterova[1], N. Gokarn[1], A. Batistatos[1], T.J. Wu[2], C.A. Stratakis[1].* 1) NICHD,SEGEN, National Institutes of Health, Bethesda, MD; 2) Program in Neuroscience, Uniformed Services University of the Health Sciences; 3) Graduate School of Nursing, Uniformed Services University of the Health Sciences.

Background: The role of the cAMP/PKA signaling in molecular pathways involved in fear and memory is well established. We recently reported that a Prkar1a heterozygote (HZ) knock-out (KO) mouse that was developed in our lab to investigate Carney complex (CNC), the disease caused by PRKAR1A mutations, exhibits an anxiety-like behavioral phenotype (Keil, 2010). Prior studies in our lab reported that transgenic mice with a down-regulated Prkar1a gene (Griffin, 2004) exhibited behavioral abnormalities including anxiety (Batista, 2005) and depression (Batista, 2006). In addition, several research groups have reported CNS effects in PKA KO mouse models for R1beta, R2alpha, and R2beta, and the PKA catalytic subunits. Methods: We measured PKA activity in brain areas involved in the stress response in HZ KO Prkar1a adult mice compared to wild-type (WT) littermates after mice were exposed to a stressor [novelty (saline) vs. predator (fox urine odor)] for 5 minutes; behavior was recorded (2x2 factorial design). Ninety minutes after stress exposure, mice were euthanized and brains were procured and immediately frozen. Biopsies of various brain nuclei were obtained for PKA analysis. Results: There were significant differences between HZ and WT in the behavioral response to stress. WT mice showed the expected response of decrease in exploratory behavior (rearing and stretch attend postures) ($p<0.02$) and an increase in defensive behavior (digging) ($p<0.03$) during predator (fox urine) vs. novelty (saline exposure) stress. However, HZ mice did not show alterations in behavior (exploratory or defensive) with exposure to different stressors (novelty vs. predator). Both HZ and WT mice showed similar pattern of decrease in grooming behavior (non-defensive behavior) during predator (fox urine) vs. novelty (saline) stress. Multivariate analysis showed that basal and total PKA activity was independently associated with genotype ($p<0.02$) and stress ($p<0.01$), with an interaction between genotype and stress ($p<0.03$). HZ mice had higher PKA activity (basal and total) in amygdala (basolateral and central), hypothalamus, and thalamus in response to predator (fox urine) vs. novelty (saline) stress. Conclusion: These results suggest that the alteration in PKA signaling in Prkar1a HZ KO mice is not ubiquitous in the brain; tissue-specific effects of the cAMP/PKA pathway are related to stress responses and fear sensitization.

2611/F

Cis-regulatory variants on chromosome 15q25 affect CHRNA5 mRNA expression and risk for smoking related illnesses. *J.C. Wang, N. Spiegel, J. Budde, L.J. Bierut, A.M. Goate.* Psychiatry, Washington Univ, St Louis, MO.

Variants within the gene cluster encoding α3, α5, and β4 nicotinic receptor subunits are major risk factors for substance dependence. The strongest impact on this risk is associated with variation in the CHRNA5 gene where we see at least two mechanisms: amino acid variation and variation in mRNA expression levels. These two mechanisms also affect risk for lung cancer. The risk allele of the non-synonymous variant (rs16969968) primarily occurs on the low mRNA expression allele of CHRNA5. The non-risk allele at rs16969968 occurs on both high and low expression alleles tagged by rs588765 within CHRNA5. When the non-risk allele occurs on the background of low mRNA expression of CHRNA5, the risk for nicotine dependence and lung cancer is significantly lower compared to those with the higher mRNA expression. Because there are 50 variants spanning 100 kb in the CHRNA5-A3-B4 gene cluster region that are highly correlated with rs588765 in European populations, it is not clear which of these variants are contributing to the changes in CHRNA5 mRNA expression. To narrow the number of putatively function variants that affect the CHRNA5 mRNA expression, we performed quantitative allele specific gene expression using brain tissue derived from European Americans. This method measures allele-specific transcript levels in the same individual, which eliminates other biological variation that occurs when comparing expression levels between different samples. With this measurement we are able to determine whether the nicotine and other substance dependence associated variants have a cis-regulatory (direct) effect on CHRNA5 transcript levels and identify variant(s) that are responsible for modulating mRNA expression. Our analysis has narrowed the number of possible functional variants from 50 to approximately 6. These variants are located in a region of ~14 kb upstream of CHRNA5, including the promoter region. This work may provide genetic information that will lead to better prevention and intervention for substance use disorders.

2612/F

Searching for functional variants in STAT3-region in Finnish multiple sclerosis patients. *V. Leppa[1,2], A.-M. Sulonen[1,2], P. Ellonen[1], H. Almusa[1], E. Jakkula[1,3], P. Tienari[4], K. Koivisto[5], I. Elovaara[6], T. Pirttila[7], M. Reunanen[8], A. Palotie[1,9], L. Peltonen[1,9], J. Saarela[1].* 1) Institute for Molecular Medicine FIMM, Helsinki, Helsinki, Finland; 2) The National Institute for Health and Welfare, Helsinki, Finland; 3) Laboratory of Molecular Genetics, HUSLAB, Helsinki, Finland; 4) Dept. of Neurology, Helsinki Univ. Central Hospital, Helsinki, Finland; 5) Central Hospital of Seinajoki, Seinajoki, Finland; 6) Dept. of Neurology, Tampere Univ. Central Hospital, Tampere, Finland; 7) Dept. of Neurology, Kuopio University Central Hospital, Kuopio, Finland; 8) Oulu University Central Hospital, Oulu, Finland; 9) The Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK.

Multiple sclerosis (MS) is a complex autoimmune disease of the central nervous system. In Southern Ostrobothnia (SO) region in Finland the prevalence of MS is 2-fold compared to other populations of Northern European descent. We previously published a GWAS using distantly related SO MS patients and identified variants in STAT3 to associate with MS (Jakkula et al. 2010). This association was replicated in a sample set of 4638 cases and 10279 controls from 7 populations (rs744166, p 2.75 x 10E-10, OR 0.87).

We targeted 111 kb of the STAT3-STAT5A region using SureSelect target enrichment method and used Illumina GAII -sequencing to refine our understanding of the association and to identify potential functional variants. We chose ten MS samples based on the homozygous genotype for the potentially protective A-allele of rs744166 and pooled the samples. Four pools with total of 34 MS samples and three pools with total of 19 controls were sequenced. We obtained on average 31 x coverage per pool. In the rs744166 AA homozygous pool the preliminary analysis identified 303 unreported variants of which twenty are missense or nonsense mutations. None of the new variants were found in all pooled MS cases homozygous for rs744166, but 13 were specific to the MS pool. We are currently validating these mutations and in addition to this we are analyzing expression of STAT3 and near-by genes in PBMCs of 30 MS and 30 control samples to monitor if any of the genes are differentially expressed and if the expression correlates with the genotypes of the associated variants.

2613/F

Haploinsufficiency of Shank3 leads to deficits in synaptic plasticity. *O. Bozdagi, T. Sakurai, J.D. Buxbaum.* Psychiatry, Mount Sinai Sch Med, New York, NY.

Haploinsufficiency of SHANK3, caused by chromosomal abnormalities or mutations, leads to a neurodevelopmental syndrome that includes absent or severely delayed speech, intellectual disability, and autism spectrum disorder. The SHANK3 protein forms a key structural part of the postsynaptic density and in vitro studies have shown that expression of SHANK3 is sufficient to trigger the formation of glutamatergic synapses. We have developed a mouse with a disruption of Shank3 and are studying synaptic structure and function, as well as behavior. We examined excitatory glutamatergic synaptic transmission in hippocampal slices from Shank3 heterozygous and wildtype littermates. We performed conventional extracellular recordings from 1-3 month old mice, as well as whole-cell patch-clamp recordings in 3-month mice. To examine the properties of basic synaptic transmission in Shank3 heterozygous synapses, we performed extracellular recordings in Schaffer collateral-CA1 synapses in hippocampal slices and further monitored spontaneous miniature postsynaptic currents using whole-cell recordings. Both the amplitude of miniature excitatory postsynaptic currents from hippocampal CA1 pyramidal neurons of Shank3 heterozygous mice and the input-output function determined by field potential recordings were significantly lower than those of control mice, indicating a reduction in basal transmission due to a postsynaptic effect. We next examined the effect of Shank3 deficiency on synaptic plasticity at Schaffer collateral/CA1 synapses. Long-term potentiation (LTP) induced either with theta-burst or high-frequency stimulation was impaired in Shank3 heterozygous mice with no significant changes in long-term depression (LTD) induced by either low frequency (LFS) or paired-pulse-LFS stimulation. To investigate the AMPA and NMDA receptor-mediated contributions to evoked excitatory postsynaptic potentials, the input-output function was analyzed in the presence of antagonists. We observed that the AMPA receptor component of the input-output curve was significantly lower in Shank3 heterozygous mice. The results are consistent with an hypothesis where loss of one functional copy of SHANK3 leads to abnormal development and function of glutamatergic synapses with associated changes in synaptic plasticity. The studies indicate that AMPA receptors and glutamatergic transmission represent therapeutics targets in SHANK3 haploinsufficiency syndromes.

2614/F

Impact of genetic variation in the serotonin system on smoking cessation: Identifying the brain mediators with functional MRI. *A.J. Jasinska[1], H.F. Chua[2], S.S. Ho[3], L. Rozek[4], T.A. Polk[5], V.J. Strecher[2].* 1) Neuroscience Program, University of Michigan, Ann Arbor, MI; 2) Health Behavior and Health Education, University of Michigan, Ann Arbor, MI; 3) Psychiatry, University of Michigan, Ann Arbor, MI; 4) Environmental Health Science, University of Michigan, Ann Arbor, MI; 5) Psychology, University of Michigan, Ann Arbor, MI.

Twin studies suggest that genetic factors account for over 50 percent of the variance in smoking cessation outcomes but the specific genetic variants involved are mostly unknown. Identification and characterization of these genetic variants, and a better understanding of the neurobiological processes through which they influence smoking behavior, could guide the design and selection of the most effective treatment for each smoker. In the current study, we combined genetics, functional MRI, and health communication techniques to examine the role of genetic variation in the serotonin system on the brain processes related to smoking cessation. We focused on the 5-HTTLPR/rs25531 and STin2 polymorphisms in the serotonin transporter gene. Because prior neuroimaging work has shown that tailored health messages preferentially activate the self-related processing network, including the medial prefrontal cortex (MPFC), we examined whether the influence of the 5-HTTLPR/rs25531 and STin2 polymorphisms on smoking cessation is mediated by the response of the self-related processing network to tailored smoking cessation messages. Ninety-one smokers interested in quitting (44 females and 47 males; mean age 37.5 ± 11.5 years) were recruited for the study. All participants completed a web-based tailored smoking cessation program. Complete data (including genotyping results, fMRI data, and a smoking cessation outcome at 4-month follow-up) were available from eighty-four participants and these results are reported. We found evidence that the response to tailored health messages in the dorsal MPFC within the self-related processing network mediated the influence of STin2 genotype on the smoking cessation outcome at the 4-month follow-up (p less than 0.05). These findings may be relevant to a broad area of research on the genetics and neurobiology of smoking cessation and may point towards genetically-tailored smoking cessation treatments in the future.

2615/F

Analyzing *in vivo* function of the *ENGRAILED 2* ASD-associated haplotype. *S. Kamdar[1,2], T. Rahman[2], P.G. Matteson[2], L. Shinkarow[3], J.H. Millonig[1,2].* 1) Dept Neuroscience, UMDNJ, Piscataway, NJ; 2) Center for Advanced Biotechnology and Medicine UMDNJ-Robert Wood Johnson Medical School; 3) James Madison University.

Autism Spectrum Disorders (ASD) is a complex neuropsychiatric disorder with a strong genetic basis. *ENGRAILED 2 (EN2)*, a homeobox transcription factor, is expressed throughout CNS development and regulates several important developmental processes including connectivity and neurotransmitter development (Millen *et al.* 1995, Baader *et al.* 1998, Brunet *et al.* 2005, Sgaier *et al.* 2007). Previous work demonstrated the common alleles (underlined) of two intronic *EN2* SNPs, rs1861972 (A/C) and rs1861973 (C/T), are associated with ASD (A-C haplotype in 518 families, 2336 individuals, P-value < .000001) (Benayed *et al.* 2005). *In vitro* molecular genetic experiments (luciferase assays, Electro Mobility Shift Assays,) demonstrate the ASD-associated A-C haplotype increases gene expression (Benayed *et al.* 2008). After observing the above results, the next important step is to test for A-C haplotype function *in vivo*. *En2* has a dynamic expression pattern throughout CNS development, which leads us to ask if A-C haplotype is functional at more than one developmental time point, in more than one cell type, and if the haplotype always affects gene expression levels during CNS development?

To investigate the *in vivo* developmental regulation of the A-C haplotype, transgenic mouse lines were generated using ~25 kb of flanking *EN2* cis-regulatory sequence, which drives expression of a fluorescent reporter instead of the functional *EN2* protein. The same DNA sequence was used to make two transgenes: one has the ASD-associated A-C haplotype (A-C) and the other has the G-T haplotype (G-T). 6 A-C and 8 G-T lines were established. RT-qPCR analyses done to measure expression level differences between the two haplotypes, revealed that A-C haplotype increases reporter gene expression in the postnatal day 7 (96% increase, P-value<0.0001, n = 18) and adult (>P30) brain (200% increase, P-value<0.00001, n = 44). Ongoing experiments are investigating the effect of the A-C haplotype on spatial/temporal expression during CNS development as well as mRNA levels at different embryonic stages (E9, E12, and E17).

Together with our *in vitro* analysis, the above data demonstrate that the A-C haplotype functions *in vivo* as a transcriptional enhancer.

2616/F

A functional link between DYT1 and DYT6 dystonia: Regulation of *DYT1* gene expression by the transcription factor activity of THAP1 (DYT6). *A. Osmanovic[1], A. Erogullari[1], A. Rakovic[2], D. Braunholz[1], N. Uflacker[2], T. Lohnau[2], M. Albrecht[1], G. Gillessen-Kaesbach[1], C. Klein[2], K. Lohmann[2], F.J. Kaiser[1].* 1) Institut für Humangenetik, Universitätsklinikum, Lübeck, Germany; 2) Section of Clinical and Molecular Neurogenetics at the Department of Neurology, Lübeck, Germany.

While dystonia is clinically characterized by involuntary twisting, repetitive movements and abnormal postures, its molecular pathophysiology is not well understood. Mutations in two genes have been associated with monogenic forms of primary (isolated) dystonia: i) mutations in the *TOR1A* gene cause DYT1 dystonia; ii) mutations in the recently identified *THAP1* gene lead to the phenotypically similar DYT6 dystonia. The *THAP1*-encoded THAP1 protein consists of 213 amino acids with a characteristic DNA-binding THAP zinc-finger domain at the N-terminus. Recent studies demonstrated that THAP1 is involved in transcriptional regulation. To gain insights into the molecular mechanisms explaining the phenotypic overlap of DYT1 and DYT6 dystonia, we tested whether the transcription factor THAP1 directly regulates *TOR1A* promoter activity. For this, a fragment of 1kb representing the predicted *TOR1A* promoter was amplified and inserted into the pGL4 luciferase reporter plasmid. Co-transfection with full-length wild-type THAP1 expression plasmids decreases the *TOR1A* promoter activity in a concentration dependent manner. By analyzing a set of different truncated TOR1A promoter constructs in reporter gene assays, we narrowed the most critical region for promoter activity down to a stretch of about 220 bps 5' of exon 1 which contains two predicted THAP-binding motifs. In addition, DYT6 dystonia-associated mutant THAP1 proteins generated by site-directed in-vitro mutagenesis showed impaired THAP1-mediated repression of the *TOR1A* promoter activity. To proof that THAP1 binds to the *TOR1A* promoter, we performed chromatin immuno-precipitation assays (ChIPs) with purified nuclear fractions of human neuroblastoma cells (SH-SY5Y). By subsequent PCR, we confirmed that THAP1 specifically binds within the *TOR1A* promoter region. Our data clearly show that THAP1 directly regulates the activity of the *TOR1A* promoter, providing experimental evidence linking the molecular pathways underlying DYT1 and DYT6 dystonia. The function of THAP1 in regulation of gene expression establishes transcriptional dysregulation as a cause of dystonia. Identification of additional target genes will further elucidate the pathophysiology of dystonia.

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Candidate Genes and Educational Attainment: Screening for Novel Associations with Nicotine Dependence. *E. Johnson[1], R. Grucza[2], P. Hipp[2], N. Breslau[3], L. Bierut[2].* 1) Behavioral Health Epidemiology, RTI International, Res. Triangle Pk, NC; 2) Department of Psychiatry, Washington University, Saint Louis, MO; 3) Department of Epidemiology, Michigan State University, East Lansing, MI.

Genome-wide association studies of smoking phenotypes have successfully identified and confirmed associations with SNPs in the in the *CHRNA5-CHRNA3-CHRNB4* cholinergic nicotinic receptor subunit gene cluster on chromosome 15q24-25. Taken together, however, these associations account for less than 10% of the phenotypic variance. It is expected that additional unidentified genetic variants and the interplay with environmental risk factors will help to account for additional phenotypic variance. Educational attainment (EDU) is an important indicator of socioeconomic status and strongly associated with a number of smoking phenotypes including nicotine dependence (ND). The purpose of this study was two-fold: 1) to use the power of SNP x EDU interaction to discover novel gene - ND associations; and 2) to better understand the interplay between EDU and genetic risk for ND. Subjects were from the Collaborative Genetic Study of Nicotine Dependence (COGEND): 797 cases ascertained for Fagerström nicotine dependence, and 811 non-nicotine dependent smokers as controls, all of European descent. The two degrees of freedom test was used to screen 3,369 SNPs from 349 candidate genes, comparing a main-effect modhael with SNP x EDU interaction models. The top 20 findings, ranked by 2 df p-value, were followed up in control subjects from the GAIN Schizophrenia study available through dbGaP. Genes that were previously associated with ND and novel gene associations were among the top 20 SNP x EDU models (2 df p-values from 0.00005 to 0.004). Of these, six SNP x EDU models showed parallel associations in the replication sample tagging four genes previously not associated with ND(secretogranin V (*SCG5*), solute carrier family 8 member 1 (*SLC8A1*), solute carrier family 12 member 6 (*SLC12A6*), and protein kinase C epsilon (*PRKCE*)) and one gene that has been associated with ND: gamma-aminobutyric acid, receptor A, subunit 4 (*GABRA4*). Application of the 2 df test of SNP x EDU interaction to assessing a panel of candidate genes for nicotine dependence identified new associations for follow-up and highlighted potential differences in specific gene associations with nicotine dependence in varying environmental contexts as measured by educational attainment.

2618/F

Gene-Environment Interactions in Psychiatry: True Results or Type I Errors? *L.E. Duncan[1,2], M.C. Keller[1,2].* 1) Department of Psychology and Neuroscience, University of Colorado at Boulder; 2) Institute for Behavioral Genetics, Boulder Colorado.

Introduction: Measured gene-environment interaction (GxE) studies have typically been conducted in a candidate GxE (cGxE) fashion, analogous to the candidate gene association studies that have been used to search for genetic main effects. Such cGxE research in psychiatry has received widespread attention and acclaim, yet cGxE findings are also controversial. We were interested in trying to understand whether positive cGxE findings reported in the psychiatric literature were robust and might help to explain some of the missing heritability in psychiatric genetics or if, in aggregate, cGxE findings were consistent with the existence of publication bias, low power, and type I errors. **Method**: We conducted analyses on data extracted from all published studies (to our knowledge, 97 studies) from the first decade (2000-2009) of cGxE research in psychiatry. **Results:** Most novel cGxE studies were significant (95%) but only a minority of replication attempts were significant (29%), findings consistent with the existence of publication bias among novel cGxE studies. There may also be publication bias among replication attempts because significant replication attempts had *smaller* sample sizes, on average, than null replication attempts. Power calculations using observed sample sizes suggest that most studies were underpowered, perhaps severely so. Low power dictates that type I errors will outnumber true results among positive findings if, as is likely to be the case, most cGxE hypotheses that could be investigated are actually null. For example, given certain realistic assumptions, cGxE studies may have a rate of ~200 type I errors for every one true result. Finally, we show that low power biases the observed form of interactions (i.e. 'crossover' vs. 'non-crossover') toward the 'crossover' variety. **Conclusion**: These results suggest reason for caution in interpreting cGxE studies in psychiatry. They suggest that the cGxE literature in psychiatry is biased toward positive reports and that many positive cGxE findings are type I errors. In addition, even if true interactions are identified, the observed form of the interaction will often be incorrect.

2619/F

Cognitive Flexibility is Modulated by BDNF and Its Interaction with Substance Dependence. *N. Li[1], H. Zhang[1], H.R. Kranzler[2], J. Poling[1], A.I. Herman[1], J. Gelernter[1].* 1) Yale Univ Sch Med, West Haven, CT; 2) Uconn, Storrs, CT.

Cognitive flexibility is the ability to switch behavioral responses based on the context of a situation. Features of this important executive function vary between individuals, with possible genetic contributions to these differences largely undetermined. Brain derived neurotrophic factor (BDNF), a member of the nerve growth factor superfamily, plays a key role in regulating complex behaviors including memory and attention. We examined the association of variation in the BDNF gene with a measure of cognitive flexibility, a key cognitive function, in 519 African Americans [AAs; 443 substance (alcohol or drug) dependent individuals] and 391 European Americans [EAs; 337 substance (alcohol or drug) dependent individuals]. Subjects' cognitive flexibility was assessed using the Wisconsin Card Sorting Test (WCST). We examined the effects of six BDNF single nucleotide polymorphisms (SNPs) [including the functional Val66Met (rs6265)] and their interaction with substance dependence on cognitive flexibility using a multivariate analysis of variance and the haplotype program HAPSTAT. Subjects' sexes, age, years of education, and ancestry proportions (based on Bayesian clustering) were used as covariates in the model. In EAs, although none of the six SNPs showed a main effect on cognitive flexibility, the interaction of three SNPs (rs1519480, rs7124442 and rs11030121) with substance dependence significantly influenced two domains of WCST performance (perseverative responses: P=0.009, 0.017, and 0.012, respectively and perseverative errors: P=0.008, 0.016 and 0.010, respectively). Moreover, two specific haplotypes, C-C (consisting of alleles of rs1519480 and rs7124442 in haplotype Block I) and Val-G-T-T (consisting of alleles of Val66Met, rs7934165, rs11030121 and rs12273363 in haplotype Block II), were associated with significantly more perseverative responses (C-C: P=0.002; Val-G-T-T: P= 0.005) and perseverative errors (C-C: P=0.002; Val-G-T-T: P=0.005). There was greater cognitive flexibility in substance dependent subjects with these two haplotypes, as evidenced by significantly fewer perseverative responses: P=0.002 for C-C and 0.006 for Val-G-T-T; and significantly fewer perseverative errors: P=0.002 for C-C and 0.002 for Val-G-T-T). In AAs, we found no evidence of a genetic or gene-substance dependence interactive effect on cognitive flexibility. We conclude that variation in BDNF can moderate an individual's cognitive flexibility in a population-specific way.

2620/F

Mitochondrial DNA Haplogroups in Patients with Multiple Sclerosis in Bogota. *W. Cardenas Cuadros[1], M. Lattig[1], M. Torres[1], C. Perea[1], C. Guio[2], J. Toro[2], H. Groot[1].* 1) Biology, Universidad de Los Andes, Bogota; 2) Grupo de Neurologia, Fundacion Santa Fe, Bogota.

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system considered as a multifactorial disorder in which both environmental and genetic factors are involved. According to Kurtzkee 1975, the prevalence of MS can be divided into three risk areas based on the disease frequency: low (<5/100.000 inhabitants), medium (5-30/100.000) and high (>30/100.000). In Bogota, an area of defined low risk for the disease, an MS prevalence rate of 4,4/100.000 was previously reported by our group. It has been widely reported that MS prevalence is high in populations of Caucasian origin. We hypothesized that in Colombia, individuals with MS might have a Caucasian origin and therefore a higher prevalence to develop the disease even if they are living in a low prevalence zone. To start exploring this hypothesis we looked at the mitochondrial DNA (mtDNA) hypervariable segment I (HVSI) in order to define the various mtDNA haplogroups present in our population. To determine the haplogroups, we sequenced the HVSI segments of mtDNA in 58 MS patients and 58 control subjects to determine their maternally inherited ethnic origin of mitochondria in a heterogeneous population of Bogotá, Colombia. The percentage of mitochondrial haplogroups found in the study where: A= 32%, B=26%, C=14% and D=7% (Amerindian); L=5% (African); T1% and J=4% (Caucasian). No statistical differences were found between the affected and control individuals and their mtDNA haplogroup, however a vast majority of Amerindian haplogroups were found in the study population. When we analyzed the female population, the haplogroup distributions in the patients were: A= 33%, B=35%, C=9% and D= 11% (Amerindian); L=4% (African); and J=4% (Caucasian) and in the controls were: A= 25%, B=11%, C=20% and D=6% (Amerindian); L=6% (African); T=2% and J=6% (Caucasian), observing a significant association (FISHER, p=0.046) between the B haplogroup and the MS patients, and also a significant association (FISHER, p=0.026) with the C haplogroup and the control population. This is the first study in Colombia in which maternal mitochondrial haplogroups together with a disease such as Multiple Sclerosis has been explored.

2621/F

Genome-wide Association Analysis of Gender Difference in Major Depressive Disorder in the Netherlands population sample from NESDA and NTR registries. *N.R. Aragam, K.S. Wang.* Dept. of Biostatistics & Epidemiology, East Tennessee State University, Johnson City, TN.

Superscript TextBackground: Major depressive disorder (MDD) is a universally prevalent, genetic, and environment dependent mental condition that disables people of every culture, race, gender, and age. The gender differences for MDD has been widely reported in literature with a prevalence in women more than twice that of men and with odds ratios estimated between 1.4 and 2.0. However, few genome-wide analyses of gender differences has been reported to date. In this study, a sample European population database (from the National Institutes of Health Genetic Association Information Network) derived from the registries of Netherlands' Twins (NTR) and study of depression and anxiety in Netherlands (NESDA), is used to conduct a genome-wide association study of gender differences for MDD. Methods: PLINK software was used to analyze the genome-wide gender differences of MDD among 3356 individuals (1726 cases and 1630 controls; 1150 males and 2206 females) with 437547 SNPs. Results: We identified a total of 66 MDD associated SNPs with p-values less than 10^{-4}. The best SNP was rs1558477 in ADCYAP1R1 gene ($p = 2.07x10^{-7}$). Furthermore, two SNPs in LAPTM4A (rs7565124 and rs12471796, $p= 1.19 \times 10^{-6}$ and $1.71x10^{-6}$, respectively) and two SNPs in PCLO (rs2522833 and rs2522840, $p = 3.06x10^{-6}$ and $5.53x10^{-6}$, respectively) showed strong associations with MDD. In addition, we found 38 SNPs showing geneder differences with p-values less than 10^{-4}. The best SNP showing gender difference was rs12692709 ($p = 5.75x10^{-6}$) in GRB14 gene located at 2q24.3. The next four best SNPs showing gender differences were rs11039588 in OR4B1 ($p = 1.16x10^{-5}$), rs6856020 in LCORL ($p = 1.47x10^{-5}$), rs1589263 in PLD5 ($p = 1.60x10^{-5}$), and rs1507061 in SNX7 ($p = 1.61x10^{-5}$). Conclusions: Our findings provide a starting point for the genome-wide study of gender differences for MDD and related phenotypes, and the gene x gender interactions.

2622/F

Speech delay modulated by the interplay between paternal age and WNT2 gene in autism spectrum disorder. *P. Lin[1,2], Y. Chien[1], S. Gau[1].* 1) Dept Psychiatry, National Taiwan Univ Hosp, Taipei, Taiwan; 2) Maryland Psychiatric Research Center, Univ Maryland School of Medicine, Catonsville, Maryland, MD, USA.

Background: Delay in speech is the hallmark feature of autism spectrum disorder (ASD). Previous evidence suggests that language development is modulated by genetic factors, such as the FOXP2 gene (e.g., Fisher et al., 1998; Lai et al., 2001; Vernes et al., 2008), and SHANK gene (Wilson et al., 2003). Some but not all previous studies have also reported that these two genes are associated with risk of ASD. Additionally, paternal/maternal age has been found to be associated with cognitive functions in ASD (Saha et al., 2009). In the current study, we aimed to explore the effect of variants in and near FOXP2 and SHANK3 genes on speech delay inherent to ASD. We also assessed if paternal/maternal age modifies these genetic effects on language development. Methods: A total of 593 individuals diagnosed with ASD were ascertained in the current study. We selected 22 single nucleotide polymorphisms (SNPs) within the FOXP2 and SHANK3 genes and nearby genes. Genotyping was performed by MALDI-TOF (matrix-assisted laser desoption/ionization - time of flight) spectrometry for SNP analysis. We used two quantitative phenotypes, age of first words (AFW) and age of first phrases (AFP), to assess the degree of speech delay in these subjects. To study the genetic effect on speech delay, we used general linear model to examine the association between AFW/AFP and 22 SNPs. Covariates including gender and intelligence quotient were adjusted. Finally, we tested the interaction between associated SNP and paternal/maternal age. Results: The results show that rs2396753 on FOXP2 gene was significantly associated with AFW (regression coefficient = -5.09, p = 0.0108). Another SNP rs2896218 on the WNT2 gene was associated with AFP (regression coefficient = -6.81; p = 0.0045). A three-locus haplotype in the WNT2 gene was significantly associated with age of first phrases (rs4727845-rs2228946-rs2896218; regression coefficient = 6.57; p = 0.00026). Finally, we detected a significant interaction effect between paternal age and rs2228946 on both AFW (p = 0.0123) and AFP (p = 0.0387). Minor allele (A allele) at rs2228946 (located in 3' UTR region of WNT2 gene) was associated with older AFW/AFP in individuals with younger paternal age, while associated with younger AFW/AFP in individuals with older paternal age. Conclusion: This study suggests that the effect of WNT2 polymorphism on speech delay may depend on paternal age in ASD.

2623/F

DNA methylation in brain in Major Depressive Disorder. *Q. Chen, L. Cheng, C. Chen, D. Zhang, G. Elliot, C. Liu.* Dept Psychiatry, University of Chicago, Chicago, IL.

Environmental stressors modulate subsequent vulnerability to depression. Epigenetic studies of DNA methylation(DNAm) and direct measurement of gene expression in brain allowed us to combine genetic and environmental study of this complex disorder. We studied cerebellum brain tissue methylation differences between 15 major depression patients and 47 healthy controls. The Nimblegen 2.1M DeLuxe Promoter Array enables identification of methylated DNA regions across biologically significant regulatory elements in the genome. This array uses methylated DNA immunoprecipitaion(MeDip) technology and tiles with the 100bp probe spacing through around 10kb region of all annotated 32k gene promoters, 28k CpG islands and 475 miRNA promoters. We used CHARM software to do microarray data quality assessment, pre-processing/normalization, probe-level percentage of methylation estimates, and identification of differentially methylated regions (DMRs). We found 689 differentially methylated regions ($p<0.05$) between depression patients and controls. None can survive multiple testing correction(FDR<0.05). The length of the DMRs is 240-2926bp and average length is 616bp. We further evaluated the location distribution of variable DNA methylation relative to genes and to CpG islands. Because epigenetic alteration may correlate with expression changes, we are analyzing the genes in promoter regions of DMRs for the correlation of methylation difference and expression change.The whole genome methylation and expression analyses may suggest some novel genes related etiology of major depression.

2624/F

The role of TpH1-218A>C, TpH2-1463G>A and SLC6A4-5HTTLPR polymorphisms in development of post partum depression: A study in Jordanian population. *O.F. Khabour[1], E.A. Bani-Hani[2], B. Amarneh[3].* 1) Medical Laboratory Sciences, Jordan Univercity of Science and Technology, Irbid, Jordan; 2) Department of Biology, Jordan Univercity of Science and Technology, Irbid, Jordan; 3) Department of Public Health, Jordan Univercity of Science and Technology, Irbid, Jordan.

Postpartum depression (PPD) is a mood disorder occurs in (20 -28)% of women who have recently given birth and may extended during the first year after delivery. The presence of PPD has many adverse effects such as, marital problems, adverse behavioral and cognitive effects on the child, so as searching for predisposing genetic and environmental elements seems an important deal. Among the genetic factors that might contribute to PPD are variations in genes that involved in serotonergic neurotransmitter system like tryptophan hydroxylase -1(TpH1), tryptophan hydroxylase-2 (TpH2) and serotonin carrier family 6 member 4 (SLC6A4). In this study we investigated the frequency of TpH1-218A>C(rs 1800532), TpH2-1463G>A and SLC6A4-5HTTLPR among postpartum depressed women compared to postpartum healthy non depressed control women in Jordan. In addition, we examined the possible correlation between certain environmental factors including socio-demographic variables like mother age, intra-partum variables like pregnancy problems and psycho-social variables like depression history with postpartum depression among the same postpartum Jordanian women. A total of 130 depressed women and 240 normal controls were randomly selected from five maternity centers from north of Jordan. Genotyping of polymorphisms were performed using PCR technology. The results showed no significant correlation in the genotype distribution or allelic frequency of the three examined polymorphisms {TpH1 (A218C), TpH2 (G1463A) and SLC6A4-5HTTLPR} between depressed and normal controls ($P > 0.05$). Both allele S and L of the SLC6A4 polymorphism are common among Jordanian women (51% & 49% respectively), while allele C of TPH1 polymorphism is more common than allele A (64% versus 37% respectively). Regarding TPH2 polymorphism allele A is absent from all the examined women. However, three socio- demographic variables were found to be significantly associated with postpartum depression: depression history, pregnancy problems and economic status ($P < 0.05$). The results suggest that the stronger influence of environmental factors on PPD might cover or minimize the weaker genetic effect.

2625/F

Development of a hiPSC model for the study of gene-environment interactions in Parkinson's disease. *A.B. Bowman, A.M. Tidball, P. Hedera, K.C. Ess, M.D. Neely.* Dept Neurology, Kennedy Ctr for Research on Human Development, Vanderbilt Univ, Nashville, TN.

The proximate cause of Parkinson's disease (PD) is the degeneration of the dopaminergic neurons in the substantia nigra pars compacta and the ensuing loss of striatal dopamine innervation. Although the discovery of genes linked to rare familial forms of PD has provided some insights into the mechanisms underlying this disease, the majority of PD cases likely result from complex interactions between genetic and environmental factors. This multifactorial nature of PD makes the development of adequate models difficult. Recently, methods to derive dopaminergic neurons from patient fibroblasts have been developed. This approach provides us with a powerful tool to assess the multifactorial nature of PD. We have derived human induced pluripotent stem cell (hiPSC) lines from two brothers who lack a functional PARK2 allele and from a control patient who shows no neurological symptoms. Interestingly, one of the brothers carrying the PARK2 mutations (now 47 years old) reported childhood onset resting tremor and dystonia. Clinical diagnosis of PD occurred at ~30 years of age. The other brother carrying the identical PARK2 mutations (now 41 years old) shows no clinical symptoms of PD. This observation highlights the multifactorial nature of the mechanism underlying PD pathogenesis. We are presently deriving dopaminergic neurons from the hiPSC-lines of both brothers and the control patient and are comparing their general characteristics and their sensitivities to different PD-relevant stressors, such as mitochondrial toxins and metals. These studies lay the foundation for the assessment of gene-environment interactions in dopaminergic neurons derived from a much larger group of PD patients (both familial and iodiopathic cases) with the goal of determining possible patterns of patient-specific sensitivities to PD-associated stressors and developing patient-specific approaches to neuroprotective therapies.

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Transcriptome and Genome-wide Association Data Implicate PCLO Gene in Bipolar Disorder. *K. Choi[1], B. Higgs[2], J. Wendland[3], J. Song[1], F. McMahon[3], M. Webster[1].* 1) Stanley Med Res Inst, Rockville, MD; 2) Elashoff Consulting, Redwood City, CA; 3) Genetic Basis of Mood and Anxiety Disorders Unit, National Institute of Mental Health, NIH, Bethesda, MD.

Genetic variation may contribute to differential gene expression in the brain of individuals with psychiatric disorders. We explored potential association between genes differentially expressed in the prefrontal cortex (PFC) of bipolar disorder subjects and single nucleotide polymorphisms (SNPs) adjacent to those genes. Postmortem brain tissue of bipolar disorder (N=40) and unaffected controls (N=43) was profiled using genome-wide expression and SNP microarrays. We identified 367 genes as being differentially expressed (fold change > 1.3 and FDR-adjusted q-value < 0.05) in the PFC of bipolar disorder subjects. We then identified local SNPs (100 kb up- and down-stream of each gene) that are associated with expression of those genes. Using those local SNPs, we tested disease association with the results derived from a meta-analysis of genome-wide association studies (GWAS) including 4,936 bipolar disorder cases and 6,654 controls. We identified 45 local SNPs that are associated with genes differentially-expressed in bipolar disorder (FDR q-value < 0.05). Expression levels of HBS1L (15 SNPs), HLA-DPB1 (15 SNPs), AMFR (8 SNPs), PCLO (2 SNPs) and WDR41 (2 SNPs) were associated with multiple local SNPs. Among those SNPs, rs13438494 in an intron of the piccolo (PCLO) gene was significantly associated with bipolar disorder (FDR-adjusted $p < 0.05$) based on the meta-analysis of GWAS. These results are consistent with recent findings implicating PCLO in mood disorder and demonstrate the utility of combining the data from postmortem brains and a large scale GWAS in order to enhance our understanding of the genetic contribution to bipolar disorder.

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Genome-Wide Analysis of Copy Number and LOH Events in Alzheimer's Disease. *S. Shams.* BioDiscovery, Inc, El Segundo, CA.

We used computational methods to identify genomic areas of interest in Alzheimer's Disease (AD). We used genome-wide SNP array data generated by Alzheimer's Disease Neuroimaging Initiative (ADNI) and processed through Nexus Copy Number version 5.1 (BioDiscovery, Inc.) to identify regions of copy number change as well as Loss-of-Heterozygosity (LOH). We used the built-in SNP-Ranks Segmentation algorithm on 617 samples consisting of 168 Normals, 188 AD, and 261 Mild Cognitive Impairment (MCI) samples. We assessed the quality of the samples using neighboring probe variance across the genome (discarding the top 3% most varied probes to compensate for expected copy number changes). Using a maximum of 0.15 variance provided 460 samples that we used for further analysis. This set contained 130 Normals, 140 AD, and 190 MCI samples. We performed a Fisher's Exact test comparing the AD group with the Normal group requiring a minimum of 1% difference and a p-value of 0.01 to identify regions of significant copy number or LOH differences between the groups. We then looked at the areas of maximum significant within these regions to identify a total of 82 genes. Using a unique Markov-Process p-value computation, we explored Gene Ontology (GO) categorize that are over represented and genomically dispersed with these 82 genes. These included "Positive regulation of calcium-dependent exocytosis" and "regulation of timing of neuron differentiation" among other terms. These terms led to the identification of some interesting genes, such as SCAMP5 which was LOH in 25% of AD cases and 10.8% in Normal and SOX5 with 18.6% deleted in AD and 13.1% in Normal. Both genes are implicated in cognitive development.

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Ashkenazi Jewish PD GWAS and combined analysis of public domain datasets. *L. Clark[1], R. Cheng[2], M. Verbitsky[1], X. Liu[1], K. Marder[1,2], J. Lee[1,2].* 1) Taub Institute for Research on Alzheimer Disease and the Aging Brain, Columbia University Medical Center, 630 West 168th street, New York, NY 10032; 2) Gertrude H. Sergievsky Center, Columbia University Medical Center, 630 West 168th street, New York, NY 10032.

We conducted a GWAS on AJ subjects with PD and controls frequency matched for age, sex and ethnicity. They were participants in a study of the genetic epidemiology of PD (GEPD, NS36630) and the Ashkenazi Jewish PD study (NS050487, NS060113) at Columbia University (CU). To replicate the most promising signals from the discovery stage we examined the positive signals in two previously published PD GWAS: The NINDS dataset (Fung et al., 2006; Simon-Sanchez et al 2009), and the joint dataset from the Progeni/GenePD studies (Pankratz et al., 2009). This provided a dataset for analysis with a combined sample size of 2,076 cases and 1,805 controls. All AJ cases (N=272) were recruited from the Center for Parkinson's disease and Other Movement disorders at CU. All met research criteria for PD . The majority of AJ controls (N=172) were recruited by random digit dialing and were frequency matched by age, gender, ethnicity and area code/exchange. The remaining AJ controls were recruited from a 50% sample of Medicare recipients aged >65 who resided in the Washington Heights community. All controls underwent the same evaluation as cases, which included a medical history, UPDRS and mMMS. Family history of PD and related disorders in first-degree relatives was obtained using a structured interview. Information on Jewish ancestry in each of the grandparents was obtained during that interview. Sixty percent of samples were genotyped using the Illumina Human 610-quad bead arrays and the remainder of samples were genotyped using the Illumina Human 660-quad bead arrays. All DNAs were derived from whole blood. Quality scores were determined from allele cluster definitions for each SNP as determined by the Illumina Genome Studio Genotyping Module and the combined intensity data from 100% of study samples. Overall, the NINDS dataset consisted of 886 PD cases and 791 controls of European American background. A total of 44 subjects (37 PD cases and 7 controls) were excluded from the analysis because population cluster analysis revealed that these individuals do not share the same ethnic background as the rest of the samples. The Progeni/Gene PD dataset consisted of 900 cases and 867 controls. None of the SNPs in our PD phenotype analysis reached genome-wide significance in the Ashkenazi Jewish PD study from CU. However, 61 SNPs with a P value <9.5x10-5 were selected for further analysis and meta-analysis with the dbGAP datasets.

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Endophenotype Ranking Facilitates Identification of Novel QTLs for Recurrent Major Depression. *D. Glahn[1,2], J.W. Kent[3], L. Almasy[3], R.L. Olvera[4], M. Carless[3], A.M. Winkler[1,2], J.E. Curran[3], J.C. Charlesworth[3], M.P. Johnson[3], H.H.H. Göring[3], T.D. Dyer[3], E.K. Moses[3], P. Kochunov[5], R. Duggirala[3], P.T. Fox[5], J. Blangero[3].* 1) Olin Neuropsychiatric Ctr, IOL & Yale, Hartford, CT; 2) Department of Psychiatry, Yale University School of Medicine, New Haven, CT; 3) Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX; 4) Department of Psychiatry, UT Health Science Center at San Antonio, San Antonio, TX; 5) Research Imaging Institute, UT Health Science Center at San Antonio, San Antonio, TX.

Although genetic studies of mental illness have made progress in recent years, identification of risk genes for psychiatric disorders has lagged behind discoveries in other complex diseases. The use of quantitative allied phenotypes or endophenotypes could significantly improve the pace of progress in psychiatric genetics. An optimal endophenotype is a highly heritable quantitative trait that is genetically correlated with disease liability, and can be measured in affected and unaffected family-members, inherently providing much greater power to localize disease-related genes than affection status alone. Unfortunately, a priori selection of the most effective endophenotype is often impossible for mental illness, a limitation that is compounded by an inability to directly compare these measures. Here, we present the Endophenotype Ranking Value (ERV), a novel index of the genetic utility of an endophenotype for any heritable illness. The ERV, an index of the standardized genetic covariance, provides an unbiased and empirically derived method for objectively ranking endophenotypes before conducting molecular genetic analyzes. To demonstrate the utility of the ERV, we ranked behavioral, neurocognitive, neuroimaging and transcriptional candidate endophenotypes for recurrent major depressive disorder (rMDD). 1122 randomly selected Mexican-Americans from 75 extended pedigrees, 215 (19%) with lifetime rMDD, participated in the study. The heritability estimate for the illness was 0.570 (p=4.2x10-4). Top-ranked endophenotypes included the Beck Depression Inventory (BDI; p=2.3X10-5), a neuroticism questionnaire (1.7X10-4), a test of declarative memory (5.4X10-2), bilateral diencephalon volume (1.3X10-3), parietal white-matter hyperintensity volume (3.9X10-3), left hippocampal volume (7.8X10-3), and the lymphocyte-based transcripts RNF123 (5.2X10-6), PDXK (1.1X10-5), and ZFP64 (2.0X10-5). Bivariate GWA analyses conducted with the BDI and rMDD diagnosis and utilizing the Illumina 550K reveals several SNPs at genome-wide significance with pleiotropic effects on both traits. These results demonstrate the utility of the ERV and provide two novel genome-wide significant QTLs for recurrent major depression.

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Identification of Novel Candidate Genes for Alzheimer Disease by Autozygosity Mapping Using Genome Wide SNP Data From an Israeli-Arab Community. *R. Sherva[1], C. Baldwin[1], R. Inzelberg[2], B. Vardarajan[1], A. Cupples[1], K. Lunetta[1], A. Bowirrat[3], A. Naj[4], M. Pericak-Vance[4], R. Friedland[5], L. Farrer[1].* 1) Boston University, Boston, MA; 2) Tel Aviv University, Tel Aviv, Israel; 3) Ziv Medical Center, Safed, Israel; 4) University of Miami, Miami, FL; 5) University of Louisville, Louisville, KY.

Wadi Ara, a genetically isolated Arab community in northern Israel, has a high prevalence of Alzheimer disease (AD) despite a low *APOE* ε4 allele frequency. The goal of this study was to exploit the genetic structure of this population to identify AD risk genes. AD cases (N=124) and controls (N=142) were genotyped for a genome-wide set of > 300,000 SNPs which were used to calculate measures of population stratification and inbreeding, and to identify regions of autozygosity. Despite its isolation we found evidence for admixture and stratification in Wadi Ara, identifying three distinct ancestry clusters. Although a high degree of relatedness was evident in both cases and controls, controls were significantly more related and contained more autozygous regions than cases (P=0.004). Eight autozygous regions on seven chromosomes were significantly more frequent in controls than cases, and 105 SNPs in these regions, primarily on chromosomes 6 and 9, were nominally associated with AD in logistic regression models. We attempted to replicate these findings in four independent GWAS datasets containing 2861 cases and 2416 controls. Three SNPs in *NOTCH4* were nominally associated with AD in both Wadi Ara and the meta-analysis: rs3132946, (OR_{meta}=1.14, P_{meta}=0.02), synonymous coding SNP rs1044506, (OR_{meta}=1.13, P_{meta}=0.04), and rs3131294 (OR_{meta}=1.14, P_{meta}=0.03). Rs3130286 in a nearby gene, *TNXB*, was also replicated in the meta-analysis (OR_{meta}=1.12, P_{meta}=0.03), as was intergenic SNP rs13271711 on chromosome 8 (OR_{meta}=1.13, P_{meta}=0.04). *NOTCH4* is a member of a gene family involved in controlling cell fate decisions during development. Proteolysis of NOTCH4 is regulated by γ-secretase, whose activity is responsible for the final cleavage of the amyloid precursor protein to release Aß peptide. SNPs in *TNXB* have previously been associated with schizophrenia. Analysis of the full Wadi Ara GWAS dataset revealed 99 SNP associations with AD at P £ 10^{-5}, but none of these were confirmed in the replication datasets. Lack of association of *APOE* in Wadi Ara afforded a unique opportunity to determine whether associations of AD with *TOMM40* and *PVRL2*, genes near *APOE*, are independent of *APOE*. However, we did not observe association with any *TOMM40* or *PVRL2* SNPs. The unique population structure of Wadi Ara enhanced efforts to identify genes not previously associated with AD that may account for some of the risk of the disorder in this and other outbred populations.

2631/F

Most reproducibly-identified genes for addiction phenotypes: dependence and smoking cessation. *G. Uhl[1], T. Drgon[1], C. Johnson[1], D. Walther[1], J. Rose[2], W. Eaton[3], N. Ialongo[3].* 1) Dept Molec Neurobiology, NIDA/NIH, Baltimore, MD; 2) Dept Psychiatry and Center for Nicotine and Tobacco Reserach, Duke University, Durham NC; 3) Dept of Mental Health, Johns Hopkins Bloomberg School of Public Health, Baltimore MD.

While classical genetics supports ca 50 percent genetic contributions to vulnerability to addiction and ability to quit smoking, no reproducible findings of genome wide significance have been identified for these phenotypes. Chromosomal regions and genes have been identified by clusters of SNPs that achieve nominal significance in each of 9 or more independent samples for each of these phenotypes. Genes that are identified much more frequently in this way than expected by chance include cell adhesion molecule (esp CDH13 and CSMD1). A splicing regulator(A2BP1)and genes involved in glutamatergic neurotransmission(GRM5) are also identified frequently. Data obtained in independent samples from the same racial/ethnic background provides more frequent identification of the same chromosomal regions than data from samples from different racial/ethnic backgrounds (racial differences are much larger than differences between studies using pooled vs indivdual genotyping). While statistical confidencein the polygenic effects at these loci does not approach the oligogenic influences of the flushing syndrome alcohol dependence results in Asians or the chr 15 nicotinic receptor loci variants on smoking quantity/frequency, the overall degree to which these results are replicated appears to be as great as those for most polygenic variants in other complex phenotypes.Seeking chromosomal regions that are identified by clustered results that achieve nominal statistical significance in multiple independent samples is likely to provide different sensitivities and specificities than SNP by SNP metaanalyses, especially in the face of allelic heterogeneity. This approach provides strong evidence for reproducible identification of a number of genes whose variants contribute to individual differences in these addiction related phenotypes, and a basis for postGWAS studies. (Support: NIDA, NIMH,unrestricted funding from PM USA to JR).

2632/F

Genetic control of sleep: Genome-wide association study of the nocturnal electroencephalogram (EEG). *M. Kawashima[1], S.C. Warby[1], O. Carrillo[1], R. Apple[2], J. Faraco[1], L. Lin[1], P. Peppard[2], T. Young[2], E. Mignot[1].* 1) Ctr Narcolepsy, Stanford University, Palo Alto, CA; 2) School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI.

In humans, sleep proceeds in predictable successive cycles of non-rapid eye movement (NREM) sleep (stage 1, 2, slow wave sleep) and rapid eye movement (REM) sleep. Each sleep stage has a distinct set of scalp electroencephalogram (EEG) features. The EEG measures the summed activity of post-synaptic currents of cortical neurons, which is influenced by deeper structures in the brain. A simple yet reliable way to analyze this complex signal is spectral analysis, which uses Fourier transform to decompose EEG into power values at various frequency ranges, typically divided into delta, theta, alpha, sigma, beta, and gamma bands. Unlike sleep duration or sleep timing, EEG features are stable within an individual over time, but highly variable between unrelated individuals (inter-individual). Twin studies have established heritability estimates of EEG within each sleep stage as high as 0.75-0.95, making EEG features one of the most heritable human traits. EEG changes have been associated with numerous disorders, and may be a useful endophenotype in complex neuropsychiatric disorders. To uncover genetic factors explaining inter-individual differences in EEG profiles, we assessed 1,876 polysomnographs (PSGs) in 1,300 adult individuals from the Wisconsin Sleep Cohort. Six hundred and sixtynine individuals had more than two PSGs separated by 4 years, so we could assess the stability of EEG features within an individual over time. Correlations of absolute and relative EEG power, and power in various frequency bands (and in various sleep stages) were examined to select suitable phenotypes. REM sleep and NREM sleep stage 1 and 2 but not slow wave sleep (known to be associated with sleep debt) were found to be most stable. Theta (θ: 4-8 Hz), alpha (α: 8-12 Hz), and sigma (σ: 12-16 Hz) frequency bands were found to have the highest correlation coefficient values. Based on this data, we selected 10 phenotypes for GWAS analysis (Affymetrix 6.0 array). A small set of SNPs, none of which have been previously linked to this phenotype, were detected with genome-wide significance. Replication study is currently underway as we extend the GWAS to additional cohorts. We believe sleep EEGs may be a useful way to study brain activity independent of sensory inputs, providing insight into normal and abnormal brain circuitry and its genetic basis.

2633/F

15q24.1 Microduplication in an Autistic Multiplex Family May Narrow a Region of Susceptibility for Developmental Disability and Autistic Disorder. *S.J. Sacharow[1,2], H.N. Cukier[1], D. Salyakina[1], S.F. Blankstein[1], J.L. Robinson[1], H.H. Wright[3], R.K. Abramson[3], R. Menon[4], S.M. Williams[5], J.L. Haines[5], M.L. Cuccaro[1], J.R. Gilbert[1], M.A. Pericak-Vance[1].* 1) John P. Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL; 2) Dr. John T. Macdonald Foundation Department of Human Genetics, University of Miami Miller School of Medicine, Miami, FL; 3) Department of Neuropsychiatry, University of South Carolina School of Medicine, Columbia, SC; 4) Department of Epidemiology and Department of Obstretrics & Gynecology, Rollins School of Public Health, Emory University, Atlanta, GA; 5) Center for Human Genetics Research, Vanderbilt University, Nashville, TN.

Copy number variations (CNVs) of 15q24.1 have been implicated in a range of phenotypes including mental retardation, developmental delay, autism and Asperger's syndrome. The majority of CNVs in this area have breakpoints near five low-copy repeat clusters. Prior studies demonstrate a minimal critical region of 0.766 Mb for deletions and 1.33 Mb for duplications. Here we report two cousins with autistic disorder who carry a ~10,000 base pair microduplication in the 15q24.1 critical region. **Case presentation:** Patient 1 has speech and language deficits, ADHD and autistic disorder. His full scale IQ is 46, and he is nondysmorphic. He has a brother with autistic disorder and mental retardation, hypotonia, motor delay, and congenital hip dislocation; however, the brother was unavailable for genetic testing. Patient 2 is a maternal first cousin of Patient 1. He has high functioning autism, mood disorder, ADHD, and dyslexia. His IQ is 99, and he is nondysmorphic. **Methods:** This multiplex autistic family was genotyped with the Illumina Human 1M Beadchip. The PennCNV algorithm using the Log R ratio and B allele frequency was used to identify CNVs which co-segregate with autism. TaqMan real-time PCR copy number assays were then utilized for validation and refinement of the CNVs. **Results and Discussion:** Two microduplications were identified to co-segregate in patient 1, patient 2, and their mothers. The first CNV is a ~10 kilobase microduplication on 15q24.1 that falls within the region previously reported in patients with autism and developmental delay. The second CNV is a novel ~348 kilobase duplication on chromosome 7p21.3. Neither duplication was identified in 676 Caucasian pediatric controls. Two other first degree relatives unavailable for genetic testing also have mental retardation and autistic features, suggesting a potentially strong but incompletely penetrant genetic component in this family. The 15q24.1 microduplication does not appear to be caused by the previously delineated low-copy repeats. This CNV contains the first 4 exons of the *ubiquitin-like 7 gene* (UBL7) and a hypothetical gene, *LOC440288*. This is the smallest duplication in the region reported to be associated with autistic disorder, potentially narrowing the critical region.

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Duplication of SLIT3 on 5q35.1 Predisposes to Major Depressive Disorder. *C.E. Kim[1], J.T. Glessner[1], K. Wang[1], P.M.A. Sleiman[1], H. Zhang[1], J.H. Flory[1], J.P. Bradfield[1], M. Imielinski[1], E.C. Frackelton[1], H. Qiu[1], F. Mentch[1], S.F.A. Grant[2], H. Hakonarson[2].* 1) Ctr Applied Genomics, CHOP, Philadelphia, PA; 2) Division of Genetics, Department of Pediatrics, The Children's Hospital of Philadelphia University of Pennsylvania School of Medicine Philadelphia, PA, 19104, USA.

Major depressive disorder (MDD) is a common psychiatric and behavioral disorder. To discover novel variants conferring risk to MDD, we conducted a whole-genome scan of copy number variation (CNV), including 1,693 MDD cases and 4,506 controls genotyped on the Perlegen 600K platform. The most significant locus was observed on 5q35.1, harboring the SLIT3 gene (P=2x10-3). Extending the controls with 30,000 subjects typed on the Illumina 550k array, we found the CNV to remain exclusive to MDD cases (P=3.2x10-9). Duplication was observed in 5 unrelated MDD cases encompassing 646 kb with highly similar breakpoints. SLIT3 is integral to repulsive axon guidance based on binding to Roundabout receptors. Duplication of 5q35.1 is a highly penetrant variation accounting for 0.7% of the subset of 647 cases harboring large CNVs, using a threshold of a minimum of 10 SNPs and 100 kb. This study leverages a large dataset of MDD cases and controls for the analysis of CNVs with matched platform and ethnicity. SLIT3 duplication is a novel association which explains a definitive proportion of the largely unknown etiology of MDD.

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Copy number variants in schizophrenia: confirmation of 5 previous findings and new evidence for association of 3q29 microdeletions and VIPR2 duplications. *D.F. Levinson[1], J. Duan[2], S. Oh[1], K. Wang[3], A.R. Sanders[2], J. Shi[4], N. Zhang[1], P.V. Gejman[2].* 1) Dept Psychiatry, Stanford Univ, Palo Alto, CA; 2) Northshore University Healthcare Research Institute, Evanston, IL; 3) University of Pennsylvania and Children's Hospital of Philadelphia, Philadelphia, PA; 4) National Cancer Institute, Bethesda, MD.

Introduction: To evaluate previously-reported and possible new associations of copy number variants (CNVs) to schizophrenia, we analyzed CNVs in the Molecular Genetics of Schizophrenia (MGS) sample and additional available data. Methods: After sample and CNV QC, 3,945 MGS cases and 3,611 controls were available for analysis using GWAS data from the Affymetrix 6.0 array. CNV analyses were carried out with Birdseye (Birdsuite 2.0) plus visual inspection and re-analysis of interesting regions with a second method. CNV call thresholds were chosen which maximized duplicate concordance in 151 duplicate specimens. Combined analyses with additional publicly-available data (ISC case-control and CHOP control datasets) were carried out where possible. Analyses of pointwise association and of genome-wide CNV counts were carried out using PLINK, and Fisher's exact tests were used to evaluate association of specific regions and of exonic CNVs in individual genes. Results: Five previously-reported associations were strongly supported (long deletions in 1q21.1, 15q13.3 and 22q11.21, duplications in 16p11.2 and exonic CNVs in NRXN1) with combined analyses producing ORs of 8 or greater. The strongest evidence for new candidate assocations were for the 3q29 mmicrodeletion region (1.6 Mb deletions containing 21 genes from TFRC to BDH1), observed in 0.1% of cases and 0 controls (p=0.004)); and 76-648kb duplications in VIPR2 (chromosome 7q), observed in 0.19% of cases and 0.03% of controls (p= 0.002). Other suggestive associations will be presented. The hypothesis of a global increase in gene-containing CNVs received modest support for large (>100kb) deletions but not for duplications. Conclusions: Association of schizophrenia with 1q21.1, 15q13.3, 22q11.21 and exonic NRXN1 deletions and with 16p11.2 duplications should be considered clearly established. It is likely that 3q29 deletions and VIPR2 duplications are also associated with moderate to high penetrances. Additional associations are likely to be confirmed from other candidate regions from this and other studies. This work was supported by NIMH grants U01MH079469 and U01MH079470. Grateful acknowledgement is made to study participants and to MGS investigators and clinical teams.

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Analysis of Heterogeneity in a Genome-wide Association of Late-Onset Alzheimer Disease Confirms Limited Heterogeneity for Strongest Associations. *A. Naj[1], R. Rajbhandary[1], G.W. Beecham[1], E.R. Martin[1], P.J. Gallins[1], E.H. Powell[1], I. Konidari[1], P.L. Whitehead[1], G. Cai[2], V. Haroutunian[2], W.K. Scott[1], J.M. Vance[1], M.A. Slifer[1], H.E. Gwirtsman[3], J.R. Gilbert[1], J.L. Haines[4], J.D. Buxbaum[2], M.A. Pericak-Vance[1].* 1) John P. Hussman Institute for Human Genomics, Dr. John T Macdonald Foundation Department of Human Genetics, University of Miami Miller School of Medicine, Miami, FL; 2) Department of Psychiatry, Mount Sinai School of Medicine, New York, NY; 3) VA Medical Center, Nashville, TN; 4) Vanderbilt Center for Human Genetics Research, Vanderbilt University, Nashville, TN.

Alzheimer Disease (AD) is highly genetic (estimated H^2~70%), but until recently, genome wide association studies (GWAS) have only observed consistent genetic associations with late-onset AD (LOAD) in APOE, possibly due to heterogeneity such as etiologic heterogeneity and ascertainment bias. To determine whether associations are being masked potentially by heterogeneity in a previous GWAS of LOAD, we used multinomial logistic regression analyses in SAS (v9.2) to re-evaluate associations of 483,399 SNPs among 931 cases and 1104 controls by two potential sources of heterogeneity, stratifying cases by autopsy confirmation of AD clinical diagnosis (370 autopsy-confirmed/561 clinically-confirmed), and stratifying cases by Ashkenazi Jewish (AJ) background in our sample (174 AJ/757 non-AJ). Stratifying by autopsy-confirmation status, we confirmed associations with SNPs in or near APOE on chromosome 19 in comparisons of both autopsy-confirmed cases (rs2075650, $P=1.63247\times10^{-22}$) and clinically-confirmed cases (rs2075650, $P=3.2\times10^{-29}$) to controls. A SNP association meeting genome-wide statistical significance in unstratified analyses, rs11754661 (OR (95% CI): 2.03 (1.58, 2.62); $P=4.70\times10^{-8}$), still demonstrated strong associations for autopsy-confirmed (OR (95% CI):1.84 (1.26, 2.68); P=0.00146) and clinically-confirmed (OR (95% CI): 2.04 (1.55, 2.70); $P=4.20\times10^{-7}$) comparisons. Stratifying by AJ/non-AJ ethnicity, we found similar patterns of peak associations in comparisons of non-AJ cases with controls, but found different peak associations comparing AJ cases with controls. The strongest among these is an association at rs10178614 on chromosome 2 (OR (95% CI): 0.346 (0.217, 0.554); $P=9.68\times10^{-6}$), located in CNTNAP5 (contactin associated protein-like 5), a gene in the neurexin family which function as cell adhesion molecules and receptors in the nervous system. In summary, stratification by autopsy-confirmation status found effect sizes of associations within stratum similar to those in non-stratified GWAS analyses of LOAD, though with reduced statistical significance, likely due to smaller sample sizes of the strata. Weak heterogeneity by autopsy-confirmation status may reflect improved accuracy of clinical AD diagnosis; further investigation is needed. Stratifying by AJ/non-AJ ethnicity identified a different set of peak associations among those with an AJ background, although additional replication in other AJ datasets is necessary to confirm this association.

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Identification and characterisation of endophenotypes for epilepsy through MRI. *G. Cavalleri[1], S. Alhusaini[1], C. Scanlon[2], L. Ronan[2], G. Borgulya[1], S. Maguire[4], P. Brennan[4], A. Fagan[5], P. Iyer[3], G.E. Boyle[6], J.F. Meaney[5], C.P. Doherty[3], M. Fitzsimons[2], N. Delanty[1,2].* 1) MCT, Royal College Surgeons, Dublin, Ireland; 2) Departments of Neurophysics and Neurology, Beaumont Hospital, Dublin 9, Ireland; 3) Department of Neurology, St James' Hospital, Dublin 8, Ireland; 4) Radiology Department, Beaumont Hospital, Dublin 9, Ireland; 5) Department of Radiology, St. James's Hospital, Dublin, Ireland; 6) Department of Medical Physics and Bioengineering, St James's Hospital, James's Street, Dublin 8, Ireland.

The experience of the research community to date has shown that endophenotypes are required to augment the power of genetic mapping studies in complex forms of epilepsy. We are applying in-vivo quantitative magnetic resonance imaging to identify subtle structural variations in the brain which are both heritable and involved in the development of temporal lobe epilepsy. Our study design involves scanning index patients with temporal lobe epilepsy together with an unaffected same gender sibling and healthy controls. We have applied numerous sets of analysis across these three groups to identify brain structures showing characteristics of good endophenotypes for epilepsy - heritable, associated with the disease and measureable in healthy controls. Results to date have highlighted several candidate endophenotypes structures including the thalamus and cingulate. These novel endophenotypes are being measured in a larger cohort of epilepsy patients for whom whole genome association data is already available.

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A Genome-Wide Association Study of Symptomatic Epilepsy in Han Chinese Detects Multiple Variants. *S.S. Cherny[1], Y.L. Guo[1], P.C. Sham[1], L. Baum[2], P. Kwan[3].* 1) Department of Psychiatry, The University of Hong Kong; 2) School of Pharmacy, The Chinese University of Hong Kong; 3) Division of Neurology, Department of Medicine & Therapeutics, The Chinese University of Hong Kong.

Epilepsy is one of the most common serious neurological disorders, affecting approximately 1% of the population. While idiopathic epilepsy has been the most studied form and has the most evidence for genetic influence, there has also been converging evidence for genetic influences on the more common symptomatic form, where the epilepsy is a result of a known brain injury or lesion. We conducted a genome-wide association study (GWAS) of 504 symptomatic epilepsy cases and a cohort of shared control samples of 1947 subjects participating in several GWAS of complex disease and an additional 1000 healthy individuals. All cases and controls were Han Chinese. We employed the Illumina HumanHap 610-Quad platform, which assays approximately 600,000 SNPs. From analysis of this initial GWAS, we identified three loci containing variants that confer epilepsy risk, in addition to confirming a previously known association with the *GRIK2* gene. One of the loci (rs4853352, $p < .000002$) on chromosome 2p12 was intragenic but near neural developmental genes, including *LRRTM1* and *LRRTM4* (SLIT proteins), which may play a role in the development and maintenance of the vertebrate nervous system. At 13q31.1, a genome-wide significant SNP (rs9519914, $p < .000000005$) is near the *SLITRK6* gene, a member of the integral membrane proteins with 2 N-terminal leucine-rich repeat (LRR) domains similar to those of SLIT proteins, which is expressed predominantly in neural tissues and has neurite-modulating activity. The strongest signal (rs12148329, $p < .00000000002$) is in the intronic region of the *FAM108C1* gene on chromosome 15q25.1, which is expressed in human atherosclerotic lesions and significantly elevated in patients with unstable angina. These SNPs and approximately 70 others are being replicated in a larger independent sample of Chinese epilepsy patients and controls.

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Genome-wide Association Study for Dementia in the Amish finds non-APOE regions. *A.C. Cummings[1], L. Jiang[1], D. Velez Edwards[2], R. Laux[1], L.L. McFarland[1], P.J. Gallins[3], L. Caywood[3], L. Reinhart-Mercer[3], D. Fuzzell[1], C. Knebusch[3], C.E. Jackson[4], J.L. McCauley[3], W.K. Scott[3], M.A. Pericak-Vance[3], J.L. Haines[1].* 1) Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 2) Vanderbilt Epidemiology Center, Vanderbilt University Medical Center, Nashville, TN; 3) Hussman Institute for Human Genomics, University of Miami, Miami, FL; 4) Scott & White, Temple, TX.

Late Onset Alzheimer disease (LOAD) is the leading cause of dementia in the elderly. While evidence for an underlying genetic etiology of AD is strong, it has proven to be quite challenging to elucidate. Polymorphisms in APOE account for less than half of the susceptibility and thus other genetic factors are likely to be involved. Genetic heterogeneity is a major complicating factor hindering further gene identification. To overcome this problem and maximize our power to identify AD risk genes, we are studying the genetically isolated and well-defined Amish populations of middle Ohio and northern Indiana. To date we have enrolled over 2200 Amish individuals with 135 of these having either probable or possible dementia. Through the use of the Anabaptist Genealogy Database, we have accurately defined the kinship coefficients and the family structure among our collected individuals. We performed a genome-wide association study (Affymetrix Human SNP Array 6.0) and successfully genotyped 830 Amish individuals (125 with LOAD). Because of the relatedness of these individuals, we employed a novel test of association using the MQLS test, which uses kinship coefficients to correct for relatedness. We performed parametric and nonparametric 2-pt and parametric multipoint linkage analysis (Merlin, MINX) after using PedCut to divide our large pedigree into smaller, more computationally tractable sub-pedigrees. We conducted parametric multipoint linkage analysis in regions in which a 2-pt lod score ≥ 3 was calculated. Following extensive QC procedures, 614,957 SNPs were analyzed. Using the MQLS test, our most significant p-value (1.7 x 10-7) was at rs1236195 on chromosome 13 in SPATA13, a spermatogenesis gene. Fourteen additional SNPs generated p-values <1x10-5, but none are near known or strongly suspected AD loci (APP, PS1, PS2, PICALM, CLU, CR1). A total of 45 regions contained at least one SNP with a lod ≥ 3. The highest multipoint peak (lod=5.82, recessive model) was calculated at 2p12. Six genes lie within the 1-lod-unit interval, none of which to our knowledge have previously been associated to LOAD. Three additional regions had a multipoint lod score >3: 3q26, 9q31, and 18p11. The GWAS results suggest novel non-APOE genetic effects for LOAD in our Amish dataset, with the most compelling evidence on chromosomes 2, 3, 9, 13, and 18. Further detailed analyses to explore these results are ongoing.

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Genome-wide linkage and association study of ADHD in adults. *D. Boomsma[1], V. Saviouk[1], G. Willemsen[1], B. Penninx[2], J. Smit[2], P. Slagboom[3], E. DeGeus[1], J. Hottenga[1].* 1) Dept Biological Psychology, Vrije Univ, Amsterdam, Netherlands; 2) Dept Psychiatry VU University Medical Centre, Amsterdam, Netherlands; 3) Dept Molecular Epidemiology, Leiden University Medical Centre, Nettherlands.

There have been several genetic linkage and GWA studies for ADHD in children, showing mixed results. The aim of our study is to detect the genomic loci that influence ADHD in (young) adults and compare these to results from studies in children. ADHD data from > 12,000 adults were analyzed with structural equation modeling, linkage (N= 752 sib pairs) and genome-wide association (GWA) tests (N = 2475 unrelated subjects). ADHD was assessed by the Conners Adult ADHD Rating Scales (CAARS; Conners CK, Erhardt D & Sparrow E (1999)) from twin families registered with the Netherlands Twin Register and subjects participating in the Netherlands Study of Depression and Anxiety. Phenotype data from over 12,000 twins, siblings and parents were analyzed with genetic structural equation modeling to estimate heritability. ADHD heritability in adults was estimated at 30% in men and women, after correction for assortative mating. All familial transmission was explained by genetic inheritance. Genome-wide linkage and association (GWA) analysis of quantitatively assessed ADHD features in adults may uncover loci influencing susceptibility to adult ADHD. We carried out linkage and association analyses in subsamples with micro-satellite data and SNP data. Genome-wide linkage analysis showed linkage to 2p25.2 - 2p25.1 (max LOD = 3.58 (p=0.037) based on parametric linkage analysis) for the ADHD index and to 18q21.2 - 18q22.1 for Inattention (max LOD = 4.58 (p=0.0026) based on VC). The ten highest p-values from the GWA study (1.51E-06 - 9.05E-06) were observed on chromosomes 8, 11, 18 and 21. We acknowledge support from NWO/ZonMW: 904-61-090; 904-61-193; 480-04-004; SPI 56-464-14192; NBIC-Bioassist; Center for Medical Systems Biology; Neuroscience Campus Amsterdam and EMGO+. the Centre for Neurogenomics and Cognitive Research (CNCR-VU); Genetics of Mental Illness (ERC-230374); the European Union (EU/WLRT-2001-01254), ZonMW (geestkracht program, 10-000-1002), NIMH (RO1 MH059160) and matching funds from participating institutes in NESDA and NTR. Genotyping was funded by the Genetic Association Information Network (GAIN) of the Foundation for the US National Institutes of Health. VS is a Marie Curie fellow supported by the EUTwinsS network (RTN, FP6).

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Variants in BDNF and CHRNA5-CHRNA3-CHRNB4 are associated with smoking persistence in African-Americans. *A. Hamidovic[1], N. Benowitz[2], K. Buttler[3], S. Buxbaum[3], N. Francescini[5], S. Gharib[6], R. Goodloe[7], B. Hitsman[1], J. Kasberger[2], D. Levy[4], S. Preiss[4], S. Redline[7], B. Spring[1], E. Tong[9], W. White[3], K. Wiggins[6], T. Young[8], E. Jorgenson[2].* 1) Northwestern University , Chicago , IL; 2) University of California San Francisco, CA; 3) Jackson State University Jackson, MS; 4) National Heart, Lung and Blood Institute Bethesda, MD; 5) University of North Carolina Chapell Hill, NC; 6) University of Washington Seattle, WA; 7) Case Western University Cleveland, OH; 8) Broad Institute Boston, MA; 9) Univeristy of California Davis, CA.

To date, most genome-wide association studies (GWAS) of smoking behavior have been conducted in populations of European ancestry, and many of these studies have focused on phenotypes that measure smoking quantity (i.e. cigarettes per day). Additional association studies in diverse populations with different linkage disequilibrium patterns can aid the search for functional variants. Furthermore, more precise phenotype measures that account for changes in smoking patterns over time may better describe smoking behavior. For these reasons, we undertook an association study of smoking persistence, as measured by pack-years of cigarette smoking, in unrelated African-American participants from The National Heart, Lung, and Blood Institute's Candidate Gene Association Resource (CARe) (n= 1490). Multiple markers in and around Brain-Derived Neutrophic Factor (BDNF) on 11p14.1 and a cluster of genes encoding nicotinic acetylcholine receptor subunits (CHRNA5-CHRNA3-CHRNB4) on 15q25.1 were associated with smoking persistence. We then imputed additional common SNPs in the 11p14.1 and 15q25.1 loci using data from the 1,000 Genomes project in order to more precisely map functional variants. Our analysis is among the first to use data from the 1,000 Genomes project in African-Americans in order to provide new information to mapping and characterizing loci underlying complex traits.

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Genome-wide association study in bipolar patients with co-morbidity - The challenge of rare variants. *B. Kerner[1], C.G. Lambert[2], B.O. Muthén[3].* 1) Department of Psychiatry, David Geffen School of Medicine, University of California, Los Angeles, CA, USA; 2) Golden Helix Inc., Bozeman, MT, USA; 3) Professor Emeritus, University of California, Los Angeles, CA, USA.

Background: Bipolar disorder is a severe psychiatric disorder with high heritability. Co-morbid conditions are common and might define latent subgroups of patients that are more homogeneous in respect to genetic risk factors. **Methodology/Principal Findings:** In the GAIN bipolar disorder sample of 1000 Caucasian cases and 1034 controls we tested the association of single nucleotide polymorphisms with patient subgroups defined by co-morbidity. The most significant association was found with rs16904801 ($p=4.5x10^{-9}$) on 8q24 in bipolar patients with low probability for substance abuse and alcohol dependence. This rare genomic variant is located in the gene Thyroglogulin precursor (TG) (HGNC:11764). Two additional rare variants in expressed sequences of unknown function approached the genome-wide level of significance in this latent class, rs1039002 ($p=1.6x10^{-8}$) on 6q27, and rs12563333 ($p=5.9x10^{-8}$) on 1q41. The genomic variants were present only in cases and absent in controls. Bipolar disorder with high probability of alcohol dependence and substance abuse was associated with rs2727943 ($p=3.3x10^{-8}$) on 3p26.3 in a non-coding region. **Conclusions/Significance:** Conceptualizing bipolar disorder as a heterogeneous disorder with regard to co-morbid conditions might facilitate the identification of genetic risk alleles. Rare variants might contribute to the susceptibility to bipolar disorder. Given the small sample size of our study and the rare nature of the variants, a replication of our results in an independent larger study or a confirmation through re-sequencing would be desirable to support our results.

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Genetics of human episodic memory: Understanding complexity through genome-wide studies. *A. Papassotiropoulos[1], C. Vogler[1], A. Heck[1], D.J.F. de Quervain[2].* 1) Molecular Psychology, University of Basel, Basel, Switzerland; 2) Cognitive Neurosciences, University of Basel, Basel, Switzerland.

Experimental work in animals has shown that memory formation depends on a cascade of molecular events. In humans, heritability estimates of ~50% suggest that genetic factors have an important impact on this fundamental brain function. Therefore, our study aims at identifying memory-related genes and gene-clusters in humans and at translating the findings to memory-related disorders. Gene identification is done by combining unbiased genome-wide association studies (GWAS), gene clustering, and functional MRI (fMRI) in populations which are carefully tested for memory performance and for the presence or absence of diseases related to impaired memory function. We show that variability of human memory performance is highly significantly related to variability in the gene encoding beta catenin-like 1 and to a cluster of genes encoding proteins of core molecular signaling cascades. Functional magnetic resonance imaging revealed that GWAS-derived genetic profiles correlate with activations in memory-related brain regions. The search for genes related to human memory processes provides new insights into the genetic basis of this cognitive ability and is promoting the targeted treatment of memory disorders by identifying relevant genetic pathways in humans.

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Genome-wide association study suggests a risk locus for pediatric-onset bipolar disorder on chromosome 5q. *D.T. Chen[1], N. Akula[1], C.J.M. Steele[1], N. Craddock[3], H.M.D. Gurling[4], J.W. Smoller[2], P.H. Lee[2], F. McMahon[1].* 1) Unit on the Genetic Basis of Mood & Anxiety Disorder, National Institute of Mental Health, National Institutes of Health, U.S. Dept of Health and Human Services, Bethesda, Maryland 20892, USA; 2) Psychiatric Genetics Program in Mood and Anxiety Disorders, Department of Psychiatry, Massachusetts General Hospital, Boston, Massachusetts 02114, USA; 3) MRC Centre for Neuropsychiatric Genetics and Genomics, Henry Wellcome Building, School of Medicine, Cardiff University, Heath Park, Cardiff CF14 4XN, UK; 4) Molecular Psychiatry Laboratory, Department of Mental Health Sciences, University College London Medical School, Windeyer Institute of Medical Sciences, London, United Kingdom.

Pediatric-onset bipolar disorder (PBPD) is a strongly familial, often chronic and disabling condition. The American Academy of Child & Adolescent Psychiatry (AACAP) defines PBPD as onset of mania <12 years. To date, no clear genetic markers of PBPD have been identified. We conducted a genome-wide association study (GWAS) of PBPD by comparing cases with age at onset < 12 years (n = 197) to controls (n = 1033) in the NIMH Genetics Initiative sample, which had been genotyped with approximately 1 million single nucleotide polymorphisms (SNPs) under the auspices of the Genetic Association Information Network (GAIN). A total of 768,000 SNPs passed quality control and were analyzed for association using PLINK. Two markers on chromosome 5q25, in the vicinity of BNIP1 and C5orf41, showed evidence of association with PBPD at the $p < 5 \times 10^{-7}$ level, with odds ratios in the range of 2.59-2.60. When case definition was restricted to first mania before age 12 in a post-hoc analysis, odds ratios at these markers increased to the range of 2.9-3.0, suggesting that the locus is important in cases meeting strict AACAP criteria for PBPD. After imputation against HapMap Phase 2 data using MACH 1.0, 2 additional SNPs were identified with case-control association p-values in the 10^{-7} range. Supportive results were obtained in a second cohort of ~1600 cases and controls, with consistent direction of association and combined p-values less than 4×10^{-8}. However, replication results in STEP-BD/UK/WTCCC were negative. Further explorations for replication are ongoing. BNIP1 is involved with apoptosis and alpha-SNAP binding, related to endoplasmic reticulum organization and membrane fusion. Future studies will focus on additional replication testing and deep resequencing. BNIP1 may be an important neurobiological target for some childhood-onset psychiatric disorders.

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A group of intracellular translocases including *TOMM40* is associated with Alzheimer disease. *M.G. Hong, J. Prince.* Medical Epi and Biostatistics, Karolinska Institutet, Stockholm, Sweden.

The output of genome-wide association studies (GWAS) which are proliferating and successfully identifying genetic determinants of traits and diseases often include data on a large number of marginally associated SNPs, which are frequently neglected by stringent alpha values at the genome-wide level. As an attempt to utilize those possibly informative data, pathway approaches have been conducted in some of GWAS, which search a group of genes with similar characteristics that are over-represented among the marginally significant markers. However, pathways have been defined with genes, not genetic markers. The process to convert to gene lists is required and we developed a tool facilitating the task and applied to a recent GWAS for Alzheimer disease (AD) in a French population. The tool reads the output of GWAS and generates a list of genes with gene-wise significance correcting for problems that longer genes are likely to have lower P-values purely by chance and that the markers are often correlated because of linkage disequilibrium (LD). With the AD study data set, a significant enrichment of genes involved in the biological process of intracellular transmembrane protein transport was observed. The products of genes of the pathway include several transport proteins across the mitochondrial membrane and nuclear envelope. The SNP, rs2075650, which was associated with highest significance with AD status is located in *TOMM40*, which encodes the channel-forming subunit of the translocase of the outer membrane complex of mitochondria. The fact that the marker is only about 16kbp away from rs429358, the key SNP of the *APOE* e4 isoform, may imply the strong association was observed because of LD between the two markers. However, considering the fact that a group of transporting proteins was also associated with AD including *TOMM40* may imply that genetic variance in the gene itself possibly contributes to the development of AD together with other translocases.

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Schizophrenia GWAS in a large Ashkenazi cohort. *T. Lencz[1], S. Guha[1], J. Rosenfeld[1], A. Malhotra[1], I. Pe'er[2], A. Darvasi[3].* 1) Dept Psychiatry Res, Zucker Hillside Hosp, Glen Oaks, NY; 2) Dept. of Computer Science, Columbia University, New York, NY; 3) Dept. of Genetics, Hebrew University, Jerusalem, Israel.

Background: Recent genomewide association studies (GWAS) in schizophrenia have, for the first time, provided strong support for several susceptibility loci. While the strongest evidence has emerged for loci in the major histocompatibility complex (MHC), disentangling the true source of this signal in heterogeneous outbred populations has proven challenging. Moreover, the overwhelming majority of genetic risk for schizophrenia, a highly heritable disorder, remains unknown. Consequently, we performed a GWAS in a large (total n>3000), ethnically homogeneous case-control cohort of Ashekenazi Jewish individuals from Israel. **Methods:** At the time of submission, genotyping of the cohort on the Illumina 1M Omni-Quad platform is nearly complete. Interim analyses were conducted using PLINK on the first half of the sample (n=649 cases with schizophrenia and 1187 controls), after eliminating population outliers identified using STRUCTURE. **Results:** In the interim analyses, signals approaching genomewide significance were observed in the MHC. Genomic inflation was low, and careful examination of population parameters appears to permit further refinement of the signal. Additionally, several other promising candidate loci emerged at genomewide ($p<10^{-7}$) or near genomewide ($p<10^{-6}$) levels. Additionally, cases were observed to have excess runs of homozygosity (ROHs), compared to controls. **Conclusions:** Full GWAS results for SNPs, ROHs, and CNVs in the entire sample will be presented. Preliminary results suggest that examination of an ethnically homogeneous founder population may increase power for detection of at least a subset of susceptibility loci.

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Meta-analysis of genome-wide association studies identifies three new multiple sclerosis susceptibility loci: *EOMES*, *MLANA*, and *2p21*. *N.A. Patsopoulos[1, 2, 3], F. Esposito[4,5], L. Ottoboni[1, 2, 3], J. Reischl[6], S. Lehr[6], D. Bauer[6], A.J. Ivinson[2], S.J. Sawcer[9], A. Compston[9], S.L. Hauser[10], J.R. Oksenberg[10], F. Boneschi[4,5], G. Comi[4,5], J. Heubach[6], R. Sandbrink[6, 7], C. Pohl[6, 8], P.L. De Jager[1, 2, 3], D.A. Hafler[11], P.I.W. de Bakker[2,3,12], the steering committees of studies evaluating IFNb-1b and a CCR1-antagonist, ANZ Consortium.* 1) Program in Translational NeuroPsychiatric Genomics, Department of Neurology, Brigham & Women's Hospital, Boston, MA 02115, USA; 2) Harvard Medical School, Boston, MA, 02115, USA; 3) Program in Medical & Population Genetics, Broad Institute of Harvard University and Massachusetts Institute of Technology, Cambridge, MA 02139, USA; 4) Department of Neurology, Scientific Institute San Raffaele, 20132 Milan, Italy; 5) Institute of Experimental Neurology, Scientific Institute San Raffaele, 20132 Milan, Italy; 6) Bayer Schering Pharma AG, 13342, Berlin, Germany; 7) Department of Neurology, Heinrich Heine University Düsseldorf, Germany; 8) Department of Neurology, University Hosptial Bonn, Germany; 9) University of Cambridge, Department of Clinical Neuroscience, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 2QQ, UK; 10) Department of Neurology, University of California San Francisco, San Francisco, CA, 94143, USA; 11) Department of Neurology, Yale University School of Medicine, New Haven, CT 06520, USA; 12) Division of Genetics, Department of Medicine, Brigham & Women's Hospital, MA 02115, USA.

Introduction Multiple Sclerosis (MS) is thought to have a complex genetic background with environmental triggers. Besides the known association with MHC, genome-wide association studies (GWASs) have identified several other loci. Here, we perform a meta-analysis of several GWASs, and explore the functional consequences of the newly identified risk-associated alleles. **Methods** Seven GWAS datasets of MS were included and, after quality checks, we imputed all datasets with HapMap phase II as reference. We analyzed each dataset using the imputed dosages, correcting for population structure. Per dataset we used the Odds Ratio (OR) and the standard error, corrected for the dataset specific inflation factor (λ), to perform a meta-analysis under the per-allele model. To leverage the list of susceptibility loci associated with inflammatory diseases, we tested known SNP from 7 inflammatory diseases (Crohn's disease (CD), ulcerative colitis (UC), celiac disease (CE), type 1 diabetes (T1D), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and psoriasis (PS)) for a role in MS. Finally, we performed eQTL analysis in 228 cases with MS and used the Ingenuity Systems software to identify pathways and networks of interacting genes. **Results/Discussion** 5,545 cases, 12,153 controls and 2,529,394 SNPs passed QC and were included in the meta-analysis. We observed 3 new loci with $p<5x10^{-8}$. $rs170934^{T}$ at locus 3p24.1 near *EOMES* (OR=1.17, P= $1.6x10^{-8}$), $rs2150702^{G}$ in the second intron of *MLANA* on chromosome 9 (OR=1.16, P=$3.3x10^{-8}$), and $rs6718520^{A}$, which maps to an intergenic region on chromosome 2p21 (OR=1.17, P=$3.4x10^{-8}$). Another 10 independent SNPs had p-value<10^{-6}, of which $rs1738074^{T}$ in *TAGAP* (OR=0.87, P= $3.7x10^{-7}$) is also associated with CE, T1D, and RA. A comprehensive comparison of *bona fide* associations with other inflammatory diseases revealed a substantial overlap of susceptibility loci. More SNPs that predispose to UC and CE are associated in MS (9 of 15 and 12 of 26 at a nominal level , respectively) than those of the other inflammatory diseases. Although eQTL analysis didn't reveal a strong *cis* effect for the newly identified SNPs, pathway and network analysis uncovered a remarkable level of interconnectedness in the transcriptomic effects of the known and novel MS susceptibility loci.

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Genome-wide supported schizophrenia-associated genes: regulation by REST/NRSF, network organization and primate-specific evolution. *M.J. SIMONNEAU[1], Y. LOE-MIE[1,2], A-M. LEPAGNOL-BESTEL[1], G. MAUSSION[1], A. DORON-FAIGENBOIM[3], S. IMBEAUD[4], H. DELACROIX[4], L. AGGERBECK[4], T. PUPKO[3], P. GORWOOD[1], J-M. MOALIC[1].* 1) INSERM U894, Paris, F, France; 2) CNRS-UPR2589, Information Génomique & Structurale, 163 Avenue de Luminy, Marseille, France; 3) Department of Cell Research and Immunology, George S. Wise Faculty of Life Sciences, Tel-Aviv University,Israel; 4) Molecular Genetics, CNRS, Gif, France.

The SMARCA2 gene encodes the ATPase BRM in the SWI/SNF chromatin-remodeling complex. SMARCA2 was recently identified as being associated with schizophrenia (SZ) in a genome-wide association study (GWAS). Polymorphisms in SMARCA2, associated with the disease, were identified as able to produce changes in the expression of the gene and/or in the encoded amino acid sequence. We showed that an SWI/SNF-centered network including the mouse Smarca2 gene was modified when REST/NRSF was down-regulated in a mouse neuronal cell line. REST/NRSF down-regulation also modifies the levels of Smarce1, Smarcd3 and SWI/SNF interactors (Hdac1, RcoR1 and Mecp2). We asked if Smarca2 down- regulation was sufficient to generate an intermediate phenotype of SZ and found that Smarca2 deregulation generated an abnormal dendritic spine morphology. We further found that 8 (CSF2RA, HIST1H2BJ, NOTCH4, NRGN, SHOX, SMARCA2, TCF4 and ZNF804A) out of 10 GWAS supported SZ-associated genes are part of an interacting network (including SMARCA2), 5 members of which encode transcription regulators. The expression of TCF4, SMARCA2 and CSF2RA out of the 10 genome-wide supported SZ-associated genes was modified when the REST/NRSF-SWI/SNF chromatin-remodeling complex was experimentally manipulated either in mouse cell lines or in transgenic mouse models. This REST/NRSF-SWI/SNF deregulation also results in the differential expression of genes that are clustered in chromosomes. This result suggests that REST/NRSF deregulation is able to induce genome-wide epigenetic changes. Finally, we studied primate-specific evolution of SMARCA2 interactors and the genome-wide supported SZ-associated genes. We found that these repertoires are considerably enriched in genes displaying positive selection in primates and in the human lineage which suggests the occurrence of novel protein interactions in primates. Altogether, these data identify the SWI/SNF chromatin-remodeling complex including SMARCA2 as a key component of the genetic architecture of SZ. [Loe-Mie et al., SMARCA2 and other genome-wide supported schizophrenia-associated genes: regulation by REST/NRSF, network organization and primate-specific evolution. Hum Mol Genet. 2010].

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Genome-wide association study of personality measures in bipolar disorder. *J.S. Strauss[1], Q. Cheng[2], A. Noor[1], S.V. Parikh[3], V. De Luca[1], J.L. Kennedy[1], W. Xu[2], J.B. Vincent[1].* 1) Centre for Addiction and Mental Health, Toronto, ON, Canada; 2) Princess Margaret Hospital/University Health Network, Toronto, ON, Canada; 3) Toronto Western Hospital, University Health Network, Toronto, ON, Canada.

Background: Much of the molecular genetic study of personality phenotypes has included candidate gene studies of monoaminergic loci, often in antisocial personality disorder and related phenotypes, such as alcohol abuse, or in traits measured by TPQ or NEO. Personality traits have been associated with symptoms of bipolar disorder. Methods: We recruited 431 adult Caucasian patients with DSM-IV/ICD-10 Bipolar I or II Disorder (BP I or II). Personality QTL data was obtained using the 90-item Eysenck Personality Questionnaire (EPQ). We used four QTLs -three from the EPQ: Extraversion(E), Neuroticism(N), Psychoticism(P), in addition to the Brief Life Events Questionnaire (BLEQ) for first episode. 523,407 SNPs were genotyped using Illumina HumanHap550 BeadChip microarrays. We checked for potential genotype errors, obtained descriptive statistics, and their LD patterns using Haploview. PLINK was applied to test for Hardy-Weinberg equilibrium (HWE) for each SNP. For genotyped polymorphisms, we excluded variants with a call rate of less than 90 percent; or strong deviation from HWE. We also excluded subjects with a completion rate of less than 90 percent; or whose reported sex did not match with the inferred sex based on the heterozygosity rate from the sex-chromosomes. Principal components (PC) analysis was undertaken to adjust for population heterogeneity using EIGENSTRAT. Population outliers were detected and subsequently excluded from the analysis. Results: After data quality control filtering, 427 subjects with 503,528 SNPs were used for analysis. The most significant PC-adjusted p-values for each of the four QTLs were-- E: 5.937x10-7 (3q28, rs4686964); N: 3.542x10-6 (15q21.3, rs12903062); P: 1.283x10-6 (3p14.2, rs7625594); BLEQ: 4.238x10-7 (1p12, rs12757996). The most significant SNP at each of three EPQ QTLs in our BP sample exists in a linkage region previously implicated in BP - linkage on 3q28 (Zandi et al. 2007), on 15q21 (Fullerton et al. 2008) and on 3p14 (Etain et al. 2006). Conclusion: While no single marker reaches genome-wide significance (p<5x10-8), given the context of previous studies, our strongest findings are suggestive of genetic overlap between personality traits and BP. Future directions include an increased sample size, with EPQ and BLEQ data, to increase statistical power.

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A Genome-wide Meta-analysis Identifies Novel Loci Associated with Schizophrenia and Bipolar disorder. *K. Wang, X. Liu, N. Aragam.* Department of Biostatistics and Epidemiology, College of Public Health, East Tennessee State University, Johnson City, TN.

Schizophrenia and bipolar disorder both have strong inherited components. Recent studies have indicated that schizophrenia and bipolar disorder may share more than half of their genetic determinants. In this study, we performed a meta-analysis for genome-wide association data of the Affymetrix Genome-Wide Human SNP array 6.0 to detect genetic variants influencing both schizophrenia and bipolar disorder using European-American samples (691 bipolar cases and 1081 controls, 1172 schizophrenia cases and 1379 controls). The best associated SNP rs11789399 ($p = 2.38x10^{-6}$, $5.74x10^{-4}$, $5.56x10^{-9}$, for schizophrenia, bipolar disorder and meta-analysis of schizophrenia and bipolar disorder, respectively) was at 9p33.1, with eight additional SNPs showed associations in meta-analysis within the haplotype block of the best SNP (p-values ranged from 9.11×10^{-6} to 1.55×10^{-8}). The next best SNP was rs12201676 ($p = 2.67x10^{-4}$, $2.12x10^{-5}$, $3.88x10^{-8}$ for schizophrenia, bipolar disorder and meta-analysis, respectively) located at 6q15, with two additional SNPs showed associations in meta-analysis within the haplotype block of this SNP ($p = 8.1 \times 10^{-5}$ and 7.75×10^{-7}, respectively). The third interesting SNP rs802568 ($p = 8.92x10^{-4}$, $1.38x10^{-5}$, $1.62x10^{-7}$ for schizophrenia, bipolar disorder and meta-analysis, respectively) was at 7q35 within CNTNAP2 while meta-analysis showed three additional SNPs within the haplotype block of this SNP (p-values ranged from 9.68×10^{-5} to 7.00×10^{-7}). Through meta-analysis, we found two additional genes NALCN (the top SNP is rs2044117, $p = 4.57 \times 10^{-7}$) and NAP5 (the top SNP is rs10496702, $p = 7.15 \times 10^{-7}$). The three genes (CNTNAP2, NALCN and NAP5) have been previously reported to be associated with psychiatric disorders such as ADHD, autism, bipolar disorder, and or schizophrenia. Haplotype analyses of above five loci indicated stronger associations than single-marker analyses in schizophrenia and bipolar disorder. These results provide evidence of common genetic variants influencing schizophrenia and bipolar disorder.

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A genome-wide association study of attempted suicide. *V.L. Willour[1], F. Seifuddin[1], P.B. Mahon[1], D. Jancic[1], M. Pirooznia[1], J. Steele[2], B. Schweizer[1], F.S. Goes[1], F.M. Mondimore[1], D.F. MacKinnon[1], R.H. Perlis[3], P.H. Lee[3], J.R. Kelsoe[4], P.D. Shilling[4], M. Rietschel[5,6], M. Nöthen[7,8], S. Cichon[8,9], H. Gurling[10], S. Purcell[3], J.W. Smoller[3], N. Craddock[11], J.R. DePaulo[1], T.G. Schulze[2], F.J. McMahon[2], P.P. Zandi[1], J.B. Potash[1], The Bipolar Genome Study (BiGS) Consortium.* 1) Department of Psychiatry and Behavioral Sciences, Johns Hopkins University, Baltimore, MD; 2) Genetic Basis of Mood and Anxiety Disorders Unit, National Institute of Mental Health Intramural Research Program, National Institutes of Health, US Department of Health and Human Services, Bethesda, MD; 3) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA; 4) Department of Psychiatry, University of California, San Diego, La Jolla, CA; 5) Department of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, Mannheim, Germany; 6) Department of Psychiatry, University of Bonn, Bonn, Germany; 7) Institute of Human Genetics, University of Bonn, Bonn, Germany; 8) Department of Genomics, Life & Brain Center, University of Bonn, Bonn, Germany; 9) Institute of Neuroscience and Medicine (INM-1), Research Center Juelich, Juelich, Germany; 10) Department of Mental Health Sciences, University College London, London, UK; 11) Department of Psychological Medicine, School of Medicine, Cardiff University, Cardiff, UK.

The heritable component to attempted and completed suicide is partly related to psychiatric disorders and also partly independent of them. While attempted suicide linkage regions have been identified on 2p11-12 and 6q25-26, there are likely many more such loci, the discovery of which will require a much higher resolution approach, such as the genome-wide association study (GWAS). With this in mind, we conducted an attempted suicide GWAS that compared the single nucleotide polymorphism (SNP) genotypes of 1,201 bipolar subjects with a history of suicide attempts to the genotypes of 1,497 bipolar subjects with no history of suicide attempts. 2,507 SNPs with evidence for association at $p<0.001$ were identified. These associated SNPs were subsequently tested for association in a large and independent bipolar disorder sample set. None of these SNPs were significantly associated in the replication sample after correcting for multiple testing, but the combined analysis of the two sample sets produced an association signal on 2p25 (rs300774) at the threshold of genome-wide significance (p= 5.07 X 10-8). The associated SNPs on 2p25 fall in a large linkage disequilibrium block containing the *ACP1* gene, a gene whose expression is significantly elevated in bipolar subjects who have completed suicide. Furthermore, the ACP1 protein is a tyrosine phosphatase that influences Wnt signaling, a pathway critical to lithium's effects. This connection to lithium is intriguing given that lithium is the best-established medication used to reduce the rate of suicidal behavior, thus making *ACP1* a strong functional candidate for involvement in the phenotype. Larger GWAS sample sets, such as the ones being compiled by the Psychiatric GWAS Consortium (PGC), will be required to confirm the signal on 2p25 and to identify additional genetic risk factors increasing susceptibility for attempted suicide.

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eQTL analysis of human brain compared to lymphoblastoid cell lines. *E.M. Schmidt[1], B.J. Keller[2], V. Strumba[3], M. Flickinger[4], J.Z. Li[5], M.H. Dai[1], F. Meng[1], R.C. Thompson[1], E. Sliwerska[1], A.F. Schatzberg[6], E.G. Jones[7], W.E Bunney[8], R.M. Myers[9], S.J. Watson[1], H. Akil[1], M. Boehnke[4], L.J. Scott[4], M. Burmeister[1].* 1) Molecular & Behavioral Neuroscience Inst, Univ of Michigan, Ann Arbor, MI; 2) Dept of Computer Science, Eastern Michigan Univ, Ypsilanti, MI; 3) Addiction Research Center, Univ of Michigan, Ann Arbor, MI; 4) Dept of Biostatistics and Center for Statistical Genetics, Univ of Michigan, Ann Arbor, MI; 5) Dept of Hum Genetics, Univ of Michigan, Ann Arbor, MI; 6) Dept of Psychiatry, Stanford Univ, Stanford, CA; 7) Center for Neuroscience, UC Davis, Davis, CA; 8) Dept of Psychiatry & Hum Behavior, UC Irvine, Irvine, CA; 9) HudsonAlpha Inst for Biotechnology, Huntsville, AL.

Our current understanding of psychiatric disorders is likely to be greatly improved by investigating the genetic control of gene expression in the human brain through genome-wide expression quantitative trait loci (eQTL) mapping. Many of the previous studies of brain eQTLs have been hampered by limited brain tissue availability and lack of brain region-specific samples. In contrast, large eQTL data sets are publicly available for lymphoblastoid cell lines (LCL). Our goal is to compare overall cortex and brain region specific eQTLs with eQTLs detected in publicly available LCL data to determine the extent to which gene regulation is the same or different between brain and LCLs, and to identify brain specific regulatory variants.

We quantified mRNA expression levels from 6 different brain regions in a single unrelated collection of approximately 40 controls and 60 cases per region from individuals with 1 of 3 psychiatric disorders including bipolar, schizophrenia, and depression. Samples were genotyped with a 550k Illumina chip, and an additional 2 million SNPs were imputed based on HapMap genotypes. We regressed the transcript expression levels for 18,187 probes in 16,254 genes on the expected SNP allele counts using linear regression with adjustment for age, gender, and disease status. For each brain region we performed 1.47×10^7 tests of SNPs in cis with genes (within the gene plus 500kb from either end) and observed 5,888 associations with $p < 3.4 \times 10^{-9}$ in 253 genes, the top 36 of which had p-values of 10^{-20}. Using LCL gene expression from 60 unrelated CEU HapMap individuals, we performed 7.5×10^6 SNP-expression association tests of SNPs in cis with LCL transcripts resulting in 553 associations with $p < 7 \times 10^{-9}$ in 46 genes. Preliminary tissue comparison revealed 6.5×10^6 SNP-probe tests for cis association in common between brain and blood. Of the significant brain associations at the 3.4×10^{-9} p-value threshold, 168 associations were observed to be in common with significant LCL cis associations at the 7×10^{-9} threshold. When comparing significant LCL cis associations with all brain cis associations, we observed 481 cis associations present in both LCLs and brain. These comparisons in combination with genome-wide association study (GWAS) results of psychiatric disorders will help bridge the gap between disease-associated SNPs identified by GWAS and their potential functional consequences on gene expression.

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An X-chromosome-wide association study identifies a novel autism spectrum disorder gene. *R.-H. Chung[1], D. Ma[1], K. Wang[2], D.J. Hedges[1], J.M. Jaworski[1], J.R. Gilbert[1], M.L. Cuccaro[1], H.H. Wright[3], R.K. Abrahamson[3], I. Konidari[1], P.L. Whitehead[1], G.D. Schellenberg[4], H. Hakonarson[2], J.L. Haines[5], M.A. Pericak-Vance[1], E.R. Martin[1].* 1) Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL; 2) Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, PA; 3) School of Medicine, University of South Carolina, Columbia, SC; 4) Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA; 5) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN.

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder with a strong genetic component. The skewed prevalence toward males compared to females and evidence of suggestive linkage to the X chromosome in some studies support the presence of X-linked susceptibility genes for ASD. We conducted replication analysis of genome-wide association study data on the X chromosome in three independent autism GWAS datasets, using two family datasets (Miami/Vanderbilt (UM/VU) and AGRE) as a discovery dataset and a case-control dataset from The Children's Hospital of Philadelphia (CHOP) as a validation dataset. Association analysis was conducted in the individual datasets using the allele-based Chi-squared tests for the case-control analysis and the X-APL in families. We defined the replication threshold for p-value<0.0025 for each of the discovery and validation datasets. Although the threshold is liberal in the individual analyses, it is expected to control the chromosome-wide type I error rate when requiring replication in both datasets. In addition to replication analysis, we also performed joint analysis on the discovery and validation datasets combined. We used a modified version of the X-APL (X-CAPL) that combines family and unrelated case-control data and accounts for differential bias and population structure between datasets for the joint analysis. The final datasets included 2,557 samples from 735 ASD families in UM/VU, 3,289 samples from 721 ASD families in AGRE, and 1,204 cases and 6,472 controls from CHOP. One SNP rs17321050 in the TBL1X gene showed chromosome-wide significance in the joint analysis (p-value=4.53×10-6) for males and was close to the replication threshold of 0.0025 in each dataset. Two other SNPs in the same gene in LD with rs17321050 also showed significance close to the chromosome-wide threshold in the joint analysis but none of the three SNPs were predicted to be functional. Studies have shown deletions in the Xp22.2-Xp22.3 region containing TBL1X and surrounding genes were associated with several genetic syndromes, mental retardation and autistic features. Our results provide evidence through both replication and joint analyses that the gene may play a broader role in ASD risk.

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Genome-wide association and linkage studies for Parkinson's Disease in the mid-western US Amish. *M.F. Davis[1], A.C. Cummings[1], L. Jiang[1], D. Velez Edwards[2], R. Laux[1], L.L. McFarland[1], P.J. Gallins[3], L. Reinhart-Mercer[3], D. Fuzzell[1], C. Knebusch[3], C.E. Jackson[4], W.K. Scott[3], M.A. Pericak-Vance[3], S.L. Lee[5], J.L. Haines[1].* 1) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN; 2) Vanderbilt Epidemiology Center, Vanderbilt University Medical Center, Nashville, TN; 3) Hussman Institute for Human Genomics, University of Miami, Miller School of Medicine, Miami, FL; 4) Scott & White, Temple, TX; 5) Dartmouth College, Hanover, NH.

Parkinson's Disease (PD) is the second most common neurodegenerative disorder of adults. Previous evidence has shown that PD has a heritable component. Mutations in five known genes [α-synuclein (PARK1, PARK4), Parkin (PARK2), PINK1 (PARK6), DJ-1 (PARK7), and LRRK2 (PARK8)] cause Mendelian inheritance of PD. Consensus regions from genome-wide linkage and association studies of the more common non-Mendelian forms of PD implicate MAPT and SNCA. Other regions have been identified but the genes remain unknown. Genetic heterogeneity complicates the verification of likely genes and the identification of other susceptibility genes. Our approach to overcome the problem of heterogeneity is to study a population isolate, the mid-western Amish communities of Ohio. To date we have enrolled over 2200 Amish individuals with 40 of these diagnosed with PD. In this dataset we see a higher average kinship coefficient among our cases (.016) compared to the rest of our dataset (.012), suggesting that PD is heritable in the Amish. We have undertaken a genome-wide association study (Affymetrix Genome-Wide Human SNP Array 6.0) and genotyped 900 Amish individuals (38 with PD). Through the use of the Anabaptist Genealogy Database, we have determined the family structure for our collected individuals. Following QC, 614,957 SNPs and 830 individuals were analyzed. SNPs were analyzed by both linkage and association analysis. Linkage analysis was conducted using Merlin after cutting the pedigree into computationally feasible sub-pedigrees using PedCut. Seven regions generated a lod score ≥ 3 in 2-pt parametric linkage analysis on chromosomes 9, 10, 11, 12, 20, and 22. Multipoint analysis was conducted on these regions, and the lod score at 9p23 increased to 3.47. Association analysis was conducted using the MQLS test, which takes into account the relatedness of the individuals. Six SNPs reached genome-wide significance (p-value £1 × 10-7). One SNP (rs3935740) is in the intron of TMC3; all others are intergenic. We further investigated to see if the 2-pt linkage and association results overlap. Fifteen SNPs are significant in both studies with the criteria of lod ≥ 2 and p-value £ 1 × 10-4. These SNPs, as well as the region at 9p23, will be followed up for confirmation to indicate possible identification of novel PD-susceptibility genes.

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Pathway Analysis of Late-onset Alzheimer Disease Genome-wide Association Data Highlights Inflammatory and Neurodevelopmental Pathways. *C.E. Humphries[1], A.C. Naj[1], Y. Edwards[1], R. Ulloa[1], G.W. Beecham[1], E.R. Martin[1], M.A. Slifer[1], E.H. Powell[1], P.H. Gallins[1], I. Kondari[1], P.H. Whitehead[1], G. Cai[2], V. Haroutunian[2], W.K. Scott[1], J.M. Vance[1], H.E. Gwirtsman[3], J.D. Buxbaum[2], J.R. Gilbert[2], J.L. Haines[4], M.A. Pericak-Vance[1].* 1) John P. Hussman Institute for Human Genomics, Dr. John T Macdonald Foundation Department of Human Genetics, University of Miami Miller School of Medicine, Miami, FL, USA; 2) Department of Psychiatry, Mount Sinai School of Medicine, New York, NY, USA; 3) VA Medical Center, Nashville, TN, USA; 4) Vanderbilt Center for Human Genetics Research, Vanderbilt University, Nashville, TN, USA.

Alzheimer disease(AD) is the most prevalent neurodegenerative disease in the developed world with heritability estimates ranging from 60-80%. Genome-wide association studies(GWAS) studies of AD have identified several genes with highly statistically significant associations, including APOE, CLU, PICALM, and CR1. Variation in these genes does not fully explain the majority of AD heritability. Pathway analysis approaches may help to identify additional genes that contribute to AD by highlighting biological pathways relevant to AD. Using P-values from logistic regression modeling of SNP associations with late-onset AD (LOAD) in a previously-described GWAS of 483,399 single nucleotide polymorphisms(SNPs) in 931 LOAD cases and 1,104 controls, all SNPs were mapped to the nearest gene within 20 kb-pairs upstream or downstream. SNP-mapped genes were then assigned to pathways from the Kyoto Encyclopedia of Genes and Genomes (KEGG). Pathway memberships for genes containing SNPs with statistically significant associations (n=5,096 with P<0.01), were compared against all pathways containing SNP-mapped genes. A hypergeometric model (as implemented in GOStats,v2.0) was used to test whether any pathways contained more genes with significant SNP associations than would have been expected. SNPs significantly associated with LOAD (P<0.01) were over-represented in several pathways: Systemic Lupus Erythematosus (KEGGID:05322, $P=2.48\times10^{-5}$) disease pathway, Cell adhesion molecule (CAM) pathways (KEGGID:04514, $P=1.38\times10^{-4}$), and Axon guidance (KEGGID:04360, $P=3.63\times10^{-4}$). Systemic Lupus Erythematosus pathway genes are predominantly involved in inflammatory response, and this may relate to AD as neuroinflammation has been associated with degeneration in AD brains, characterized by overexpression of acute phase proteins and proinflammatory cytokines in tissues proximal to lesions. Likewise, CAM pathways include neuronal cell-cell adhesion pathways, which have demonstrated significant roles in memory and learning. Pathway analyses of GWAS data have identified excess association with LOAD of variants in inflammatory and neurodevelopmental pathway genes highlighting potential pathways and gene families for functional genomic investigations. Future directions include validation of this pathway analysis with additional analytic approaches, including the SNP Ratio Test and PARIS pathway analysis, and further evaluation of the SNPs associated with LOAD among the top pathways identified.

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Web-based, genome-wide association studies of Parkinson's Disease. *N. Eriksson, C. Do, J.Y. Tung, A. Kiefer, K. Marton, L. Dorfman, D. Hinds, B. Naughton, J.M. Macpherson, S. Saxonov, A. Wojcicki, J. Mountain.* 23andMe, Mountain View, CA.

Over the past year, we have gathered over 3,500 participants who self-reported Parkinson's disease into an online community. Participants were genotyped, optionally filled out questionnaires on their diagnosis, symptoms, and medical history, and were provided with an opportunity to view their (interpreted) genetic data. We have performed a genome-wide association study using these participants as well as over 900 cases from NINDS and over 20,000 population controls from the 23andMe database. We have found 9 regions associated with PD in this study, including 4 that are novel (all with p-values under 1e-7): SNCA (rs356220), LRRK2 (rs34637584, rs33939927, and rs10878246), GBA (N370S, V394L, and R496H), MAPT (rs2316765), SLC41A4/PARK16 (rs708730), RIT2 (rs4130047), MCCC1/LAMP3 (rs10513789), DGKQ/GAK (rs11724804), and STBD1/SCARB2 (rs6812193). We have substantial confidence that the subjects actually have PD for two reasons. First, estimated odds ratios for replicated SNPs agree closely with those in previous papers. Second, most participants reported a history that met clinical definitions of PD, including gradual and unilateral onset, persistent asymmetry, a progressive course, and response to levodopa. We have collected age of onset and symptom data (using the UPDRS), allowing us to perform analysis of association studies with age of onset and with disease subtype and to investigate disease progression for various high penetrance mutations.

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Meta-analysis in genome-wide scans identified BIN1 locus associated with Alzheimer disease susceptibility. *X. Hu[1], E. Pickering[2], Y. Liu[3], S. John[1], S. Hall[1], E. Katz[1], H. Soares[4].* 1) Molec Med, Pfizer Global R&D, Groton, CT; 2) Research Statistics, Pfizer Global R&D, Groton, CT; 3) Research Statistics, Pfizer Global R&D, Shanghai, China; 4) Translational Medicine, Pfizer Global R&D, Groton, CT.

Alzheimer's disease (AD) is the leading cause of dementia in the elderly population. Apart from APOE, recent GWAS studies nominated promising candidate loci near APOJ, CR1 and PICALM. In this study, we conducted a genome-wide association study in an independent set of 1301 AD cases and 1381 controls using Illumina platforms coupled with meta-analysis with available GWAS data from GenADA and Harold et al. 2009 to identify and replicate genetic markers with significant associations with AD disease etiology. We replicated the variants from APOJ (rs11136000), PICALM (rs3851179) and CR1 (rs3818361) in at least one independent sample set and identified a marker in the BIN1 locus (rs744373) below the genome-wide significance level (p less than 10-9). These four variants were further tested for association with disease progression in 467 LEADe trial and 181 ADNI AD patients where longitudinal cognitive measures are available for up to 24 months. The PICALM variant showed significant association with cognitive decline as measured by change of CDR score from the baseline suggesting it might play a role in both disease susceptibility and disease progression. However, combination of these genetic markers may have limited clinical utility for progression prediction.

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Genome-wide meta-analysis of pragmatic communication skills. *B. StPourcain[1], A. Whitehouse[2], N. Warrington[3], J. Golding[4], C. Steer[4], J. Kemp[1], G. MacMahon[1], N.J. Timpson[1], D.M. Evans[1], S.M. Ring[1], P. Deloukas[5], L. Palmer[6], C. Pennell[3], G. Davey Smith[1].* 1) MRC CAiTE/Dept Social Medicine, University of Bristol, Bristol, England, United Kingdom; 2) Telethon Institute for Child Health Research, University of Western Australia, Perth, Western Australia, Australia; 3) School of Women's and Infants' Health, The University of Western Australia, Perth, Australia; 4) Centre for Child and Adolescent Health, Department of Community Based Medicine, University of Bristol, Bristol, United Kingdom; 5) Wellcome Trust Sanger, Institute, Cambridge CB10 1SA, UK; 6) Centre for Genetic Epidemiology and Biostatistics, University of Western Australia, Perth, Australia.

Recent research showed that pragmatic communication deficits in the general population are associated with the same common genetic variant on chromosome 5p14 that also enhances risk for Autism Spectrum Disorder (ASD). This suggests the existence of underlying quantitative trait loci (QTL) that influence the entire autistic spectrum, and strongly supports theories that posit autism as a dimensional disorder. Our study performed a meta-analysis of genome-wide association study (GWAS) results on an index of pragmatic aspects of communication that was derived from the Children's Communication checklist (CCC). Severe problems with pragmatic aspects of language appear to be universal in ASD and are usually ascribed to an impaired theory of mind, i.e. an impaired understanding of other people's mind and mental state. Study participants were ~1100 9-year old children from the Western Australian Pregnancy Cohort (RAINE) Study and ~2850 10-year old children from the Avon Longitudinal Study of Parents and Children (ALSPAC) with imputed genome-wide data. We identified several meta-GWAS signals that met suggestive evidence for association (meta-p £ 1.0E-06). The strongest association was observed for a common variant near the *Cordon-Bleu (COBL)* gene, which is part of an imprinted region on chromosome 7p12 (meta-p < 3.4E-07). *COBL* is a conserved gene that plays a role in neural tube development and influences actin filament polymerisation. As a next step, the identified Meta-GWAS signals will be examined for replication within a further ~4000 10-year old ALSPAC children with available phenotype data and extracted DNA.

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Genome Wide Association Study Identifies DACH1 Gene Associated with Epilepsy. *H. Zhang[1], K. Wang[1], M.R. Sperling[2], D.J. Dlugos[3], W.D. Lo[4], C. Hou[1], J.T. Glessner[1], J.P. Bradfield[1], P. Sleiman[1], Y. Guo[1], C. Kim[1], R. Chiavacci[1], F. Mentch[1], H. Qiu[1], B. Keating[1], S.F. Grant[1], M. Privitera[5], J.A. French[6], S.C. Schachter[7], P. Cossette[8], F.W. Lohoff[9], W. Berrettini[9], H. Basehore[10], T.N. Ferraro[9], R.J. Buono[10], H. Hakonarson[1].* 1) Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, PA; 2) Department of Neurology, Thomas Jefferson University, Philadelphia, PA; 3) Division of Neurology, Children's Hospital of Philadelphia, Philadelphia, PA; 4) The Ohio State University and Nationwide Children's Hospital, Columbus, Ohio; 5) Department of Neurology, University of Cincinnati, Cincinnati, Ohio; 6) Department of Neurology, New York University, New York, NY; 7) Department of Neurology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; 8) Department of Medicine, University of Montreal, Montreal (Quebec), CANADA; 9) Department of Psychiatry, University of Pennsylvania, Philadelphia, PA; 10) Coatesville Veteran's Affairs Medical Center, Coatesville PA.

Epilepsy is a common chronic neurological disorder affecting more than 3 million people in the United States and having an overall incidence of about 1%. A major obstacle impeding the development of new treatments is lack of understanding of the neurobiological mechanisms that produce recurrent seizures, the hallmark of epilepsy. In an attempt to better characterize the genetic risk factors for epilepsy, we performed a genome-wide association study of 295 cryptogenic focal epilepsy patients and 2,282 healthy control subjects of European ancestry, as well as a second cohort of 412 idiopathic generalized epilepsy (IGE) patients and 3,876 control subjects of European ancestry genotyped by the Illumina HumanHap550 BeadChip. Genome wide association analysis identified DACH1 (dachshund homolog 1) on 13q22 as a susceptibility gene for epilepsy, with the most significant marker being rs9572727 (p=0.001, OR=1.93 in focal cohort and p=3.46x10-13, OR=2.89 in IGE cohort and p=1.71x10-14, OR=2.48 in combined cohort). Other candidate genes include MYH11 (p=1.3x10-9) and MMP8 (p=6.3x10-7) for epilepsy, ZNF695 and VGLL3 for focal group, C6orf103, ENPP2 and C7orf41for IGE. In addition, we have performed preliminary analysis on large copy number variation (CNV) on the subjects of European ancestry. Two IGE patients carry the 1.5Mb deletion on 15q13.3 previously associated with epilepsy, while one focal patient carries the reciprocal duplication. Furthermore, two unrelated generalized patients carry 1.2Mb deletion on 16p13.11. In conclusion, our results confirmed previously reported candidate CNVs in epilepsy susceptibility, but also identified potential novel genes worthy of further investigation and replication.

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Common genetic variants near TRANK1 are associated with bipolar disorder in European and Asian ancestry populations. *J.R. Wendland[1], T.G. Schulze[1], A. Cheng[2], C.-H. Chen[2], N. Akula[1], J. Steele[1], M. Nöthen[3], S. Cichon[3], M. Rietschel[4], BiGS. Consortium[5], F.J. McMahon[1].* 1) Genetic Basis of Mood and Anxiety Disorders, NIMH, Bethesda, MD; 2) Inst. of Biomedical Sciences, Academica Sinica, Taiwan; 3) Dept. of Human Genetics, University of Bonn, Germany; 4) Central Institute of Mental Health, Mannheim, Germany; 5) Bipolar Genome Studies (BiGS) Consortium.

Bipolar disorder (BD) is a common neuropsychiatric disorder marked by recurring episodes of mania and depression. Although there is compelling evidence of high heritability, few genetic findings for BD have been consistently replicated. Here, we conducted a meta-analysis of six BD genome-wide association studies originating from five European ancestry and one Han Chinese population (N = 13,853 individuals in total). Genotypes for individuals or association statistics for genome-wide markers were obtained through collaborations, public databases and publications. In total, 893,707 autosomal markers were observed or imputed in at least four of the six populations and meta-analyzed with MetAL after careful quality control. We identified a genomic region on chromosome 3p22 harboring TRANK1 that was associated with BD. The lowest P-value (1.08E-10; odds ratio = 1.2; 95% confidence interval, 1.1-1.3) was observed at rs9834970, a common variant located 12.3 kbp telomeric of TRANK1, previously implicated at suggestive levels of significance in the Wellcome Trust Case-Control Consortium BD sample. The second-most significant association (P = 2.76E-9) was at rs4789, a polymorphism in the 3'-untranslated region of TRANK1, in linkage disequilibrium with rs9834970. For both markers, the major allele (T) was consistently overrepresented in cases of BD in all study populations. Genome-wide suggestive association signals were detected at SNPs near PBRM1 (McMahon et al., 2010), LMBR1L, and a region of 8q12 containing many genes.TRANK1 (also known as lupus brain antigen 1, LBA1) is the human ortholog of murine Lba1, which encodes a cell surface target of brain-specific autoantibodies observed in experimental models of neurobehavioral systemic lupus erythematosus. We conclude that common genetic variation in TRANK1 influences risk for BD in multiple populations.

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Clinical and Genetic studies in Kleine Levin Syndrome. *T. Rico, J. Faraco, L. Lin, J. Hallmayer, E. Mignot.* Stanford University Center for Narcolepsy, Stanford, CA.

Kleine-Levin Syndrome (KLS) is a very rare disorder (quoted prevalence~1 for 1million) characterized by relapsing-remitting episodes of profound hypersomnia accompanied by specific cognitive and behavioral disturbances. Episodes typically last 1-3 weeks and recur every few weeks to few months with no symptomatology between episodes. The disease affects primarily adolescent males, with onset in the teens, and subsides within 8-12 years (median duration), with significant impact on social and educational development. A viral prodrome frequently precedes episodes, but no specific infectious trigger has ever been identified. An increased risk in first and second degree relatives of KLS cases (5 of 105 cases had an affected family member), and increased prevalence in the Ashkenazi Jewish population suggest the implication of genetic risk factors. We hypothesize that KLS results from an abnormal response to a pathogenic trigger acting on a susceptible genetic background. We performed a pilot genome-wide association (GWA) in 225 KLS patients of various ethnic backgrounds and 617 matched controls genotyped on the Affymetrix 6.0 array. We identified SNP variations in three genomic regions (Chromosomes 8, 10, and 14) with genome-wide significant associations. These findings are currently being replicated and extended in additional patients. To do so, we are currently recruiting and forming collaborations. In addition to blood samples, nasal and throat swabs are being collected from controls and patients both during and between episodes to identify potential pathogenic triggers or other factors associated with episode onset. Recruitment is a significant challenge due to the rarity of the disease: to date we have an additional 134 patients of various ethnic backgrounds pending analysis. Results of genotyping will direct our follow up of susceptibility loci, versus extending our GWA with the larger and more statistically powerful overall cohort. Acknowledgments: We thank our other collaborators not listed here for contributing samples, cohort genotypes and participating in the genetic analysis. Funded by MH080957-03.

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The microtubule network and bipolar disorder: biological theme of differentially expressed genes in lymphoblastoid cells from ill individuals. *H. Chen, M.G. McInnis.* Department of Psychiatry, University of Michigan Medical School, Ann Arbor, MI.

Cumulative evidence suggests that bipolar disorder is caused by multiple genetic variants interacting with environmental factors. We hypothesize that manifestation of this interaction may be "imprinted" in cellular systems and subsequently leads to altered gene expression. We measured the abundance of 22,184 genome transcripts in lymphoblastoid cell lines (LCL) from a sample of 19 bipolar individuals without psychosis features and 24 controls using Illumina's Refseq8-v2 BeadChips. This experiment identified 11,372 transcripts expressed in LCLs among the 22,184 transcripts assayed. Using the two-group unpaired algorithms implemented in the significant analysis of microarrays (SAM) statistics package, we identified 250 unique gene transcripts differentially expressed in LCLs from bipolar individuals, given a false discovery rate (FDR) cutoff point of 0.1 as statistical significance. 249 of the 250 transcripts show decreased levels, and one increased in LCLs from bipolar individuals compared to controls. Forty-one of the 250 transcripts show expression alteration in postmortem brains affected with bipolar disorder (35 in same direction change) based on results from our recent microarray analysis of postmortem brains for the same 11,372 transcripts. Biological path analysis of the 250 transcripts identifies six Gene Ontology terms with enrichment of genes involved in metabolic process (FDR < 0.01). Notably, the 41 genes commonly altered in expression in bipolar LCLs and postmortem brains are significantly enriched under "regulation of microtubule depolarization/polarization" (FDR < 0.01). For examples, MAP4 and CLASP2 encode microtubule-associated protein 4 and cytoplasmic linker associated protein 2, respectively, both show decreased levels in bipolar LCLs and postmortem brain. MAP4 and CLASP2 are best known for their microtubule-stabilizing activity and for proposed roles regulating microtubule networks in axon guidance and growth. Our results suggest that dysregulation of the microtubule network may contribute to the molecular basis of bipolar disorder, and provide candidate genes for further exploration of gene interaction networks and pathways involved in the etiology and pathogenesis of bipolar disorder.

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Characterization of SGCE isoforms in human brain. *K. Ritz[1], B.D.C van Schaik[2], M.E. Jakobs[1], A.H. van Kampen[2,5], E. Aronica[3], M.A. Tijssen[4], F. Baas[1].* 1) Department of Neurogenetics, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105AZ AmsterdamAcademic Medical Center, Amsterdam, Amsterdam, Netherlands; 2) Bioinformatic Laboratory, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105AZ Amsterdam; 3) Department of Neurpathology, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105AZ Amsterdam; 4) Department of Neurology, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105AZ Amsterdam; 5) Biosystems Data Analysis, Swammerdam Institute for Lice Science, University of Amsterdam, Nieuwe Achtergracht 166, 1018WV Amsterdam, The Netherlands.

Myoclonus-Dystonia (M-D) is an autosomal-dominant inherited movement disorder characterized by jerky movements and dystonic features. About 50% of M-D patients have a mutation in the epsilon-sarcoglycan (SGCE) gene, which encodes a transmembrane protein that is widely expressed in several tissues. The function of SGCE and the pathophysiology of the disease with its brain-specific phenotype remains unknown. Recently, new brain-specific SGCE exons coding for a different C-terminal of the protein have been identified suggesting a alternative functions for those isoforms in the brain. We investigated SGCE exon structure with ultra deep sequencing (454 GS FLX, Roche) to characterize all alternatively spliced SGCE exons and their qualitative and quantitative distribution in different human brain regions. Further analysis of the major brain-specific isoform by qPCR showed a high expression in the cerebellum. Isoform-specific ISH confirmed its expression pattern and showed a predominant neuronal expression. We show that ultra deep sequencing is a powerful tool to study exon structure of a gene. We propose that loss of function of the major-brain specific SGCE isoform mainly affecting the cerebellum explains the specific M-D symptoms.

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PRESENILIN ENHANCER-2 GENE MUTATIONS AND FAMILIAL ALZHEIMER'S DISEASE. *V. Andreoli, F. Trecroci, A. La Russa, M. Caracciolo, G. Di Palma, P. Spadafora, R. Cittadella.* Institute of Neurological Sciences, National Research Council, Piano Lago di Mangone, Cosenza-Italy.

During the last few years, much progress has been made in identifying new proteins involved in Alzheimer's disease (AD), the most prevalent form of progressive dementia in the elderly. It is characterized by extracellular amyloid plaques and neurofibrillary tangles in affected brains. Many studies have confirmed that beta-amyloid (Aβ) is the core ingredient of β-amyloid plaques; Aβ is produced in a process of proteolysis of amyloid precursor protein (APP) in which the γ-secretase complex plays a crucial role. Presenilin enhancer 2, a small integral membrane glycoprotein, is one of four components of the γ-secretase complex which cleaves APP to generate Aβ. Moreover, its gene (PEN-2) maps to a highly significant linkage region on chromosome 19q13. PEN-2 is an essential cofactor of this secretase complex, whose dysfunction could influence γ-secretase formation, reduce presenilins levels and impair nicastrin maturation. It is possible that a sequence variation of PEN-2 could accelerate AD pathology. To better assess the genetic contribution of PEN-2 to familial AD (FAD), we performed a systematic mutation analysis of this gene in 90 Italian patients with FAD. The whole PEN-2 promoter and its exons were screened by denaturing high performance liquid chromatography (DHPLC). Patients'chromatograms for each PCR fragments were compared with corresponding normal controls and the amplicons showing a variant DHPLC profile were sequenced in both directions. The sequencing analysis of the PEN-2 promoter identified a novel heterozygous 3 bp deletion mutation (—328_—326delGAA) in a subject with early-FAD. The patient was a 55-year-old woman, with a 3-year history of mild memory deficit. This deletion occurs directly in the proximal promoter of PEN-2 gene, which apparently contains important structural elements, indispensable for its function. The coding region of the PEN-2 was sequenced in this patient, which was also analyzed for mutations in PS1, PS2 and APP genes, in order to ensure that a second mutation will not be missed. This deletion was excluded in 150 aged cognitively intact controls. We conclude that —328_—326delGAA might represent the first pathogenic mutations in the promoter region of the PEN-2. This screening of the PEN-2 gene revealed a very rare mutation in a FAD patient, although the identification of additional mutations together with functional studies will be necessary, to further understand the pathogenesis resulting from PEN-2 mutations.

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Panthothenate kinase associated neurodegeneration: clinical description and genetic study in 3 large algerian familes. *H. Azzedine[1], S. Assami[2], S. Mahoui[2], E. Mundwiller[1], S. Nouioua[2], D. Grid[3], A. Brice[1,4], M. Djemai[5], G. Stevanin[1,4], M. Tazir[2].* 1) INSERM, U975 (formerly U679), Paris, France and UPMC Univ. Paris 6, UMR_S975, CNRS 7225, Centre de Recherche de l'Institut du Cerveau et de la Moelle épinière, Hôpital Pitié-Salpêtrière, Paris, France; 2) Department of Neurology CHU Mustapha and Laboratoire de Neurosciences, Université Benyoucef Benkhedda, Algiers, Algeria; 3) Généthon, Paris, France; 4) APHP, Department of Genetics, and Cytogenetics, Hôpital Pitié-Salpêtrière, Paris, France; 5) Service de Neurologie, CHU Bab el Oued, Algiers, Algeria.

We described 10 Panthothenate kinase associated neurodegeneration (PKAN) patients from 3 different kindreds with a mean age at onset in the first decade (5 ± 1.9, range 3 to 8 years) and peculiar clinical picture. Hyperkinetic syndromes with concentration difficulties were the first and striking features. They were followed by abnormal falls, gait or postural difficulties. Later on, examination showed dysarthria, oromandicular-facial and axial dystonia, pyramidal signs and cognitive impairment. Progression was rapid in the first decade with loss of independent ambulation at a mean age of 11.7 ± 1.9 years with generalized painful dystonia. Pigmentary retinopathy was observed in all the patients. T2-weighted brain MRI showed the specific pattern known as the eye of the tiger. We identified two homozygous truncating PANK2 mutations (c.846_847delAG / p.S282SfsX3 and c.1171_1174dupATTG / p.G392DfsX11) segregating with the disease in all patients in family 1 and 2, respectively. Interestingly, in the second branch of family 3, all the patients are double homozygous for two missense variants (c.457A_T / p.I153F and c.519C_G / p.H173Q). These variants were absent in 898 control chromosomes. However, the single patient of the first branch of this family, presents a particular genotype as he is a triple heterozygous. Indeed, he carries the truncating mutation (c.1171_1174dupATTG / p.G392DfsX11) identified in family 2, in a tans position to the missense variants present both in cis position in the second branch of family 3. The c.1171_1174dupATTG mutation found in family 2 as well as in the first branch of family 3 was associated with similar flanking haplotypes suggesting that these 2 families have a common ancestor. In conclusion, we report 4 mutations, 2 truncating mutations already described and 2 novel missense PANK2 mutations. These mutations were associated with severe PKAN. All patients presented pigmentary retinopathy and cerebellar ataxia, while hyperactivity was observed in some of them, extending the clinical profile of this disease.

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A comprehensive mutation analysis of the PINK1 gene in Southern Italian patients with early- and late-onset parkinsonism. *E.V. De Marco[1], V. Scornaienchi[1], P. Tarantino[1], F.E Rocca[1], V. Greco[1], G. Provenzano[1], D. Civitelli D[1], F. Annesi[1], G. Nicoletti[2], A. Uncini[3,4], A. Quattrone[2,5], G. Annesi[1].* 1) Institute of Neurological Sciences, National Research Council, Cosenza, Italy; 2) Institute of Neurology, University of Magna Graecia, Catanzaro, Italy; 3) Neuromuscular Diseases Unit, Centre for Excellence on Aging, "G. d'Annunzio" University Foundation, Chieti, Italy; 4) Department of Human Motor Sciences, University "G. d'Annunzio", Chieti, Italy; 5) Neuroimaging Research Unit, National Research Council, Catanzaro, ItalY.

Mutations in the PINK1 gene are associated with both familial recessive and sporadic early-onset Parkinson's disease (EOPD) (onset £50 years) and have been found in populations of different geographical origins. Data on Italian EOPD patients show mutations in a relatively high percentage (8-9%). Although mutations in PINK1 predominantly involve EOPD patients, heterozygous or compound heterozygous mutations were also reported in a small number of late-onset PD patients (LOPD). In this study, we aimed at assessing the frequency and possibly the pathogenic role of PINK1 mutations in familial and sporadic patients with EOPD and LOPD coming from Southern Italy. We selected 115 patients, including 102 cases with EOPD, 9 of which had a positive family history, and 13 familial cases with LOPD. All the eight PINK1 exons and intron-exon boundaries were analyzed by PCR and sequencing. The MLPA method was used in the heterozygous patients in order to detect exon dosage changes, caused by genomic rearrangements, of the known PD genes. Four already know different mutations (three homozygous: Q126P, W437X , Q456X and one heterozygous: E476K) and one novel heterozygous mutation (R207Q) were found in five patients, giving a 4.3% frequency of PINK1 mutations in our total PD cohort. In particular, 4 out of 103 EOPD patients (3.9%) carried PINK1 mutations. This frequency is lower than previous studies on Italian EOPD have suggested (8-9%). Furthermore, a frequency of 1 out 13 (7.7%) in familial LOPD patient was found, thus confirming a certain influence of the PINK1 gene also in LOPD as observed in other studies. In conclusion, our study confirms the role played by the PINK1 gene mutations both in heterozygous and homozygous condition in PD development.

2667/F

Molecular analysis of *REEP1* gene mutations in patients with familial and sporadic spastic paraplegia. *D. Di Bella[1], E. Sarto[1], M. Plumari[1], L. Brighina[2], C. Ferrarese[2], L. La Mantia[3], S. Baratta[1], F. Taroni[1].* 1) Genet Neurodegen Metab Dis, IRCCS Ist Neurol Carlo Besta, Milan, Italy; 2) Unit of Neurology, University of Milan-Bicocca, AO San Gerardo, Monza, Italy; 3) Movement Disorders, Neurology Unit 1, IRCCS Ist Neurol Carlo Besta, Milan, Italy.

Hereditary spastic paraplegias (HSP) are a clinically and genetically heterogeneous group of neurodegenerative disorders. Pure and complicated forms of the disease have been described. About half of HSP cases result from autosomal dominant mutations in spastin (SPG4) or atlastin-1 (also known as SPG3A). Mutations in the receptor expression-enhancing protein 1 (REEP1) gene have recently been reported to be associated with autosomal dominant (AD) pure or complicated HSP phenotypes (SPG31) with a variable frequency ranging from 2.3% to 6.5%. Recent data indicate that the HSP proteins atlastin-1, spastin, and REEP1 interact within the tubular ER membrane in corticospinal neurons to coordinate ER shaping and microtubule dynamics. We have analyzed a large cohort of Italian patients with spastic paraparesis for REEP1 mutations, including both point mutations and micro-rearrangements. The REEP1 gene was analysed by high-resolution melting analysis (HRM) and/or direct sequencing in 175 unrelated index cases, including 80 AD cases, and 95 sporadic cases, negative for SPG4 mutations. Moreover, a subgroup of patients was also screened for micro-rearrangements in the REEP1 gene by multiplex ligation-dependent probe amplification (MLPA) to assess the frequency of deletions/duplications. We have identified two novel different pathogenic mutations were found in the AD-HSP group (2.5%). No REEP1 mutations were identified in the sporadic group. No micro-rearrangements were identified in the analyzed patients. One deletion of 16 nucleotides (c.256_271del16) causing a frameshift and a premature stop codon was identified in exon 4 in two affected brothers and in their mildly affected mother while a splice site mutation (c.182+1 G>T) predicted to abolish the splice donor site was identified in exon 3 in another proband from an AD-HSP family with an uncomplicated spastic paraplegia phenotype. In conclusion, our data indicate that REEP1 mutations account for 2.5% of cases with autosomal dominant inheritance, suggesting that this form is relatively uncommon in an Italian population of spastic paraplegia patients. Clinically, the probands of our SPG31 families present with a pure spastic phenotype.

2668/F

Rare copy number and sequence variants in DOCK8 are involved in autism. *S.C. Lund, A.D. McGrew, J.S. Sutcliffe.* Department of Molecular Physiology & Biophysics and Psychiatry, Centers for Molecular Neuroscience and Human Genetics Research and Vanderbilt Kennedy Center. Vanderbilt University, Nashville, TN, USA.

Autism spectrum disorder (ASD) is a neurodevelopmental condition affecting approximately 1 in 110 individuals, predominantly males. ASD is characterized by deficits in reciprocal social interaction, communication, and patterns of repetitive behaviors and restricted interests. Twin and family studies indicate high heritability in ASD, and we now understand that copy number variation (CNV) is an important class of rare variation underlying risk and/or causation in ASD. We and others hypothesize that genes disrupted by de novo and/or inherited CNV harbor discrete functional (e.g. coding) variants that cause or increase risk for disease. We initially screened a series of 89 probands using the Affymetrix 6.0 platform. CNVs were predicted using multiple algorithms to maximize sensitivity and specificity of CNV detection. Among qPCR-validated variants, we identified DOCK8 (dedicator of cytokinesis 8) as disrupted in two probands. Moreover, the Autism Genome Project has detected two cases of de novo deletion of DOCK8, lending further support to this gene as a candidate. DOCK8 is a guanidine-exchange factor (GEF) of the Rho-family GTPase, identified in a yeast two-hybrid assay screening for Cdc42-interacting proteins. Compelling evidence shows that DOCKs have important roles in fundamental neurodevelopmental processes, moreover, DOCK8 gene disruption was reported in two unrelated patients with mental retardation. DOCK8 exons (n=48) were screened for novel variants in 183 unrelated probands and 200 controls using Sanger sequencing. Variants were validated and segregation determined by sequencing independent PCR products. Multiple bioinformatic tools were used to predict the functional effect of synonymous and nonsynonymous variation. Novel variants detected in more than one proband were subsequently genotyped using TaqMan ~950 families. To date, 15 novel nonsynonymous and 7 synonymous variants were detected in 20 and 8 probands, respectively, compared with 9 nonsynonymous and 2 synonymous variants in 11 and 2 ethnically matched controls, respectively. These data show trend in nonsynonymous variants in cases vs controls (p=0.061) and reveal a pronounced increased of all transcribed variants in cases vs. controls (p= 0.0075), indicating a putative increase in burden of exonic variants in ASD cases compared with a non-clinical comparison sample. The biological significance of these variants is under study and will help us to further interpret these data.

2669/F

Whole genome sequencing in schizophrenia: the first genomes. *A. Need[1,2], J. McEvoy[2], H. Meltzer[3], D. Goldstein[1].* 1) Center for Human Genome Variation, Duke Univ, Durham, NC; 2) Department of Psychiatry, Duke Univ, Durham, NC; 3) Department of Psychiatry, School of Medicine, Vanderbilt University, Nashville, TN.

Genetic studies of neuropsychiatric illnesses such as schizophrenia have indicated that the common disease common variant hypothesis contributes less to the etiology of the disorders than previously thought. Studies of large copy number variants provide evidence for a much larger role of rare, highly penetrant genetic variants in these disorders, which can only be fully explored using next generation sequencing. Here we present whole genome and whole exome data from 50 schizophrenia patients, selected because either a strong family history or a treatment-resistant form of the illness indicates a highly penetrant genetic predisposition. We compare the whole genome and whole exome data, discuss candidate variants and outline analytical and technical challenges encountered when working with these novel data.

2670/F

Molecular analysis of folate receptor genes in patients with Cerebral Folate Deficiency. *K. Segers[1], J. Hanson[3], V. Ramaekers[2], V. Bours[1].* 1) Dept Human Gen, CHU Sart Tilman, Liege, Belgium; 2) Neuropediatrie, CHU, Liege, Belgium; 3) Andre Vesale College, Liege, Belgium.

Folates are essential cofactors for a multitude of biological processes such as synthesis and repair of DNA, regulation of gene expression and synthesis of amino acids. Different inherited disorders of folate transport and metabolism are presently known, all of which cause systemic folate deficiency. Steinfeld (2009) identified an inherited brain-specific folate transport defect caused by mutations in the folate receptor 1 (FOLR1). Patients carrying FOLR1 mutations developed progressive movement disturbance, psychomotor decline, epilepsy and showed severe reduced folate concentration in the cerebrospinal fluid (CSF). In this study, we performed molecular analysis of the two folate receptor genes, FOLR1 and FOLR2 in 27 patients showing neurological symptoms and reduced folate concentration in CSF. Both genes (fetal and adult form) were studied by direct sequencing. Analysis was restricted to coding region and intronic bounderies. Three variants, including one splice variant, were identified but none of them affect the coding sequence. Familial study was performed for all the variants. The results lead to the conclusion that none of these variants were pathogenic. Despite the confirmed cerebral folate deficiency, we were not able to explain this deficiency by inactivation of the folate receptor genes.

2671/F

Whole exome sequencing identifies novel, nonsynonymous variants in a large pedigree with Tourette Syndrome. *S.K. Sundaram, A.H.M. Huq, B.J. Wilson, Z. Sun, W. Yu, L. Bennett, H.T. Chugani.* Pediatrics and Neurology, Wayne state university, Detroit, MI.

Objectives: To identify the causal mutation(s)/polymophisms in a large pedigree with Tourette Syndrome using whole exome sequencing. **Methods:** Nine members of a 3 generation pedigree with five showing Tourette Syndrome/chronic tic phenotype were evaluated with whole exome sequencing. Nimblegen 2.1M human exome microarray was used to prepare the exome library. The library contains approximately 180,000 exons targeting 34 million bases of the genome. Sequencing was performed with the Illumina Genome Analyzer II. Approximately 35 trillion bases of sequence data were obtained from the 9 subjects. The ELAND algorithm was used to align the reads to reference human genome (UCSC hg18) and single nucleotide polymorphisms (SNPs) were identified by filtering genomic positions with greater than 8x coverage and phred-scaled consensus quality greater than 20. Those variants that are known in dbSNP database were excluded. Novel, nonsynonymous SNPs that perfectly segregate with chronic tic phenotype under the assumption of autosomal dominant inheritance were identified. **Results:** We identified a list of 17 novel, nonsynonymous variants (clustered into 5 genomic regions) that segregate with chronic tic phenotype. While these variants were present in all the affected members, only 2 variants were not present in any of the unaffected members. The remaining 15 variants were present in all of the affected members and in one of the unaffected members. The inability to reduce to a single variant could be due to chance segregation of non-pathogenic variants, base calling/alignment errors or complex/oligogenic inheritance. **Conclusion:** Exome sequencing identifies 17 novel, nonsynonymos candidate variants in a multigenerational family with Tourette syndrome. Further studies in a larger sample, functional studies and improved base calling/alignment algorithms will clarify the importance of these variants in Tourette Syndrome.

2672/F

A case-control study focusing on rare variants obtained by comprehensive resequencing of the causative genes for familial amyotrophic lateral sclerosis (ALS) to identify disease-relevant alleles for sporadic ALS. *Y. Takahashi[1], J. Goto[1], A. Toyoda[2], A. Fujiyama[2], S. Tsuji[1].* 1) Dept Neurology, Univ Tokyo, Tokyo, Japan; 2) Research Organization of Information and Systems, National Institute of Genetics, Shizuoka, Japan.

[Background] Molecular pathogenesis of the majority of SALS patients remains unknown. Accumulating evidences suggested that resequencing of disease-related genes in patients might reveal multiple rare variants potentially associated with disease risks. However, resequencing of controls should also be necessary in order to conduct unbiased evaluation of the relevance of these rare variants to disease pathogenesis. [Object]To identify causative mutations and rare variants potentially associated with disease risks for SALS. [Subjects and Methods] One hundred and sixty SALS patients and 260 controls were enrolled in this study. All the exon and flanking intron sequences of *SOD1*, *ALS2*, *DCTN1*, *VAPB*, *CHMP2B*, *ANG* and *TARDBP* were analyzed employing a DNA microarray-based resequencing system TKYALS02. Resequencing of these genes in controls and that of *FUS* in patients and controls were conducted employing direct nucleotide sequence analysis. [Result]Three known causative mutations including *SOD1* S134N, *TARDBP* N352S and *FUS* R521C were found in 4 patients. In addition, twelve novel nonsynonymous variants were found, including those in *SOD1* (C6Y), *ALS2* (Q435L, P1016T, F1453C), *DCTN1* (D431G, G462E, Q738R, T778A, R997W), *CHMP2B* (E173A), *ANG* (N49S) and *FUS* (G206S). Resequencing of controls also revealed 13 novel nonsynonynous variants, including those in *ALS2* (P522L, R1330H, T1472M), *DCTN1* (A173V, R225W, Q488R, L615I, Q738R, R1261Q), *VAPB* (K188R), *CHMP2B* (P178H) and *FUS* (S147R, F353L). [Discussion]This study revealed unexpected abundance of novel nonsynonymous variants in controls as well as in SALS patients in the causative genes for FALS. This implied that the interpretation of the rare nonsynonymous variants specifically identified in SALS except for the three known causative mutations remained elusive, because the enrichment of multiple rare variants in these genes was not observed in SALS patients. Three novel nonsynonymous variants (*SOD1* C6Y, *ANG* N49S and *FUS* G206S) identified in SALS patients are likely disease-relevant alleles, because they were not identified in controls and located in mutation-clustered regions. Large-scale resequencing-based case-control studies or functional studies of identified variants will be necessary to confirm the implications of these rare nonsynonymous variants in SALS pathogenesis.

2673/F

Copy Number Variations may be associated with the Chinese Autism patient. *ZM. Hu[1,3], GL. Xun[2], Y. Peng[1], XP. Li[1], LW. Long[1], Q. Pan[1], ZH. Zhang[1], XJ. Zhang[4], JP. Zhao[2], K. Xia[1,3].* 1) State key Laboratory of Medical Genetics, Central South University, 110 Xiangya Road, Changsha, Hunan, China; 2) Department of Psychiatry, Xiangya 2nd Hospital, Central South University, Changsha, Hunan, China; 3) The School of Biological Science and Technology, Central South University, Changsha, Hunan, China; 4) Institute of Dermatology and Department of Dermatology at No.1 Hospital, Anhui Medical University, Hefei, Anhui 230022, China.

Autism spectrum disorder (ASD) is a highly heritable neurodevelopmental disorder, recently study reveals copy number variations (CNVs) play an important role in pathogenesis of ASD. To assess the association between rare CNVs and autism in Chinese population, we screened 991 subjects (431 cases, 280 trio pedigrees) using HumanCNV370-Quad v3.0 SNP arrays. 1084 control samples were also genotyped with HumanCNV370-Quad v3.0 (90 samples) or Human610-Quad bead chip (994 samples). Four different copy number states using PennCNV software were detected. For the CNV calls from all ASD subjects, we identified 1037 homozygous deletions (CN=0), 14751 hemizygous deletions (CN=1), 4959 duplications (CN=3), and 77 duplications (CN=4). For the CNV calls in controls (90/994), we identified 109/1129 homozygous deletions (CN=0), 1116/12761 hemizygous deletions (CN=1), 341/12633 duplications (CN=3), and 8/1936 duplications (CN=4). In total, 21 autism-specific CNVs (>1M) were identified in ASD patients. Two duplications of 15q11-q13 known to be associated with ASD were identified. Duplications of 3p26.3 were confirmed in 2 ASD subjects. This variable region includes contactin 4 isoform c precursor (*CNTN4*) gene, which may play a role in the formation of axon connections in the developing nervous system. The other 17 CNV include 5p14, 20q13.31-q13.33 etc. Our study supports a role for rare CNVs in autism susceptibility.

2674/F

A genome-wide evaluation of exonic copy number variations (eCNVs) in autism spectrum disorders. *D.Q Ma[1], H.N Cukier[1], A.J Griswold[1], D. Salyakina[1], J.M Jaworski[1], S. Williams[2], R. Menon[3], I. Konidari1[1], P.L Whitehead[1], J.R Gilbert[1], M.L Cuccaro[1], J.H Haines[2], M.A Pericak-Vance1[1].* 1) Hussman Institute for Human Genomics, University of Miami, Miller School of Medicine, Miami, FL;; 2) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN; 3) Emory University (Department of Epidemiology and Department of OB & Gyn, Rollins School of Public Health, Emory University, Atlanta, GA.

Recent studies have shown consistent association of rare, large CNVs with autism spectrum disorders (ASDs) although the functional impact of common CNVs in ASDs remains uncertain. A comprehensive genome-wide evaluation of CNVs was carried out in an ASD case control study using samples of European ancestry, genotyping on the Illumina Human 1M Beadchip and utilizing PennCNV for CNV detection. A total number of 817 unrelated ASD cases and 593 pediatric controls survived the stringent quality control of sample call rate, intensity quality and population stratification. CNVs falling into centromeric, telomeric and DNA-origin artifact segment regions were excluded. Our preliminary results indicate a significantly heavier eCNV burden in cases rather than the global CNVs. This is mainly from exonic deletions (eDels) of all sizes (p=2.7e-05[10 vs 8], 10e-06[571kb vs 375 kb], 0.009[55 vs 47 kb] for the number of Dels per sample, total length spanned and average segment size respectively). Thus, the CNVs having ≥ 5 contributing SNPs and overlapping exonic regions were included in the analysis. We first examined large CNVs and found 15 Dels larger than 1Mb exclusively in ASD implicating potential novel CNV regions at 4q12,10q11,13q12 and 7p13. Secondly, we examined eCNVs of all sizes. Overall, 36 eCNV regions (27 eDels and 9 eDups) presented significant associations with empirical P-values ranging from 10e-06 to 0.047 using genome-wide permutation correction. Several novel candidate genes were revealed including Neurofibromatosis 1 (NF1) and ADAM8, however, both merit further validation. The final analysis targeted on rare case-unique eCNVs, of which 4418 were identified. These included CNVs in previously reported candidate gene regions NRXN1(Del: N=5), SHANK3(Del: N=2), CNTN4(Dup: N=1), PTEN(Del: N=6), EN2(Del: N=6), DISC1(Dup: N=1), GABRG1(Del: N=2), and PARK2(Del: N=3). However, no cases carry either exonic or intronic CNVs in recently reported candidate genes CNTNAP2, SHANK2, BZRAP1, SYNGAP1 and DLGAP2. In addition, our analysis reveals an enrichment of case-unique eCNVs disrupting novel candidate genes involved in signal transduction (DOCK1, SDCBP2), cytoskeleton arrangement (NCAM2, SLITRK5) and cell adhesion (COL27A1, CDH8, CDH13, CDH17). In conclusion, our results suggest the possible involvement of common eCNVs in specific biological pathways as contributing to autism risk and target more novel potential ASD candidates for further validation.

2675/F

Enrichment of rare inherited and de novo copy number variants in autism. *A. Nord[1], W. Roeb[2], D. Dickel[1], T. Walsh[2], M. Kusenda[3,4], K. Lewis[5], D. Malhotra[6], S. McCarthy[3], C. Rippey[1], S. Stray[2], S. Taylor[2], J. Sebat[6], B. King[7], M-C. King[1,2], J. McClellan[8], STAART Psychopharmacology Network.* 1) Department of Genome Sciences, University of Washington, Seattle, WA; 2) Department of Medical Genetics, University of Washington, Seattle, WA; 3) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; 4) Graduate Program in Genetics State University of New York, Stony Brook, NY; 5) National Institute of Standards and Technology, Gaithersburg, MD; 6) Department of Psychiatry, Department of Cellular and Molecular Medicine, Institute for Genomic Medicine, University of California, San Diego, La Jolla, CA; 7) Seattle Children's Hospital, University of Washington, Seattle, WA; 8) Department of Psychiatry, University of Washington, Seattle, WA.

Previous studies have demonstrated that individuals with autism are significantly more likely than controls to harbor large de novo copy number variants (CNVs). We tested whether individuals with autism are also more likely to harbor extremely rare, smaller CNVs (≥ ~10 kb), either de novo or inherited. Subjects were 41 probands with autism and their parents and 367 NIMH controls. Controls were split into a population copy number polymorphism discovery set (n=244) and a rare CNV analysis set (n=123). Based on evaluation of genomic DNA by high-resolution array comparative genomic hybridization (Nimblegen HD2 platform), 63% of cases and 41% of controls carried a rare deletion that impacted a gene or genes (OR = 2.52, P=0.02). Enrichment in autism cases was greater for deletions than for duplications and increased with CNV size. Rare CNVs in cases disproportionately included genes associated with DNA binding and transcriptional regulation, nervous system development, and signaling. De novo deletions and rare inherited CNVs in patients disrupted genes and genomic regions previously linked to autism, including PRODH, CNTNAP2, GRIK2 and chromosomes 15q11 and 11p15. Collectively these results implicate smaller, rare, inherited CNVs, as well as large, de novo CNVs, in the pathogenesis of autism.

2676/F

Exploring the correlations between mutations in EFHC1 and the phenotype of patients with juvenile myoclonic epilepsy. *F. Conte[1], T. Peluzzo[1], L. Betting[2], F. Cendes[2,3], I. Lopes-Cendes[1,3].* 1) Dept Medical Genetics, UNICAMP, Campinas, SP, Brazil; 2) Dept of Neurology, UNICAMP, Campinas, SP, Brazil; 3) CInAPCe Program, Brazil.

Purpose: Mutations in EFHC1 were implicated with some types of idiopathic generalized epilepsies (IGEs), mainly juvenile myoclonic epilepsy (JME), in rare patients from Central America, Europe and Japan. It was recently shown that EFHC1 associates with microtubules, being a component of the centrosome, the mitotic spindle and of flagella and cilia. In neurons, it was observed that EFHC1 loss-of-function interferes with the organization of the mitotic spindle, arrests cell cycle, induces the aggregation of microtubules and increases the rate of apoptosis. Moreover, it was shown that during the development of central nervous system, EFHC1 participates in the process of radial migration, controlling both the division of neuronal progenitors as well the locomotion of post-mitotic neurons. Thus, EFHC1 loss-of-function could lead to changes in cortical and sub-cortical architectures, which may be one of the causes of IGEs. The aim of this study was to search for mutations in EFHC1 in a large group of unrelated patients with JME and to explore the presence of genotype-phenotype correlations. Method: Genomic DNA of 52 unrelated patients with JME was submitted to direct sequencing. The chromatograms were analyzed for the presence of polymorphisms and/or mutations by the alignment with EFHC1 sequences available in the public databases. Results: We found mutations in 4 out of 52 patients, which give a frequency of 7.7%. There were two different mutations among the 4 patients. The first was a previously described thymine to cytosine transition in position 685 of exon 4, resulting in a phenylalanine to leucine change. This mutation was observed in 3 patients, corresponding to a frequency of 5.7%, almost 4 times greater than the one reported in a previous work (1.5%). The second mutation has never been reported previously and was found in only 1 patient. It is an adenine to guanine transition in position 896 of exon 5, resulting in a lysine to an arginine (K229R) change. Although there is a change between two positively charged amino acids, its relevance to the pathogenesis of epilepsy is unclear. In addition, we did not find any significant phenotypic difference between patients with and without EFHC1 mutations. Conclusion: We found a higher frequency of EFHC1 mutations than previously reported, including a new missence mutation; however, no clinical differences between patients with and without EFHC1 mutations were observed.

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Exome-sequencing of medically-intractable juvenile myclonic epilepsy patients. *E.L. Heinzen[1], G.L. Cavalleri[2], M. McCormack[2], S. Alhusaini[2], G. O'Connor[3], R.A. Radtke[4], C. Depondt[5], S.M. Sisodiya[6], N. Delanty[2], D.B. Goldstein[1], EpiGen Consortium.* 1) Center for Human Genome Variation, Duke University Medical Center, Durham, NC; 2) The Department of Clinical Neurological Sciences and Molecular and Cellular Therapeutics, RCSI Research Institute Royal College of Surgeons in Ireland, and Division of Neurology, Beaumont Hospital, Dublin, Ireland; 3) Department of Clinical Neurological Sciences, Division of Neurology, Beaumont Hospital, Dublin, Ireland; 4) Department of Medicine (Neurology), Duke University Medical School, Durham, NC; 5) Department of Neurology, Hoêpital Erasme, Universite' Libre de Bruxelles, Brussels, Belgium; 6) Department of Clinical and Experimental Epilepsy, UCL Institute of Neurology, Queen Square, London, UK.

Juvenile myoclonic epilepsy (JME) is a common form of idiopathic generalized epilepsy. While the majority of patients with JME are well-controlled on antiepileptic medications, a small fraction of patients continue to have seizures regardless of pharmacotherapeutic interventions. Despite high estimates of heritability of this disorder, few genetic variants have been identified that clearly confer risk of JME. In this study we sought to identify genetic variants that increase JME susceptibility using next-generation sequencing. Agilent's All Exon capture technology was used to sequence the exomes of 50 refractory JME patients. One hundred exome or whole-genome sequenced controls, not enriched for seizure phenotypes, were used to evaluate the frequency of candidate rare variants and to ascertain the likelihood of the variants increasing the risk for JME. Alignments of the sequenced fragments to the reference genome were performed using BWA software. Approximately 165000 exons (~18500 genes) were targeted with this technology, and on average we successfully sequenced >96% of these exonic regions (success defined by >80% of the bases comprising the exon sequenced with >5-fold coverage). Single nucleotide variants (SNVs) and small insertion-deletions (indels) were called from the sequence data using SAMTools. Annotation and statistical analyses were performed with Sequence Variant Analyzer. On average, 23638 high-quality SNVs and 1908 indels were identified in each sequenced JME exome, including approximately 600 SNVs and 165 indels predicted to alter the coding sequence of the protein and not observed in any control sample. Similar numbers of variants, both rare and common, were observed in control exomes. Targeted analysis of *GABRA1* and *EHFC1*, Mendelian genes known to harbor susceptibility variants to JME, identified no rare, functional mutations in patients with JME. One rare, intronic, heterozygous variant, not observed in any sequenced control, was observed in *GABRA1* in one JME patient. To evaluate the likelihood of all candidate susceptibility variants identified in the exome-sequencing data to impact susceptibility to JME, we genotyped the mutations in 200 non-refractory JME patients, in more than 2000 neuropsychiatrically-normal controls, and also in a subset of affected and unaffected family members of exome sequenced JME patients. We continue to explore the possibility that rare variants likely contribute to the susceptibility of refractory JME.

2678/F

A familial whole-genome sequencing approach to identify genetic variants influencing epilepsy susceptibility. *E.K. Ruzzo[1,3], E.L. Heinzen[1,3], R. Ottman[2,3], D.B. Goldstein[1,3].* 1) Center for Human Genome Variation, Duke University School of Medicine, Durham, North Carolina 27708, USA; 2) G. H. Sergievsky Center and Departments of Epidemiology and Neurology, Columbia University, and Division of Epidemiology, New York State Psychiatric Institute, New York, NY, USA; 3) Epigen Consortium.

Epilepsy susceptibility has clear a genetic component, but, despite years of research, the etiology of the vast majority of cases remains elusive. We have a unique cohort of multiplex epilepsy families that has enabled a new approach to primary discovery of epilepsy variants. We postulated that relatives harboring a rare, highly penetrant contributor to epilepsy explain the high incidence of non-acquired epilepsy in most of these families (average of 8 affected per family). Under this model, screening whole-genome sequences (WGS) for rare variants shared by related coaffecteds will identify a small set of candidates. To reduce the number of nonpathogenic shared variants, we selected the two most distantly related coaffecteds from each family. We obtained WGS data from 18 epilepsy patients-two in each of nine multiplex families. All 18 genomes were sequenced to an average coverage of ~31x using the Illumina GAIIx, and aligned to the reference genome using BWA software. Single nucleotide variants and small insertion-deletions were called using SAMTools software. We annotated and analyzed all identified variants using Sequence Variant Analyzer software. The genomes of affected relative pairs from each family were analyzed simultaneously to identify rare shared functional variants. We defined functional variants as nonsynonymous, protein truncating, or splice site-disrupting variants, and rare variants as those present at a frequency lower than the cumulative incidence of epilepsy in the general population (~3%) based on comparison to HapMap and a set of 80 whole-genome or whole-exome sequenced controls from our laboratory. No variant was present in all 18 epilepsy genomes and no shared functional variants were found in any of 12 genes already known to play a role in Mendelian forms of epilepsy. The number of rare, shared functional variants averaged 108 (min 49, max 201) across the 9 families. Very few of the shared variants were found in multiple families, indicating that different genetic factors are responsible for epilepsy in most of the families. This work defines a set of 974 candidate variants that can be followed up by genotyping in larger cohorts, and in other members of the same families. This work demonstrates that sequencing of coaffecteds in multiplex families is a powerful strategy for reducing the number of candidate variants emerging from sequencing studies.

2679/F

Novel mutation in the NF1 gene associated with an atypical NF1 clinical presentation. *S.L. Sawyer[1], L. MacLaren[1], C. White[2], O. Suchowersky[1,2].* 1) Department of Clinical Medical Genetics, Alberta Children's Hospital, Calgary, Alberta, Canada; 2) Department of Clinical Neurosciences, University of Calgary, Alberta, Canada.

Care Presentation: A 45 yo patient was seen in the Neurogenetics clinic for assessment of possible Neurofibromatosis. He had multiple intraspinal and plexiform neurofibromas and bilateral Lisch nodules, but no café au lait macules or axillary freckling. He also has a peripheral neuropathy, for which no other cause could be identified. Other affected family members had similar findings. The NFI gene was sequenced in this patient, and an unclassified missense mutation was found that was not present in 2000 controls. Bioinformatic analysis suggested that the mutation was likely pathogenic; it was highly conserved evolutionarily and Polyphen predicted the amino acid changing variant to be probably damaging. Sequencing of the NF1 gene in his unaffected sister and his affected mother showed that his mother carried the same c.6905T>C polymorphism, and his healthy sister did not, thus demonstrating that the mutation is segregating with the disease in this family and lending further evidence for its' pathogenicity. To our knowledge this is the first report of a NF1 phenotype associated with the c.6905T>C mutation. In our family, intraspinal neurofibromas and a peripheral neuropathy are the main clinical features.

2680/F

The development of high-throughput gene scanning system for autism spectrum disorders by a PCR coupled high-resolution melting curve analysis. *K. Yanagi[1], T. Kaname[1], K. Morita[2], S. Ikematsu[2], H. Maehara[1], Y. Fukushima[3], K. Naritomi[1].* 1) Dept Medical Genetics, Univ Ryukyus, Nishihara, Japan; 2) Dept Bioresources Engineering, Okinawa Natl College Technol, Okinawa, Japan; 3) Dept Medical Genetics, Shinshu University, Graduate School of Medicine, Matsumoto, Japan.

It is important to establish an easy and reliable system to detect mutations or variations for gene examination and genetic association study. High-resolution melting curve analysis (HRM analysis) is a method, which allows simple and rapid detection of gene variations. We developed a system for scanning gene variations in *NLGN3*, *NLGN4X*, and *c-MET*, which mutations were found in the patients with autism spectrum disorders (ASDs). There is a possibility that those variations are associated with ASDs. Since it is, however, estimated that small number of patients will be affected by variations in those genes, a scanning system is needed to investigate correlations between the gene variations and ADS patients, which allows rapid detection of variations in large scale. We set up the PCR/HRM system for exons of the *NLGN3*, *NLGN4*, and *c-MET* gene using combination with SYTO9 fluorescent dye (Invitrogen), Ex-Taq polymerase (TAKARA), and LightCycler 480 Instrument (Roche). Evaluation of the PCR/HRM system for those genes in 48 Japanese controls by confirming direct sequencing, resulted complete detection of all the known and novel variations. Next, we scanned variations in those genes in 62 ASD patients. The novel three variations were found in each gene. The system is a valuable method for rapid and reliable detection of variations in ASD patients.

2681/F

Deep resequencing in a family with X-linked ataxia dementia. *N. Pankratz[1], B. Craig[2], B. Marosy[2], K. Hetrick[2], J. Rosenfeld[1], L. Padgett[1], E. Pugh[2], H. Ling[2], M. Barnhart[2], S. Griffith[2], M. Econs[1], M. Farlow[1], K. Doheny[2], T. Foroud[1].* 1) Indiana University School of Medicine, Indianapolis, IN; 2) Center for Inherited Disease Research (CIDR) and Genetic Resources Core Facility (GRCF) High Throughput Sequencing Center (HTS), John Hopkins School of Medicine, Baltimore, MD.

X-linked ataxia dementia (XLAD) is a neurodegenerative disease with early-onset ataxia, pyramidal tract signs, and adult-onset progressive dementia. Linkage analysis using the only known kindred with this syndrome yielded a maximum LOD score of 5.29 with DXS1231 at θ=0. Key recombinants delineated a 24 Mb region using microsatellite markers. Illumina Omni1-Quad array data ruled out the presence of a large causative CNV and narrowed the region further to ~19 Mb. Next generation sequencing of the X chromosome for two affected cousins and one of their unaffected siblings was then performed in collaboration with the Center for Inherited Disease Research (CIDR). The Agilent SureSelect Human X Chromosome Demo Kit was used to capture exons on the X chromosome and was followed by 75 bp paired-end sequencing using the Illumina GAIIx. BWA was used to align the data, and consensus base calling was performed with SAMtools. Average capture specificity was 79.0%, and ≥10X depth was achieved for 98.7% of targeted bases. Variants were filtered for presence in both affected cousins and absence in the unaffected brother. Given the rarity and severity of the phenotype, all SNP and indel variants present in dbSNP were excluded from further consideration. Four short indels (i.e. <10 bp) were reviewed using the Integrative Genomics Viewer (IGV); however all are present in the pilot 1 dataset of the 1000 Genomes Project. Three nucleotide substitutions located in an exon or UTR were present in the 19 Mb region in both affected cases. One is a synonymous variant in exon 8 of NOX1, chrX:99992932C->T, and is predicted by SKIPPY (http://research.nhgri.nih.gov/skippy/) to introduce 3 exon splicing silencers and thus may cause exon skipping during mRNA processing. Knocking out NOX1 in a mouse model of a neurodegenerative disorder modifies disease severity. Another variant, 102932576G->A, is in the 3' UTR of PLP1, which is associated with Pelizaeus-Merzbacher disease (PMD) where childhood ataxia and cognitive impairment are typical. These variants were not identified in 182 HapMap samples available from pilot 1 of the 1000 Genomes Project. We are currently genotyping these variants in 2000 control subjects and will have additional data to present at the meeting. Identification of the mutated gene in this unique family has the potential to increase our understanding of nervous system function, pathogenesis of neurodegenerative conditions, and possible treatments for such conditions.

2682/F

Comprehensive resequencing of *PEX5* gene in patients with X-linked adrenoleukodystrophy (ALD) and association studies with the phenotypes of ALD. *T. Matsukawa[1], Y. Takahashi[1], J. Goto[1], Y. Suzuki[2], N. Shimozawa[2], O. Onodera[3], M. Nishizawa[3], S. Tsuji[1].* 1) Dept Neurology, Univ Tokyo, Tokyo, Japan; 2) Gifu University, Gifu, Japan; 3) Niigata University, Niigata, Japan.

Background & Purpose: Adrenoleukodystrophy (ALD) is an X-linked disorder affecting primarily the white matter of the central nervous system occasionally accompanied with adrenal insufficiency. The ages at onset and the clinical presentations of ALD are substantially broad. There are various kinds of phenotypes, like Childhood cerebral ALD (CCALD), Adult cerebral ALD (AdultCer), Adrenomyeloneuropathy (AMN). Despite the discovery of the causative gene, *ABCD1*, clear genotype-phenotype correlations have not been established. *ABCD1* gene encodes adrenoleukodystrophy protein (ALDP), which is localized to the peroxisomal membrane. *ABCD1* mutant mice show a milder AMN phenotype. On the other hand, mice lacking *PEX5* gene expression, which is related to formation process of peroxisome, in their oligodendrocytes develop cerebral demyelination involving the corpus callosum and the anterior commissure as occurs in CCALD. Association studies based on SNPs identified by comprehensive resequencing of gene related to *ABCD1* may reveal genes modifying the phenotypes of ALD. The purpose of this study is to investigate genotype-phenotype correlations and to explore the possibility of *PEX5* as the disease modifying gene. **Methods:** We analyzed 51 Japanese patients with ALD (CCALD 14, Adlescent Cerebral ALD 3, AdultCer ALD 6, AMN with cerebral (AMN-Cer) ALD 4, Cerebellobrainstem ALD 2, AMN 16, Addison disease 1, asymptomatic 2 and Unknown 3). Sequences of all of the exons, introns around the exon-intron boundary, 5'UTR and 3'UTR were analyzed by direct nucleotide sequence analysis. **Result:** 2 novel non-synonymous SNPs in exon, 1 novel SNP in intron, 2 novel SNPs in 3'UTR and 2 known SNPs in 3'UTR were detected. SNPs identified by comprehensive resequencing of *PEX5* were used for association studies. There were no significant associations between these SNPs of *PEX5* and ALD phenotypes in the Japanese population. **Conclusion:** The present study indicates that *PEX5* are less likely the disease modifying genes, necessitating further studies to identify genes modifying the phenotypes of ALD.

2683/F

Familial Schizophrenia, Complete Genome and Exon Sequence Analysis. *C.T. Caskey[1], S. Sims[1], M.L. Gonzalez-Garay[1], A.J. Marian[1], A. Wassef[2], A. Swann[2], N. Williams[2].* 1) UT - IMM, Houston, TX. 1825 Pressler Street, Ste. 205 Houston, TX 77030; 2) Harris County Psychiatric Center 2800 South MacGregor Way Houston, TX 77021.

The genetic basis of schizophrenia is well established clinically and by molecular population studies using SNP and copy number variant (CNV) analysis. Heterogeneity of the genetic basis of the schizophrenia phenotype is anticipated. We have studied families with multiple affected individuals from the Harris County Psychiatric Center, in Houston, by complete genome sequencing with two approaches - 1) whole exome capture and deep sequencing (Illumina GAIIx), and 2) whole nuclear genomic DNA sequencing (Complete Genomics, Inc., Science- 1 January 2010 Vol 327). The observed sequence changes are being validated by comparison of results from both sequencing approaches. Where appropriate any candidate variants found are further confirmed by still a third method (genotyping or Sanger sequencing). Our study utilizes human genetics and complete genome sequencing to identify disease gene candidates for individual families. Families have been selected and the sequencing has been completed. Bioinformatic analysis focuses on non-synonymous coding and splice junction alterations, in addition to CNV analysis. We correlate any sequence variants discovered with known SNPs, CNVs, and regional brain gene expression of candidate alleles. Thus, we utilize a systems biology approach to candidate gene identification, taking into account family pedigrees, sequence variants, knowledge of pathways involved and expression information. The studies identify gene candidates for schizophrenia. New disease/gene candidates are the focus of our presentation.

2684/F

Comprehensive variant discovery in the new Late-Onset Alzheimer Disease susceptibility gene MTHFD1L using next-generation sequencing technology. *M.A. Kohli[1], J.M. van Baaren[1], N.C. Naj[1], G.W. Beecham[1], J.L. Haines[2], J.R. Gilbert[1], M.A. Pericak-Vance[1].* 1) John P. Hussman Institute for Human Genomics, Miller School of Medicine, University of Miami, FL, USA; 2) Vanderbilt Center for Human Genetics Research, Vanderbilt University, Nashville, TN, USA.

Genome-wide association studies (GWAS) of Late-onset Alzheimer disease (LOAD) have consistently observed associations with APOE variants, and until recently, few statistically significant associations at other loci have replicated across studies. We recently reported a statistically significant association of rs11754661 with LOAD ($P=4.70\times10^{-8}$; Bonferroni-corrected P=0.022), a chromosome 6q25.1 single nucleotide polymorphisms (SNP) in the gene MTHFD1L (encoding the methylenetetrahydrofolate dehydrogenase 1-like protein) and we replicated this finding from our discovery GWAS of 932 cases and 1,104 controls in an independent replication GWAS dataset of 1,338 cases and 2,003 controls at P=0.002 ($P=1.90\times10^{-10}$ in combined analysis of discovery and replication sets).While this strongly associated SNP (odds ratio (OR) = 2.1 (95% confidence interval (CI): 1.7, 2.6) is located in an biological candidate gene which may influence homocysteine levels (a significant risk factor for LOAD), the risk allele of rs11754661 is rare (minor allele frequency = 0.07) and the SNP falls into an intronic region with linkage disequilibrium (LD) extending for only 32.6 kb of the genes total 236.7 kb length. To identify potential LOAD-related functional variants, we initially sequenced the MTHFD1L LD block containing rs11754661 using high-throughput next-generation sequencing (NGS). We captured the target region using amplicons of ~8kb length generated by long-range PCR in 43 LOAD cases and 59 controls, which are homozygotes or heterozygotes for the rs11754661 risk allele and negative for the APOE ε4 allele. Equimolar amounts of each PCR product per individual DNA sample were multiplexed using up to 12 individuals per single lane on an Illumina Genome Analyzer II sequencer to provide sufficient sequence depth for new variant discovery (>1,000-fold).

2685/F

Analysis of genetic inheritance in autism spectrum disorders by whole exome sequencing. *H. Kuwabara[1], T. Shimada[3], M. Bundo[2], T. Sasaki[4], C. Kakiuchi[3], Y. Kano[1], K. Iwamoto[2], M. Tochigi[3], K. Kasai[3].* 1) Department of Child Psychiatry, Graduate School, Tokyo, Japan; 2) Department of Molecular Psychiatry, Graduate School of Medicine, University of Tokyo; 3) Department of Neuropsychiatry, Graduate School of Medicine, University of Tokyo; 4) Office for Mental Health Support, University of Tokyo.

Introduction: Although heritability of autism spectrum disorders (ASD) is up to 90%, genetic factors remain largely unknown. Genome-wide association studies revealed that common genetic variants explain a modest fraction of heritable risk for ASD, suggesting the important role of rare variants to the unexplained heritability. Recently, whole-exome sequencing combined with a series of refined filtering methods has proven to be effective to identify the gene underlying a Mendelian disease (Ng et al., Nature Genetics 2010). Furthermore, such approach is likely to provide important insights into the pathogenesis of complex disorders such as ASD. Method: We selected a multiplex family with Autism spectrum disorder, consisted of members across four generation, for exome analysis. The family includes a son and a mother with Asperger's disorder, grandfather and great grandfather with pervasive developmental disorders not otherwise specified (PDDNOS). Exome sequencing is performing on a son with Asperger's disorder and non-affected father and grandmother. Results: Identification of the pathogenic mutations is underway. To identify the pathogenic mutations, we are focusing on novel and nonsynonymous variants. In addition, we are comparing the variants between a patient and non-affected family members to scrutinize the pathogenicity. Conclusion: We discuss the availability of exome sequencing in a selected patient to identify the pathogenic mutations in the complex neuropsychiatric disorders such as ASD.

2686/F

Extra-cerebral biallelic expression of the UBE3A gene: contribution to Angelman syndrome (AS) diagnosis. *C. Flament, J. Amiel, G. Royer, S. Lyonnet, J. Wong, A. Munnich, JP. Bonnefont, S. Gobin-Limballe.* INSERM U781, Paris Descartes University, Necker Hospital, Paris, France.

UBE3A anomalies, such as 15q maternal deletions, paternal 15q uniparental disomies, 15q imprinting center mutations, and mutations of UBE3A itself, are responsible for AS. The UBE3A gene (15q11-q13) is an imprinted gene, maternal monoallelic expression of which is restricted to specific cerebral areas, while its expression pattern is biallelic in other tissues. We took advantage of this particular pattern of expression to establish AS diagnosis in two unrelated children carrying a same atypical UBE3A sequence variant. 15q FISH and methylation profile at the SNRPN locus were normal in each of them. We identified in both a same heterozygous 14-bp deletion of UBE3A with a proximal break point at 21 nt downstream of the stop codon. This 3' UTR sequence variation had not previously been described as a disease-causing mutation or a neutral polymorphic variant, and no other sequence abnormality could be detected in coding exons and exon-intron junctions as well. Predicting softwares indicated that this deletion could alter hnRNPA1 protein binding to the UBE3A mRNA and therefore cause RNA instability. While the disease-causing effect of this deletion could neither be confirmed nor be excluded in Family 1, based on its familial transmission (inheritance from the healthy mother and maternal grandfather), it was finally excluded in Family 2 owing to its presence in the asymptomatic father and brothers. In an attempt at identifying a mutation undetected at the genomic level, we then analyzed the UBE3A cDNA, using RNA extracted from blood sample. In Family 2, cDNA sequencing detected the deletion at heterozygous state thus confirming the biallelic expression of UBE3A in white blood cells, but failed to detect any deleterious anomaly. In Family 1 however, the 14-bp deletion was no longer present in the proband's cDNA, suggestive of mRNA decay and therefore of the presence of a truncating mutation in cis of the 14-bp deleted allele. This putative mutation could however not be detected. This observation illustrates that the lack of extra-cerebral imprinting of UBE3A provides an easy access to both maternal and paternal co-expressed RNA species, thus enabling detection of mutations that escape analysis at the genomic DNA level.

2687/F

Rare Variants in Nicotinic Acetylcholine Receptors are Associated with Nicotine Dependence. *G. Haller[1], J. Budde[1], F. Vallania[2], T. Druley[2], R. Mitra[2], D. Hatsukami[3], J. Rice[1], L. Bierut[1], A. Goate[1].* 1) Department of Psychiatry, Washington University , Saint Louis, MO; 2) Center for Genome Sciences, Washington University, Saint Louis, MO; 3) Tobacco Use Research Center, University of Minnesota, Minneapolis, MN.

Genome-wide association studies (GWASs) have identified variation in both the *CHRNA5-CHRNA3-CHRNB4* and *CHRNA6-CHRNB3* gene clusters that contribute to nicotine dependence (ND). However, variation in these and the other nicotinic receptor genes have not been studied in depth in African American populations. Sequencing individuals at the extremes of a phenotypic distribution can be a powerful approach to identify novel associated variants. Rare (minor allele frequency <5%) variants, while individually infrequent, make up a large proportion of genetic diversity when taken in aggregate. To identify novel variants associated with nicotine dependence in African Americans we pooled DNA samples from individuals based on their Fagerström Test of Nicotine Dependence (FTND) score and the number of cigarettes smoked per day (CPD). In total, 176 nicotine dependent heavy smokers (FTND>5, mean CPD = 29) and 176 light smoking controls (FTND<1, mean CPD = 5) from the Collaborative Genetic Study of Nicotine Dependence (COGEND) were studied. The coding regions of each of the neuronally expressed nicotinic acetylcholine receptor (CHRN) genes (*A1-7, A9, A10, B1-4, G*, and *D*) were sequenced for each pool using Illumina sequencing. Variant locations and allele frequencies were then determined using algorithms that utilize large deviation theory to differentiate between polymorphisms and sequencing errors. Individuals comprising the sequenced pools were subsequently genotyped at all loci predicted to be polymorphic to confirm the sequencing results. Twenty-five novel missense SNPs in 11 genes (*A1,A2,A4,A5,A9,A10,B1,B2,B4,D,G*) were identified by this method and validated by Sequenom genotyping. To increase analytical power, we collapsed missense variants, both known and novel, in each gene into one aggregate genotype before running tests of association. Using a linear regression model, we find that carriers of missense variants in *CHRNA1* and *CHRNB4* have decreased CPD compared to non-carriers (*CHRNA1* P= 0.002, *CHRNB4* P=0.006), suggesting a protective role of variants in these genes with respect to the development of nicotine dependence. We tested each gene with at least two missense variants (8 genes) for association in this manner, making both of the above associations significant after correcting for multiple tests (Bonferroni P-value cutoff = 0.00625). This is the first example of an association between either *CHRNA1* or *CHRNB4* and a nicotine dependence phenotype.

2688/F

Genetic variation in APOE and neighbouring genes and risk of Alzheimer's disease. *S. Cervantes[1], Ll. Samaranch[1], J.M. Vidal[2], M.J. Bullido[3], F. Coria[4], A. Lleó[5], J. Clarimón[5], P. Pastor[1].* 1) Neurogenetics Laboratory, Center for Applied Medical Research, Pamplona, Navarra, Spain. Department of Neurology, Clínica Universidad de Navarra, Pamplona, Spain; 2) Pharmacology Unit, Pathology, Pharmacology and Microbiology Department, University of Barcelona School of Medicine, Barcelona, Spain; 3) Molecular Biology Department and CIBERNED, Instituto de Salud Carlos III, Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Madrid, Spain; 4) Clinic for Nervous System Disorders and Service of Neurology, Hospital Universitario Son Dureta, Palma de Mallorca, Spain; 5) Department of Neurology, Hospital Santa Creu i Sant Pau, Barcelona, Spain CIBERNED, Instituto de Salud Carlos III, Spain.

Background: Genetic factors are responsible for 80% of Alzheimer's disease (AD) risk. Lipid metabolism has been involved in AD pathogenesis. In fact, APOE ε4 is the most important genetic risk factor for AD described thus far, with estimates of late-onset AD risk owing to APOE ε4 being around 50%. In this study we focused on APOE and genes located near APOE locus, such as APOC1, APOC4 and APOC2 and TOMM40. TOMM40 is a channel-forming protein of the outer mitochondrial membrane that could interact with Aβ in AD pathogenesis. Therefore, variants at these genes could alter the normal function of protein products and modulate AD risk. Methods: We performed long-range sequencing of promoter and regulatory regions of APOE, APOC1, APOC4, APOC2 and TOMM40 genes, in 29 subjects with MCI that progressed to dementia. In addition, we sequenced TOMM40 exons. We detected 49 variants, 19 of them novel. We analyzed with MatInspector (www.genomatix.de) effect of variants in transcription factors binding sites (TFBS), and Human Splicing Finder (www.umd.be/HSF/) was used to analyze their influence of splicing sites. Thirty-six variants (6 novel) with a potential regulatory effect were genotyped in 627 healthy controls and 619 subjects with AD. We used PLINK software for association analysis between AD cases and healthy controls. Results: Ten of the genotyped variants located in promoter regions (3 of them novel), had a potential effect in TFBS. Two variants (one of them novel) modified TOMM40 protein sequence. One variant showed a potential effect in modifying splicing of TOMM40 protein. In the single locus analysis, rs439358, which differentiates APOEε4 from non-ε4 individuals showed the strongest association with AD (OR=4.9; IC= [3.9-6.2]; p=1.42x10-46; q= 5.12x10-45). Independent effects analysis suggested that the SNPs rs5158 and rs10413089 could increase risk of AD independently from APOEε4 allele (p=0.009 and 0.01, respectively). Conclusions: Fine sequencing of APOE cluster genes revealed novel variants with a potential effect in transcription regulation and in splicing. Association analysis suggested that two variants near the 3' end of the region analyzed may increase risk of AD independently from APOE ε4 influence. TOMM40 variants analyzed showed no independent effect for AD risk.

2689/F

Exome sequencing of multiplex autistic families to define a risk region on chromosome 12. *H.N. Cukier[1], D.Q. Ma[1], D. Salyakina[1], H.H. Wright[2], R.K. Abramson[2], J.L. Haines[3], M.L. Cuccaro[1], J.R. Gilbert[1], M.A. Pericak-Vance[1].* 1) John P. Hussman Institute for Human Genomics, University of Miami, Miami, FL; 2) University of South Carolina School of Medicine, Columbia, SC; 3) Center for Human Genetics Research, Vanderbilt University, Nashville, TN.

There is a strong genetic component to autism, but studies to date have demonstrated that the underlying genetic architecture is complex, with numerous genes potentially involved. One strategy for uncovering these genes is the use of large, extended families with multiple, distantly related, affected individuals. Our previous genome-wide linkage study identified a 4 centimorgan region (75-79 cM) on chromosome 12 that demonstrated significant linkage (HLOD = 4.51) across eleven extended multiplex families with only male affected individuals. Our goal is to comprehensively evaluate the chromosome 12 candidate region in order to identify the genetic abnormalities that underlie the strong autism linkage peak. To test candidate genes for autism in these families we performed next-generation sequencing using the Illumina Genome Analyzer II to identify potential causative variants. We used the Agilent SureSelect Human All Exon kit in order to capture 38 Mb corresponding to the CCDS defined exons. Eight individuals, two affected pairs from four distinct multiplex autistic families, were selected and sequenced in a 2x76 paired end run for an average of 150x coverage. Using the MAQ program (Mapping and Assembly with Qualities), 43,000-53,000 single nucleotide polymorphisms (SNPs) were identified in each sample, with approximately 16,000 exonic variations per individual. We also identified between 3,200-7,000 insertion/deletions in each of our samples. When comparing SNP variants that fall in the chromosome 12 region of interest (60,000-68,000 Mb) in related patients, each family has 37-54 identical alterations between their two autistic individuals. Thus far, we have identified eight SNP variations that are identical across all four extended families, including four missense mutations that fall within *GRIP1*, *IRAK3*, and the hypothetical *C12orf66* gene. *GRIP1* (*glucocorticoid receptor-interacting protein 1*) is a particularly interesting candidate due to its presence throughout the nervous system and role in long-term potentiation. Additional analysis will be performed to determine if variants may cluster across families around a gene of interest. We will also evaluate variations that fall within regions across the genome that have previously been linked to autism. Further cataloguing of variations in these extended autistic families is currently underway and studies are ongoing to determine if any identified alteration play a role in the etiology of ASD.

2690/F

Targeted re-sequencing of a 150kb contiguous genomic region of chromosome 5p14.1 associated with autism. *J.R. Gilbert[1], A.J. Griswold[1], D.Q. Ma[1], H.N. Cukier[1], I. Konidari[1], W. Hulme[1], P. Whitehead[1], J. Jaworski[1], H.H. Wright[2], R.K. Abramson[2], M.L. Cuccaro[1], J.L. Haines[3], M.A. Pericak-Vance[1].* 1) John P. Hussman Institute for Human Genomics, University of Miami, Miami, FL; 2) University of South Carolina School of Medicine, Columbia, SC, USA; 3) Center for Human Genetics Research, Vanderbilt University, Nashville, TN.

Genome-wide association studies (GWAS) have identified and validated in independent datasets a region with significant association to autism on chromosome 5p14.1. The 150kb peak region of association (25.9-26.1Mb) is flanked by the Cadherin 9 and Cadherin 10 genes located approximately 1Mb proximal and 1.5 Mb distal, respectively. The peak region itself lacks any annotated genes or known regulatory sequence elements, though the clustering of significant association signals suggests that a common variant(s) is responsible for the association. The goal of this study was to sequence the entire peak region of association in an attempt to identify a causative variant(s). We have amplified 145kb of the peak region using long-range PCR amplicons ranging from 5 to 11kb in 49 autism cases and 49 pediatric non-autistic controls. A 5kb region (chr5:26,030,927-26,035,873) was sequenced using traditional Sanger sequencing. Amplicons were pooled in equimolar amounts to generate sequencing libraries for each individual and were sequenced using multiplexed 2x75 paired-end sequencing on the Illumina Genome Analyzer IIx. Sequencing generated an average of 2.8 million reads per individual, corresponding to a depth of coverage of over 2000X over the 145kb target region. The sequencing reads were aligned to the human genome and variants were called using the Mapping and Assembly with Quality (MAQ) software package. Overall, MAQ identified 584 novel variants, 174 unique to cases, 227 unique to controls, and 183 common to both groups. Of the 174 novel variants found only in cases, 136 were identified in only a single case, while 21 were identified in more than 5 cases indicating significant overrepresentation of these variants using the Fisher Exact test. In addition, MAQ found variation at 611previously identified (dbSNP) sites, 20 only in cases, 49 only in controls, and 542 common to both groups. Of the 20 known variants unique to cases, 5 were frequent enough to be statistically significant. An additional 9 novel and 33 known variants, common to cases and controls, show statistical overrepresentation in cases. Cluster analysis of these overrepresented variants show that 19 of the 52 known variants cluster between rs1748297 and rs1835055 (chr5: 25937365-25940689), though no similar clustering is evident in novel variants. Overrepresented variants are being genotyped in a larger dataset and functionally assessed to determine their role in autism risk.

2691/F

Comprehensive re-sequencing of the autism associated GABRα4 to identify potential autism risk functional variants. *A.J. Griswold[1], D.Q. Ma[1], P. Whitehead[1], J. Jaworski[1], J.L. Haines[2], M.L. Cuccaro[1], J.R. Gilbert[1], M.A. Pericak-Vance[1].* 1) John P. Hussman Institute for Human Genomics, University of Miami, Miami, FL; 2) Center for Human Genetics Research, Vanderbilt University, Nashville, TN.

Previous studies have demonstrated association of autism with gamma-aminobutyric acid (GABA) receptor subunits, including *GABRα4* on chromosome 4p12. The GABAergic system is responsible for synaptic inhibition in the adult brain and is the key neurotransmitter directing neuron development. To identify functional variants within *GABRα4* contributing to autism risk, we comprehensively re-sequenced the entire gene and its regulatory regions in 84 Caucasian autism cases, homozygous for a previously associated allele (G/G at rs1912960), and 84 non-autistic pediatric Caucasian controls, 55 of which were homozygous for the associated allele. We amplified 88kb covering the entire transcript and 5kb up and downstream of the gene with 10 tiled long-range PCR amplicons. The amplicons were pooled in equimolar amounts to generate sequencing libraries for each individual and were sequenced using multiplexed 2x50 paired-end sequencing on the Illumina Genome Analyzer IIx. Sequencing generated 2.5 million reads per individual, corresponding to a depth of coverage of over 2000X across the 88kb target region. The sequencing reads were aligned to the human genome and variants were called using the Mapping and Assembly with Quality (MAQ) software package. Overall, MAQ identified 342 novel variants of which 87 varied only in cases and 177 only in controls. In addition, MAQ detected 421 previously identified (dbSNP) variants, 7 varied only in cases and 82 only in controls. When analyzing only those homozygous associated allele individuals (84 cases, 55 controls), there were 256 novel variants, 110 only in cases and 91 only in controls, and 175 known variants, 29 varied only in cases and 2 only in controls. In order to correct for genetic background effects, only these individuals were used for subsequent analysis. Since there is no statistical difference in the global burden of variation in cases and controls, and all identified variants were in non-coding regions of the gene, we analyzed individual variants for statistical overrepresentation in cases. Among the variants unique to cases, 6 novel variants were statistically overrepresented using the Fisher Exact test: 3 were within the 13.5kb first exon, 2 in the 3kb third intron, and the sixth located 7kb 5' upstream of the gene. Genotyping of these overrepresented variants in a larger dataset, and assessment of their functional impact is currently underway to determine their role in contributing to autism risk.

2692/F

The role of KIF gene rare variants in the severity of multiple sclerosis. *A.E. Handel, G. DiSanto, G.C. Ebers, S.V. Ramagopalan.* WTCHG, University of Oxford, Oxford, United Kingdom.

Multiple sclerosis (MS) is a complex disease whose aetiology is generally thought to be a result of autoimmune processes primarily directed against myelin in the central nervous system. However, it is now clear that axonal loss is also a key aspect of MS pathogenesis and underlies the progressive disability seen in most people afflicted with this disease. The majority of genes identified thus far from genome wide association studies in MS have been immunological but recent studies are now identifying single nucleotide polymorphisms (SNPs) in neuronal genes that would be expected to contribute to axonal loss. In particular the kinesin family members KIF1B, KIF5A and KIF21B have been implicated in MS susceptibility. These are attractive candidates for involvement in MS axonal degeneration as they are involved in the transport of vesicles along axonal microtubules. We sequenced the entirety of KIF1B, KIF5A, KIF21B and KIFAP3 in 30 patients with an extremely severe form of MS and compared them to a group of patients with an extremely mild disease phenotype and healthy controls drawn from the HAPMAP project to establish whether any novel/rare variants influenced the clinical course of MS. We identified multiple new rare variants in all of the KIF genes. The frequency of some of these rare variants showed nominally significant differences between the MS and control cohorts. We genotyped further cohorts of MS patients and controls for these rare variants. Sequencing of multiple sclerosis candidate genes offers a powerful method to identify rare variants involved in disease susceptibility. By using an extremes-of-outcome approach we have been able to investigate the role of several candidate regions in the phenotypic expression of multiple sclerosis.

2693/F

Promoter-wide DNA methylation analysis in neuronal nuclei derived from brains of patients with psychiatric diseases. *K. Iwamoto[1], M. Bundo[1], J. Ueda[2], K. Kasai[3], T. Kato[2].* 1) Department of Molecular Psychiatry, Graduate School of Medicine, University of Tokyo, Japan; 2) Laboratory for Molecular Dynamics of Mental Disorders, RIKEN Brain Science Institute, Japan; 3) Department of Neuropsychiatry, Graduate School of Medicine, University of Tokyo, Japan.

Epigenetic factors are believed to be important for the pathophysiology of major mental disorders such as schizophrenia, bipolar disorder and major depression. Although several studies have tried to identify the epigenetic mutations in brains of patients, interpretations of their results are usually difficult due to many confounding factors. One of main factors would be the cellular complexity in the brain. We have previously developed the method for the separation of neuronal and non-neuronal nuclei from the fresh-frozen postmortem brain, and have successfully performed genome-wide DNA methylation analysis. Here we performed a large-scale neuronal and non-neuronal methylation analyses using brains of patients with bipolar disorder (N = 35) as well as controls (N = 35). Neuronal and non-neuronal nuclei were obtained with the NeuN-based cell sorting using the brain samples obtained from the Stanley Medical Research Institute. Methylation profile was obtained using an Affymetrix promoter tiling array. Using the control subjects, we confirmed our previous finding of higher inter-individual variations in neuronal nuclei compared to non-neuronal nuclei. We did not observe the significant effects of sample pH, age and postmortem interval on the number of methylated genomic regions. In bipolar disorder, we identified several DNA methylation difference compared to control subjects. Some of differences were only found in neuronal nuclei. Our study revealed the importance of considering the cellular heterogeneity in brain in epigenomic studies using brains of patients with psychiatric disorders.

2694/F

Exome capture for targeted resequencing in French Canadian patients with familial Essential Tremor (ET). *N. Merner[1], C. Bourassa[1], S. Girard[1], J. Gauthier[1], S. Diab[1], S. Chouinard[1], N. Dupre[2], P. Dion[1], G. Rouleau[1].* 1) Centre de Recherche du CHUM, Université de Montréal, Montreal, Canada; 2) Department of Neurological Sciences, CHA - Enfant-Jésus, Quebec City, Canada.

ET is one of the most common neurological disorders. It is characterized by postural tremor, which worsens with movement. The clinical phenotype is variable and a lack of consensus on diagnostic criteria has resulted in 30-50% of patients previously diagnosed with ET to be misdiagnosed. Most studies indicate that ET is hereditary in 50-70% of patients. Studies of large ET families have shown that a family history of ET usually means an early age of onset, and the phenotype is usually fully penetrant by the age of 65. Linkage studies on families with ET have identified three ET loci, but no causative gene has been identified. More recently, common sequence variants in LINGO1 have been associated with ET but the significance of these findings remains unclear. Exome sequencing has been recently validated as a method to identify rare coding variants that cause Mendelian disease. This approach allows the use of only a few selected affected individuals and controls to identify disease genes, which is beneficial when large families are not available for linkage. Large ET families are available however misdiagnosis hinders effective linkage analysis; the Rouleau lab has recruited 84 French Canadian ET families and linkage studies have been performed on several of them but the results are inconclusive. In order to overcome the diagnostic barrier associated with gene identification we will be carrying out 'exome' sequencing on affected individuals within ET families with a 'definite' ET diagnosis and age of onset under 40 years of age. We will perform the exome capture of genomic DNA using the Agilent Technology SureSelect Human All Exon Kit, and resequencing by massive parallel sequencing using microarrays specific for the ABI SOLiD3 DNA sequencer. The Mapping and Assembling Qualities software will be used for the assembly and base-calling, and Novoalign for the detection of small indels. The dbSNP database will provide a good filter for all the common SNPs, and additional computational tools will identify rare, potentially pathogenic variants. Any variants that are shared by all affected family members and not in control samples and variants common to a particular gene between families will be flagged. Variants will be validated through Sanger sequencing by screening healthy controls and additional affected individuals. This study has the potential to identify the first ET gene, which will provide the first insights towards disease mechanism.

2695/F

Frequency of common LRRK2 mutations and identification of two novel variants in Mexican patients with Parkinson's disease. *N. Monroy[1,2], P. Yescas[1], M. López[2], M.C. Boll[1], M. Rodríguez-Violante[1], U. Rodríguez[1], A. Ochoa[1], M.E. Alonso[1].* 1) Department of Genetics, division of Neurology, and Clinical Neurodegenerative Disease Research Unit. Instituto Nacional de Neurología y Neurocirugía Manuel Velasco Suárez, Mexico City, Mexico; 2) Department of Biological Systems, Universidad Autónoma Metropolitana-Xochimilco. Mexico City, Mexico.

Mutations in leucine-rich repeat kinase 2 gene (*LRRK2*) are estimated to account for as much as 5-6% of familial Parkinson's disease (PD) and 1-2% of sporadic PD. Although these mutations represent the most frequent cause of autosomal dominant PD, particularly in certain ethnic groups, there was no report about *LRRK2* mutations in Mexicans. For this purpose, we screened 319 consecutive PD cases (mean age at onset: 52.4 years) for *LRRK2* mutations in exons 31 and 41 and for the Y1699C mutation in exon 35. Seven additional subjects, relatives of PD mutated probands, were enrolled. Only six (1.88%) heterozygous patients, five sporadic and one familial case, with disease mean age at onset 50 years had *LRRK2* mutations. Five patients had four different mutations in exon 31 and another patient carried the G2019S mutation in exon 41. Two of the exon 31 mutations have been previously described (R1441G and R1441H) and the remaining two are novel variants (T1452R and K1471R). They are both located in the Roc domain of the LRRK2 protein and T1452R is probably pathogenic. The Y1699C mutation was absent from this PD sample. None of the five *LRRK2* mutations identified was present in 200 neurologically healthy Mexican control individuals. These findings have important implications for diagnosis and genetic counseling in PD Mexican patients.

2696/F

Mutational analysis of optineurin (*OPTN*) in familial amyotrophic lateral sclerosis in the Japanese population. *H. Naruse, Y. Takahashi, J. Goto, S. Tsuji.* Dept Neurology, Univ Tokyo, Tokyo, Tokyo, Japan.

[Objectives] To elucidate genetic epidemiology of familial amyotrophic lateral sclerosis (ALS) with *OPTN* mutations in the Japanese population. [Background] Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by progressive motor neuron degeneration. About 5-10% of ALS patients are familial, where mutations in *SOD1* account for about 20% of FALS. Mutations in *TARDBP* or *FUS/TLS* account for about 5%, whereas mutations of other causative genes are rare. Very recently, *OPTN*, the gene encoding optineurin, previously identified as the causative gene for rare autosomal-dominant familial glaucoma, has been reported as the causative gene for autosomal-dominant as well as autosomal-recessive familial ALS (FALS). Comprehensive mutational analysis of *OPTN* in independent FALS pedigrees should be mandatory to establish the genetic epidemiology and to delineate the clinical characteristics of ALS with *OPTN* mutations. [Materials and Methods] Thirty-six FALS pedigrees were enrolled in this study. Previous screening employing a DNA microarray-based resequencing system and direct nucleotide sequencing method revealed 14 pedigrees with *SOD1* mutations, 1 with *TARDBP*, and 2 with *FUS/TLS*, all of whom were excluded in this study. The remaining 19 pedigrees included 14 pedigrees with autosomal-dominant mode of inheritance, 2 pedigrees with affected sibs with consanguinity and 3 pedigrees with affected sibs without consanguinity. In addition, four sporadic ALS (SALS) patients with consanguinity were also enrolled. All the coding exons of *OPTN* were amplified with genomic PCR using specific primers for each exon and further subjected to direct nucleotide sequence analysis. [Results] Five variants were identified, including 4 known SNPs and a novel synonymous variant in exon 16. Neither novel nonsynonymous variants nor known mutations for ALS were identified in this cohort. [Conclusion]This study suggests that mutations in *OPTN* were not the frequent cause for FALS in the Japanese population. Further investigation in large-scale cohorts or in cohorts with different ethnic backgrounds should be necessary to establish genetic epidemiology of ALS with *OPTN* mutations.

2697/F

Exome Sequencing in Cases of Sporadic Autism. *B.J. O'Roak[1], L. Vives[1], A.P. MacKenzie[1], S. Girirajan[1], E. Karakoc[1], C. Lee[1], A. Adey[1], S. Ng[1], C. Baker[1], B. King[2], R. Bernier[2], J. Shendure[1], E.E. Eichler[1,3].* 1) Department of Genome Sciences, University of Washington, Seattle, WA; 2) Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA; 3) Howard Hughes Medical Institute, University of Washington, Seattle, WA.

Autism is the most genetic of all neuropsychiatric syndromes. However, identifying the genetic factors involved has proved difficult in light of widespread genetic and phenotypic heterogeneity. Several lines of evidence support the hypothesis that sporadic cases of autism are enriched for causal *de novo* mutation events (O'Roak and State 2008). To evaluate this directly, we are performing global identification of *de novo* protein-altering events in parent-child trios. Specifically, we selected 20 trios with idiopathic autism, each showing a strong likelihood of sporadic disease, and subjected each of the 60 individuals to exome sequencing. *Methods*: In brief, standard Illumina sequencing libraries are prepared and hybridized in-solution to probes complementary for the CCDS exome (Nimblegen). After PCR enrichment of captured product, one lane of paired-end 76bp sequencing is performed on an Illumina GAII sequencer. Raw sequences are aligned to the build36 reference with BWA and variants called with samtools (Li et al. 2009). Proband variants meeting an 8X and phred-like quality 30 (q30) threshold are compared to the parents for non-Mendelian inheritance and against dbSNP, 1000 Genomes, and other sequenced exomes to identity potential novel *de novo* mutations. Variant reads are then visually inspected for artifacts and likely true mutations confirmed by Sanger sequencing. *Results*: Preliminary data from the first six trios completed shows an average Mendelian error rate of 0.75% for all variants and 0.52% for on-target variants. Target coverage has been ~90% at 8X/q30 for individual exomes, with ~88% of the target bases covered across the trio. Each trio has ~10 potential novel *de novo* mutations. As expected, many of these potential mutations are found in segmental duplication regions and likely reflect cross-talk from paralogous sequences. We have identified potentially *de novo* missense variants in two brain expressed transcripts, including a G protein-coupled receptor and ankyrin repeat domain-containing protein, as well as several inherited disruptive rare variants. *Conclusions*: Given the high coverage concordance, relatively small number of candidates (<10) per family, and low Mendelian error rates, our preliminary results show that exome sequencing in select families is a tractable method to identify novel candidate genes for autism and other complex disorders. These candidates can then be further evaluated in a much larger number of cases/controls.

2698/F

Chimeric Genes in Schizophrenia. *C.F. Rippey[1], A.S. Nord[1], J. McClellan[2], M-C. King[1,3].* 1) Department of Genome Sciences, University of Washington, Seattle, WA; 2) Department of Psychiatry, University of Washington, Seattle, WA; 3) Department of Medicine, University of Washington, Seattle, WA.

Our lab has shown that individually rare, gene-disrupting copy number variants (CNVs) are significantly more prevalent in individuals with schizophrenia than in unaffected controls. Several groups have replicated and extended this finding, yet it remains an open question as to which individual events are pathogenic. A subset of CNVs connects components of two separate genes into novel, often deleterious chimeric genes. We hypothesize that some rare CNVs create brain-expressed chimeric genes that contribute to schizophrenia pathogenesis. To test this hypothesis, we screened DNA from 124 individuals with schizophrenia using the Nimblegen HD2 array comparative genomic hybridization platform. We scanned genome-wide for CNVs >30 kb in length that were present in at least one case and no controls (n=367, same platform), and did not coincide with events in the database of genomic variants. From these events, we selected only those predicted to delete or duplicate the 5' end of one gene and the 3' end of another, as these CNVs should produce chimeras. We detected four such events in cases, three resulting from tandem duplications and one from a deletion. We confirmed the breakpoints of each of these events by PCR and sequencing. We also confirmed that in all cases, at least one of the two genes in the predicted chimera was expressed in brain. To test whether these events produced stable chimeric transcripts in the patients' lymphoblasts, we targeted PCR primers to the predicted chimeric mRNA. In three cases we were able to detect a stable transcript, confirmed by sequencing. In the fourth case, the gene contributing the 5' end of the chimera had no detectable expression in lymphoblasts, but was highly expressed in brain, so we expect the transcript to be brain-specific. For the chimeras with lymphoblast expression, we are performing western blots to ascertain protein stability and relative expression levels. We will then transfect neural cell lines with chimeric transcripts to test the effect of these fusion proteins on molecular and cellular phenotypes. In conclusion, we have detected rare CNVs that lead to stable expression of chimeric mRNA in individuals with schizophrenia. We propose that these events are likely to lead to aberrant expression of fusion proteins in the brains of these patients, and contribute to schizophrenia. This represents a novel genetic mechanism for major mental illness, as well as implicating new genes and pathways in schizophrenia.

2699/F

Whole Exome Sequencing of Two Unique Autism Pedigrees. *S.P. Strom[1], Z. Chen[1], S.P. Taylor[1], B. Harry[1], D.H. Geschwind[1,2,3,4], S.F. Nelson[1,2,3].* 1) Department of Human Genetics, University of California Los Angeles, Los Angeles, CA; 2) Center for Neurobehavioral Genetics, University of California Los Angeles, Los Angeles, CA; 3) Department of Psychiatry and Biobehavioral Sciences, University of California Los Angeles, Los Angeles, CA; 4) Department of Neurology, University of California Los Angeles, Los Angeles, CA.

While strongly heritable, few common alleles have been unambiguously identified as conferring risk to autism spectrum disorder (ASD). Attempts to significantly increase the sample size of ASD genome-wide association studies are ongoing, under the hypothesis that such studies to date have lacked sufficient statistical power to detect associated alleles of weak effect. Alternatively, the lack of common variant association signals in ASD suggests rare variation may contribute to genetic susceptibility more strongly than previously thought. Significant enrichment of rare genic copy number variants in ASD compared to neuropsychiatric normal control samples supports this alternate hypothesis. To comprehensively assess the spectrum of rare genic variation in two autism pedigrees, whole exome sequencing was performed. Using a combined approach employing commercially available target enrichment products and massively parallel DNA sequencing, over 32 million bases of targeted loci were sequenced at ten times or greater coverage depth in twelve individuals from two uniquely structured ASD pedigrees. Coverage, target enrichment, and variant analysis for both pedigrees are presented here as a proof of concept that exome sequencing of ASD families is a valuable approach to rare variant discovery in autism.

2700/F

Recessive paraplegin (SPG7) mutations in spastic paraplegia families with dominant pattern of inheritance. *F. Taroni[1], M. Plumari[1], S. Magri[1], V. Fracasso[1], C. Gellera[1], E. Salsano[2], S. Baratta[1], D. Di Bella[1].* 1) Unit of Genetics of Neurodegenerative & Metabolic Diseases, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy; 2) Unit of Neurology 8, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy.

Hereditary spastic paraplegias (HSP) are a clinically and genetically heterogeneous group of neurodegenerative disorders primarily affecting the corticospinal motor tracts. About half of HSP cases result from autosomal dominant mutations in the *SPG4* gene. Mutations in the *SPG7* gene are responsible for autosomal recessive HSP with both “pure” and “complex” phenotypes and, in our patients series, account for >10% of recessive or sporadic HSP patients. The *SPG7* gene encodes paraplegin, a component of the mitochondrial *m*-AAA metalloprotease. We report 3 unrelated families presenting an HSP phenotype with an autosomal dominant pattern of inheritance. Families 1 and 2 had a consanguineous marriage in one generation. Family 3 was compatible with autosomal dominant transmission with reduced penetrance. Molecular analysis of the *SPG4* gene was negative for point mutations and dup/del. Immunoblot analysis demonstrated absence or severe reduction of paraplegin protein in lymphocytes from the index cases. Molecular analysis of the *SPG7* gene revealed pathogenic mutations [2 missense mutations (A510V, T655P), 2 nonsense mutations (L78X, R457X), and deletion of exon 2] on both alleles in all the affected subjects analysed. In family 1, the proband presented with a pure HSP phenotype and was compound heterozygous for A510V and exon 2 deletion. In family 2, the two affected sibs, born to consanguineous parents, one of whom affected with spastic paraplegia, differed in genotype and clinical presentation (A510V/T655P, mild, and T655P/T655P, severe). Expression analysis of SPG7 mutants in yeast showed that the T655P mutation has a more drastic impact on paraplegin function. Interestingly, paraplegin protein levels were found to correlate with the severity of clinical phenotype. Analysis of paraplegin levels also allowed to exclude a pathogenic role for a third heterozygous variant (I297T) identified in one sister heterozygous for the pathogenic T655P but with no sign of HSP. In family 3, the proband (A510V/L78X) had spastic ataxia with a predominantly cerebellar phenotype. His half-nephew was similarly affected but exhibited a different genotype (A510V/R457X). Our findings indicate that recessive *SPG7* mutations are not an exceptional cause of spastic paraplegia in families with an apparently dominant pattern of inheritance and suggest that this might be due to the relatively high frequency of the A510V allele in the general population. [Telethon grant GGP09301 to FT].

2701/F

The genetic signatures of transposable elements (TEs) in schizophrenia. *F. Macciardi[1,2], E. Osimo[2], M. Vawter[1], F. Torri[2], A. Calabria[2], B. Lerer[4], S. Gaudi[3].* 1) Dept Psychiatry & Human Behavior, UCI, Irvine, CA; 2) Fondazione Filarete, Universita’ degli Studi di Milano (Italy); 3) Istituto Superiore di Sanità, Roma (Italy); 4) Hadassah Medical Organization / Hebrew University, Jerusalem (Israel).

Introduction. Understanding how the information in the human genome is utilized is one of the central question to unravel the genetics of complex diseases. Once completed, the human genome sequence has revealed being composed by transposable elements (TE) for about 45% and by coding genes in only 2%. Many genome wide association studies (GWAS) in complex diseases have mapped disease-associated variations to non-coding regions, presumably regulatory and endogenous retroviral. There are also recent evidences that not coding DNA sequences (known as “junk DNA”) tend to be enriched near genes involved in brain-cell function than in non-CNS genes, specifically in genes building connections between brain cells. **Methods.** Our aim is to investigate the functional relationship between TE-related sequences and genes identified as “best” candidates via SNP mapping in genome wide association studies (GWAS) of schizophrenia. We use gene expression data to identify and detect the expression level of TEs in blood and brain samples from schizophrenia samples and matched controls. We also extend our analyses to Transcriptional Start Sites (TSS) for the same genes. These analyses are then complemented with target-resequencing of the entire genomic area, to look for differences between subjects who do or do not present the associated risk SNPs. **Results and conclusion.** For a prototypal gene, AHI1 (Torri et al, 2010) we found an excess of Alu, Line1 and Line2 elements across the AHI1 genomic region, with a clear pattern of tissue-specific expression that looks regulated by CNS-specific TSS, with gene expression data on post mortem schizophrenic brain increased compared to LCL cells of the same patients. Initial sequencing in extreme subjects with and without the AHI1 risk haplotype shows marked differences in their respective sequences. We also observed differences in term of coverage and reads distance between the two patients. Within the 5’ of PDE7B we found that the patient with the schizophrenia risk haplotype displays a significantly different distance between the reads compared to the risk-haplotype-absent patient. This finding may suggest that these two extreme patients differ also in terms of genomic architecture. Our results strongly suggest that a detailed analysis of risk genes in complex disorders must include TE distribution and expression, and that it is perhaps only through inclusion of these elements that psychiatric diseases will be fully understood.

2702/F

Normal ATXN3 allele but not CHIP polymorphisms modulates age at onset in Machado Joseph Disease. *M.C. França Jr[1,2], V. Emmel[3], A. D’Abreu[2], C.V. Maurer-Morelli[1], R. Secolin[1], L.C. Bonadia[1], M.S. Silva[1], A. Nucci[2], L.B. Jardim[3], M.L. Saraiva-Pereira[3], W. Marques Jr[4], H. Paulson[5], I. Lopes-Cendes[1].* 1) Department of Medical Genetics, University of Campinas (UNICAMP), Campinas, Brazil; 2) Department of Neurology, University of Campinas (UNICAMP), Campinas, Brazil; 3) Department of Internal Medicine and Biochemistry, Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, Brazil; 4) Department of Neurology, University of São Paulo (USP) at Ribeirão Preto, Ribeirão Preto, Brazil; 5) Department of Neurology, University of Michigan, Ann Arbor, MI,USA.

Length of expanded CAG at the ATXN3 gene accounts for part of the variability in the age at onset (AO) of patients with Machado-Joseph disease (MJD), but other genetic factors are probably involved. Experimental evidence indicates that the normal ATXN3 allele and the C-terminal heat shock protein 70 (Hsp70)-interacting protein (CHIP) may take part in the pathogenesis of MJD, thus making these two candidates for genetic modifiers of AO in patients with MJD. To investigate this, we determined the length of normal and expanded CAG repeats at the ATXN3 gene in 210 unrelated patients with MJD. In addition, we genotyped 5 single nucleotide polymorphisms (SNPs) within the CHIP gene. We first compared the frequencies of the different genotypes in two subgroups of patients who were highly discordant for age at onset after correction for length of the expanded CAG allele. The possible modifier effect of each gene was then evaluated in a stepwise multiple linear regression model.Mean AO was 35.7 (SD = 11.3) years. Mean length of expanded and normal CAG repeats were 72 (SD = 3.6) and 21 (SD = 5.3), respectively. AO was associated with length of the expanded allele CAG ($r2 = 0.596$, $p<0.001$). Frequencies of the normal CAG repeats at the ATXN3 gene and of CHIP polymorphisms were not significantly different in groups with highly discordant ages at onset. However, addition of the normal allele improved the model fit ($r2 = 0.601$, $p=0.014$). Therefore, we conclude that the normal CAG repeat at the ATXN3 gene has a small but significant influence in AO of MJD.

2703/F

Expansion bias for transmission of expanded CGG-repeat murine Fmr1 alleles. *K. Kim[1,4], S. Taylor[1], B. Ta[4], P.J. Hagerman[2,4], R.F. Berman[3,4].* 1) Division of Biostatistics, Department of Public Health Sciences; 2) Department of Biochemistry and Molecular Medicine; 3) Department of Neurological Surgery; 4) the Neurotherapeutics Research Institute, School of Medicine, University of California Davis. Davis. CA.

The fragile X mental retardation 1 (FMR1) gene harbors a non-coding CGG repeat that is normally between 5-41 repeats in length. For larger alleles (gray zone, 41-54 CGG repeats; premutation, 55-200 repeats) the CGG is increasingly unstable upon transmission to the next generation, with expansions to between ~70-200 repeats resulting in increasing susceptibility to Fragile X-associated Tremor/Ataxia syndrome (FXTAS). To investigate the mechanisms underlying (CGG)n instability upon transmission, we conducted repeat instability analysis in expanded CGG-repeat knock-in (KI) mice to determine whether (1) the probability of expansion or contraction of CGG repeat numbers is functionally related to the parental number of CGG repeats, and (2) whether the parental origin of the repeat allele affects the magnitude or direction of instability. We have bred 2,354 KI mice over several generations to establish transgenic lines with varying expanded alleles up to 350 CGG repeats. These CGG-repeat KI mice showed moderate intergenerational instability upon paternal and maternal transmission, and both expansions and contractions were observed. To assess the probability of repeat instability, and any attendant bias towards expansion or contraction, as a function of parental CGG repeat length and parental origin of transmitting allele, we conducted multinomial logistic regression analyses built upon a generalized additive model to estimate the underlying function relationships. Our results showed an increasing relationship between the probability of instability in CGG repeat numbers and the parental repeat numbers. While a paternal bias for transmission of expansions was not evident, the magnitude of expansion increased with repeat sizes of alleles with maternal origin. As maternal repeat numbers increased, repeat numbers were more likely to expand than contract. More interestingly, at a maternal allele repeat number of about 100, the probability of expansion started to increase and continued to gradually increase up to 200 repeats. Supported by NIH NINDS RL1 NS062411, NIH Roadmap Consortium NIDCR UL1 DE19583 & NIA AG032119.

2704/F

Modeling SCA31 non-coding pentanucleotide expression in cultured cells. *Y. Niimi, N. Sato, K. Ishikawa, H. Mizusawa.* Neurology, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, Japan.

Background Spinocerebellar ataxia type 31 (SCA31) is caused by a pentanucleotide insertion expansion located in an intron shared by two genes (*BEAN* and *TK2*) transcribed in opposite directions (Sato N. and Amino T. et al. Am J Hum Genet 85: 544-557, 2009.). This insertion is a 2.5-3.8 kb-long genomic fragment containing (TGGAA)n, (TAGAA)n and (TAAAA)n stretches, among which (TGGAA)n is considered crucial for pathogenesis. The mechanism by which the complex insertion including (TGGAA)n repeat leads to neuronal dysfunction remains elusive. **Object** Our goal is to establish a SCA31 cell model for discovering pathogenesis of complex insertion. In parallel with creating cell models with insertions, we aimed to clarify whether intron sequences flanking the insertion are also involved in RNA foci. **Method** As an initial step, SCA31 insertion was cloned into a dual-expression vector, which could monitor the expression of gene-of-interest with recombinant green fluorescent protein (Cycle 3 GFP) driven by a different promoter. Besides insertions from SCA31 patients, control insertions without (TGGAA)n repeats were also cloned. Because the insertion is predicted to be transcribed in two directions (i.e., *BEAN* and *TK2* transcription orientations), two different clones corresponding sense and anti-sense transcripts were made from each insertion. Lipofection method was used for introducing the vector, and toxicity of the gene was assessed by MTS and LDH assays. For in situ hybridization (ISH), probes were designed to detect repeat sequences or intron sequences. RT-PCR was used to confirm expression in cultured cells. **Result** Insertion sequences were confirmed to be expressed as RNA in cultured cells by RT-PCR. When toxicity was assessed by MTS and LDH assays, UGGAA sequences derived from patient-specific (TGGAA)n repeat appeared toxic than control insertion sequences. We are currently examining on different cell lines to see whether (UGGAA)n on RNA level, is indeed toxic in cells. In patients' brains, we observed RNA foci include repeat sequences. Further studies are now underway to evaluate whether flanking intron sequences are also included in RNA foci besides transcribed repeat sequences.

2705/F

A mouse model of CUG RNA toxicity in the brain. *J. Pruitt[1], D. Rudnicki[1], K. Pate[2], D. Swing[3], L. Tessarollo[3], R. Margolis[1].* 1) Psychiatry, Johns Hopkins University, Baltimore, MD; 2) Pathology, Johns Hopkins University, Baltimore, MD; 3) National Cancer Institute, National Institute of Health, Fredrick, MD.

Huntington's disease-like 2 (HDL2) is an autosomal dominant disorder with clinical and pathological features nearly indistinguishable from Huntington's disease (HD). HDL2 is a progressive adult onset neurodegenerative disorder caused by a CAG/CTG expansion mutation on chromosome 16q24.3, where the repeat falls within a variably spliced exon of junctophilin-3 (JPH3) in the CTG orientation. We have recently discovered that HDL2 brains contain intranuclear RNA foci, detectable by riboprobes against either CUG repeats or against JPH3 sequence flanking the repeat. These foci closely resemble the RNA foci detected in DM1 brains. We have also shown that overexpression of an untranslatable JPH3 construct containing an expanded CUG repeat is toxic to cultured cells. We therefore hypothesized that transcripts with an expanded CUG repeat may be toxic to the mammalian brain. To directly test this hypothesis, we have generated transgenic mice expressing an untranslatable fragment of JPH3 exon 2A, with either 10 or 100 CTG triplets, driven by the mouse prion promoter (PrP) vector. Preliminary data indicates that (CTG)100, but not (CTG)10, mice develop RNA foci by seven months. Behavioral analysis using an array of tests including: rotarod, open field, inversion, reach, and clasping suggest a modest impairment of motor function in the (CTG)100 mice compared to the (CTG)10 mice by six months of age. This phenotype is milder than observed in transgenic mice expressing polyglutamine repeats of a similar length under the same promoter, and suggests that factors in addition to CUG RNA toxicity, including loss of JPH3 expression, are likely to contribute to HDL2 pathogenesis.

2706/F

Pathogenic significance of the complex penta-nucleotide repeat insertion containing $(TGGAA)_n$ in spinocerebellar ataxia type 31. *N. Sato[1], Y. Niimi[1], T. Amino[1,2], T. Ishiguro[1], T. Makoto[1], K. Ishikawa[1], H. Mizusawa[1].* 1) Dept. of Neurology & Neurological Science, Graduate School, Tokyo Medical & Dental University, Tokyo, Japan; 2) Dept. of Neurology, Musashino Red Cross Hospital, Tokyo, Japan.

Background Spinocerebellar ataxia type 31 (SCA31) is a form of adult-onset neurodegenerative disorder that manifests pure cerebellar ataxia and slow progression. We previously mapped the SCA31 locus to 16q22.1, and identified a complex penta-nucleotide repeat insertion containing $(TGGAA)_n$ in every SCA31 patient's genome. The pathogenic effect of this insertion, however, does not appear straightforward; it is located in a non-coding region, and in rare occasions, control individuals have similar insertions lacking $(TGGAA)_n$ at the same position.

Purpose To clarify the pathogenic effect of the non-coding penta-nucleotide repeat insertion in SCA31.

Materials and Methods Fifty-six consecutive SCA31 patients, including 39 patients previously reported (Am J Hum Genet 2009;85:544-57), whose detailed clinical information had been obtained were enrolled in the study. The genomic DNA fragment spanning the insertion was amplified by PCR and the length of the insertion was measured by electrophoresis as described in our pilot study. The length of the insertion was compared with the patient's age of onset. The component of the insertion consisting of pure $(TGGAA)_n$ stretch was also amplified by PCR and its length was compared with the age of onset.

Results The length of the insertion was inversely correlated with the age of disease onset, with Pearson's product moment coefficient being -0.38 ($p=0.0044$). The length of pure $(TGGAA)_n$ component was also associated with earlier disease onset.

Conclusions Patients with longer penta-nucleotide repeat insertions tend to develop the disease earlier, and the pure $(TGGAA)_n$ component seems to be important for the development of SCA31.

2707/F

Mice lacking glutamate receptor 3 associated with significantly increased aggressive behaviors. *A. Adamczyk[1], R. Mejías-Estévez[1], J. Calderon[1], K. Takamiya[2], R. Huganir[2], M. Pletnikov[3], T. Wang[1].* 1) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Department of Neuroscience, Johns Hopkins University, Baltimore, MD; 3) Department of Psychiatry, Johns Hopkins University, Baltimore, MD.

Glutamate mediates the majority of excitatory neurotransmission in the brain and is implicated in the modulation of behavioral phenotype. The AMPA glutamate receptor family has four different subunits (GluR1-4) that assemble into a tetrameric receptor complex consisting mainly GluR1/2 and/or Glu2/3 heteromers to form ion channels of distinct functional properties. Lack of GluR1 subunit in mice is associated with reduced levels of anxiety and aggressive behaviors while lack of GluR2 subunit in mice results in increased aggression. The genomic region encoding GluR3 (Xq24-27) has been implicated as a quantitative locus for male aggression in mice in a genome-wide scan. Genetic defects of GluR3 in humans cause moderate intellectual disability, bipolar or autistic phenotype. To understand the potential role of GluR3 in behavioral modulation, we studied GluR3-deficient mice generated by classical gene targeting and back-crossed to C57BL/6 background using a standard battery of mouse behavioral tests. Compared to their wt littermates (n=14), this cohort of GluR3-deficient male mice (n=13) had a significant increase in aggressive behaviors (total time of aggression, p=0.011 and number of attack, p=0.015) in the home cage intruder-resident test, a moderate increase in peripheral activity in Open Field test (p=0.037), but no significant deficit in learning and memory function in Morris Water Maze and Y-maze tests. These studies suggest an important role of AMPA glutamate receptor 3 in modulating behavioral phenotype in mice.

2708/F

Differential microRNA expression in AD hippocampus compared to AD cerebellum, control hippocampus and control cerebellum. *L.M. Bekris[1], C.E. Yu[2].* 1) Dept Med, Univ Washington, Seattle, WA; 2) Geriatric Research, Education, and Clinical Center (GRECC) VA Puget Sound Health Care System, Seattle, WA.

MicroRNAs play an essential role in gene regulation in the brain. However, little is known about their role in neurodegenerative diseases, such as late-onset Alzheimer's disease (AD). Characterizing expression profiles of microRNAs in AD brain may help elucidate the role of microRNA in the pathophysiology of AD and may lead to identification of AD specific biomarkers. The aim of this investigation was to demonstrate that AD hippocampus microRNAs are expressed differentially compared to AD cerebellum, control hippocampus or control cerebellum. MicroRNA arrays were utilized to measure post-mortem brain microRNA levels in AD hippocampus (n=21) compared to AD cerebellum (n=21), control hippocampus (n=21), or control cerebellum (n=21). In addition, in silico analyses were performed to predict target genes for differentially expressed microRNAs. We found several microRNAs to be differentially expressed in the AD hippocampus compared to controls. Of these microRNAs, several are predicted to target AD relevant genes. Two of these AD relevant genes (PSEN1, BACE2) contain genetic variants (SNPs) within the predicted microRNA binding site. In conclusion, in this investigation we demonstrate that hippocampus specific microRNAs are expressed differentially in AD compared to controls and are predicted to target AD relevant genes. SNPs within predicted microRNA binding sites may be of interest for further study into their contribution to AD risk. In addition, brain region and AD specific microRNAs identified in this study warrant further investigation into their specificity as biomarkers of neurodegenerative disease.

2709/F

Influence of Genomic Methylation on Brain Structure and Function. *M.A. Carless[1], D.C. Glahn[2,3], T.D. Dyer[1], R.L. Olvera[4], J.E. Curran[1], R. Dugirala[1], J. Blangero[1].* 1) Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX; 2) Department of Psychiatry, Yale University School of Medicine, New Haven, CT; 3) Olin Psychiatric Research Center, Institute of Living, Hartford, CT; 4) Department of Psychiatry, University of Texas Health Science Center San Antonio, San Antonio, TX.

Epigenetic regulation is rapidly becoming a well-recognized regulatory mechanism contributing to the development of complex diseases. Given that many psychiatric disorders are poorly understood and currently known genetic factors only account for a small proportion of the phenotype, it is likely that epigenetic regulation plays an important role in the development of mental illness and this may be made apparent through testing of brain-related phenotypes. Using Illumina GoldenGate technology, we interrogated genomic methylation at 1,505 CpG sites, representing 807 genes, in lymphocytes derived from 95 Mexican Americans within the San Antonio Family Study (SAFS), for which we have extensive neurocognitive and neuroanatomical data. We have previously shown that approximately 25% of CpG sites exhibit relative methylation variation that is significantly heritable (median heritability = 0.11), reinforcing the biological validity and relevance of such epigenetic changes. We used SOLAR to identify correlations between neurocognitive and neuroanatomical traits and genomic methylation at CpG sites within highly relevant genes. One of the strongest associations detected was for methylation within *PDGFB*, a growth factor that stimulates fetal neural precursor cells, and face memory delay test (6.00×10^{-6}). Methylation within other genes demonstrated association with multiple phenotypes, such as the *SOX1* gene, which is involved in inhibition of neural differentiation and *FGFR1*, which gives rise to the auditory epithelium. Methylation in *SOX1* was associated with cortical thickness of the inferior parietal ($p=6.18 \times 10^{-5}$), precentral ($p=7.87 \times 10^{-4}$) and superior parietal ($p=9.59 \times 10^{-4}$) regions of the brain. Methylation within *FGFR1* was associated verbal intelligence estimates based on the reading of irregular words (Wechsler Test of Adult Reading Full-Scale IQ, $p=8.51 \times 10^{-5}$) and a measure of vocabulary from the Wechsler Abbreviated Scale of Intelligence ($p=8.42 \times 10^{-4}$). Further, methylation within *EGF*, previously implicated in schizophrenia, showed association with ventral diencephalon volume ($p=5.11 \times 10^{-5}$). These results suggest that genomic methylation may play an important role in a number of neuroanatomical and neurocognitive endophenotypes and may also have a significant impact on psychiatric disease.

2710/F

Nicotine Dependence and Five-Factor Model of Personality: Examination of Specific Genetic Variants in Nicotinic Receptor Genes. *L. Chen[1], H. Xian[2], R.A. Grucza[1], N.L. Saccone[3], E.O. Johnson[4], N. Breslau[5], D. Hatsukami[6], L.J. Bierut[1].* 1) Department of Psychiatry, Washington University School of Medicine, St. Louis, MO; 2) Department of Medicine, Washington University School of Medicine, St. Louis, MO; 3) Department of Genetics, Washington University School of Medicine, St. Louis, MO; 4) Research Triangle Institute International, Reseasrch Triangle Park, North Carolina, NC; 5) Department of Epidemiology, Michigan State University, East Lansing, MI; 6) Department of Psychiatry, University of Minnesota, Minneapolis, MN.

Introduction: The associations between nicotine dependence and specific variants in the nicotinic receptor CHRNA5-A3-B4 subunit and CHRNA6-B3 subunit genes are irrefutable with replications in many studies. The relationship between the newly identified genetic risk variants for nicotine dependence and five-factor model of personality is unclear. We examined whether personality characteristics modified the genetic risk of nicotine dependence and whether these genetic variants were associated with personality characteristics. Methods: In a case control study of nicotine dependence with 2047 subjects of European descent, we used a systematic series of logistic and linear regression models to examine the risk moderation and pleiotropy. Nicotine dependence was assessed using Fagerstrom Test for Nicotine Dependence (FTND) and personality characteristics were assessed using neuroticism extroversion openness-five factor inventory (NEO-FFI). Results: When compared to non-dependent smokers, individuals with nicotine dependence were associated with high neuroticism (N), low extroversion (E), low openness (O), high agreeableness (A), and low conscientiousness (C), adjusting for age and gender. No evidence suggested that the risks for nicotine dependence associated with the genetic variants vary with personality characteristics in general, but the power was limited in detecting interactions. No evidence supported the associations between the genetic variants and these personality characteristics (pleiotropy). Conclusions: The risks for nicotine dependence associated with these genetic variants are not modified by personality characteristics measured by the five-factor model. The genetic risks of nicotine dependence associated with the CHRNA5-A3-B4 subunit and CHRNA6-B3 subunit genes were specific, and not shared with any specific personality characteristics. However, the power is an important limitation in studying the interplay of personality and genetic variants, and a meta-analytic approach would be needed to reach the appropriate sample size.

2711/F

Regulation of human tyrosine hydroxylase by the male-specific factor SRY in the brain. *D. Czech[1, 2], H. Sim[1], F. Bagheri-Fam[1], P. Bernard[1], J. Lee[1], V. Harley[1].* 1) Human Genetics & Development Divison, Prince Henry's Institute of Medical Research, Clayton, Melbourne, Victoria, Australia; 2) Department of Biochemistry and Molecular Biology, Faculty of Medicine, Monash Univeristy, Clayton, Melbourne, Victoria, Australia.

SRY is the Y-chromosome switch gene which directs gonads to differentiate into testes rather than ovaries. SRY is a well characterised architectural transcription factor, binding and bending DNA though its high mobility group domain and activating downstream male-pathway targets. Recent studies in our laborartory using immunhistochemical staining localized SRY in catecholaminergic brain regions in rodents such as the ventral tegmental area (VTA), substantia nigra compacta (SNc) and locus coeruleus (LC). In the SNc, SRY localizes in tyrosine hydroxylase (TH) positive neurons. TH is the key rate-limiting enzyme controlling dopamine production in the striatum. Surprisingly, transient inhibition of SRY production in the SNc in rats, by injection of SRY antisense oligonucleotides, has been shown to reduce TH expression and consequent motor deficits reminiscent of Parkinson's disease. Others have now shown that SRY directly targets monamine oxidase A (MAOA), a key enzyme involved in breaking down dopamine in the SNc. Taken together, the evidence suggests that SRY has a significant role outside of testis development. We hypothesise that in the male brain, SRY is a transcriptional activator of the TH gene. The present study seeks to elucidate the role of SRY using human dopaminergic cell line models and human brain sections. A human SRY antibody was purified and tested. Dot blot, western blot, immunocytochemistry and peptide competition assays concluded that the peptide-affinity, column-purified antibody was specific to both transfected and endogenous SRY protein in multiple cell lines. Immunohistochemistry of post-mortem, male brain sections confirmed that SRY is also co-localised with TH positive cells in the human SNc. To investigate whether SRY affects TH transcription, a human pluripotent cell line NTera2, was differentiated into dopamine neurons with retinoic acid. Over 28 days, both SRY and TH mRNA and protein levels increased, as demonstrated by qRT-PCR and immunocytochemistry. A positive linear trend is observed between increasing SRY and TH protein levels. Overexpression of the SRY gene in transfected, FAC-sorted NT2N neurons resulted in a significant 2-fold upregulation of TH and MAOA mRNA levels. Similarly, overexpression of SRY in a human dopaminergic, neuroblastoma cell line, BE(2)-M17, showed the same level of TH and MAOA upregulation. Current studies are aimed at mapping putative SRY-responsive regions in the 11kb human TH promoter.

2712/F

Molecular networks for lithium and valproate regulated genes highlight a marked enrichment of apoptosis functional clusters. *S. Detera-Wadleigh[1], T. Schulze[1], A. Gupta[1], N. Akula[1], V. Nagarajan[2], X. Jiang[1], W. Corona[1], N. Hunter[1], F. McMahon[1].* 1) NIMH/NIH, Bethesda, MD; 2) NIAID/NIH, Bethesda, MD.

The biological mechanisms by which lithium and valproate (VPA) stabilize mood in bipolar disorder are not fully understood. The role of inositol depletion and Wnt signaling in lithium action, and the inhibitory effect of VPA on histone deacetylases are well-documented. Published literature seems to suggest that lithium and VPA perturb disparate biological processes. Alternatively, the similar therapeutic effects of these drugs could indicate shared pathways. To explore this question, we identified genes that were differentially regulated by both lithium and VPA in gene expression studies. Twelve genes met the criteria for inclusion in subsequent analyses: a) significant differential expression of at least 1.4 fold, and b) identical direction of change in expression with both lithium and VPA in at least two studies. To explore the functional relationships of these genes, molecular networks were generated by two network-building tools which created complex interaction networks comprising multiple hubs. To determine the most-supported interactions we identified nodes that were identically recruited in both networks, upon which functional annotation was performed by using DAVID Bioinformatics Wizard. Apoptosis clusters were shown to be the most highly enriched functional clusters in the networks. This analysis further displayed significant enrichment of >20 canonical signaling pathways including neurotrophin, MAPK and ErbB. The predominant pathway constituent nodes were Akt1 and MAPK, suggesting a vital role in both lithium and VPA action. These findings also suggest a functional convergence of lithium and VPA involving the regulation of apoptosis which may feature prominently in the therapeutic efficacy of both medications.

2713/F

Clinical non-motor manifestations in Parkinson disease patients carrying GBA mutations. *G. Lopez[1], J. Choi[1], B. McElroy[3], C. Crews[2], M. Brooks[3], N. Gupta[1], A. Velayati[1], A. Britton[2], M. Hallett[3], E. Sidransky[1].* 1) NHGRI/NIH, Bethesda, MD; 2) NIA/NIH, Bethesda, MD; 3) NINDS/NIH, Bethesda, MD.

The association between parkinsonism and mutations in the glucocerebrosidase gene (GBA) is now well established. Patients with parkinsonism have a five-fold increased likelihood of carrying a mutation in GBA . Non-motor symptoms described in Parkinson disease include depression, sleep disorders, fatigue, olfactory problems, and cognitive difficulties. Non-motor symptoms are considered non-dopaminergic and therefore, refractory to dopaminergic supplementation. Non-motor manifestations often precede motor symptoms, and tend to intensify with disease progression, dominating the clinical picture in late-stages of the disease. Systematic evaluation of non-motor manifestations in subjects with Gaucher disease and GBA mutation carriers has not been extensively studied. We performed a uniform cross-sectional clinical study of 155 consecutive patients evaluated at the Parkinson disease clinic at the National Institutes of Health by a single neurologist. All 11 exons of GBA were sequenced and subjects were screened for the three most common LRRK2 mutations (G2019S, R1441H/C, and Y1699C). Eight patients were found to be heterozygous for a GBA mutation. Clinical evaluations included a detailed history and physical examination, neurological and UPDRS evaluation, detailed review of systems interview, Geriatric Depression Scale, Fatigue Scale, Epworth Sleep Scale, and smell evaluation using the University of Pennsylvania Smell Identification Test. Our study showed that patients with Parkinson disease who carried a GBA mutation were more likely to complain of memory problems when compared to Parkinson disease patients without mutations, although objective measurements with clinical cognitive screening tools did not demonstrate a cognitive deficit. While the small sample size limits generalization of this finding, more formal neurocognitive assessments are warranted. Both patients with Gaucher disease and GBA carriers are being prospectively followed at the National Human Genome Research Institute in order to study motor and non-motor symptoms in patients at increased risk of parkinsonism.

2714/F

Copy Number Variations in Autism Spectrum Disorders. *F. Mari[1], M.A. Mencarelli[1], M. Mucciolo[1], V. Disciglio[1], R. Canitano[2], F.T. Papa[1], A. Marozza[1], L. Radice[2], C. Castagnini[1], L. Dosa[1], M. Pollazzon[1], J. Hayek[2], A. Renieri[1].* 1) Med Gen - Mol Biol, Univ Siena, Siena, Italy; 2) Child Neuropsychiatry, University Hospital Siena, Siena, Italy.

Autism and related traits present a complex and heterogeneous aetiology with a strong evidence of a genetic involvement. The identification of copy-number variants (CNVs) by the application of comparative genomic hybridization (CGH) is beginning to provide some insights into the underlying genetic causes of neurodevelopmental disorders. Recently, recurrent microdeletions at 16p11.2, 16p13.1 and microduplications at 15q13.3 have been associated with susceptibility to autism spectrum disorder (ASD) in up to 1% of patients. Using oligonucleotide arrays with 44,000 probes with an average resolution of approximately 100 Kb (44K, Agilent), we have analyzed 95 unrelated subjects with ASD. Inheritance, whenever possible, was determined using the same technique. Patients were thoroughly investigated by both child neuropsychiatrists and clinical geneticists. Sixty patients were negative for rearrangement while 35 patients exhibited at least one private rearrangement. In these 35 patients, a total of 49 rearrangements were identified, 22 were deletions and 27 were duplications. Published data and gene content analysis indicated that 14 CNVs were in strong disease candidate regions while the remaining were assigned an unknown significance (detection rate: 15%). Among them, two were de novo CNVs (del16p11.2; delXq12) and 5 were CNVs in known susceptibility regions (dup16p13.11, dup15q13.3, dup17q12, del11p12, del15q11.2). Three patients presented two inherited rearrangements each, one inherited by the mother and the other inherited by the father, leading to hypothesize the possibility of a digenic or multigenic inheritance. In conclusion, our study has confirmed the array-CGH as an important diagnostic tool in ASD patients and it has provided additional data on genetic causes of ASD. Our most important finding from the clinical investigation was that CNVs are more common among the subgroup of patients with multiple congenital anomalies (40% versus 22%) and surprisingly, epilepsy was absent in the cohort of patients with pathogenic CNVs. Furthermore, these findings underscore a challenge in genetic counselling related to risk assessment of each CNV. Given the rapidly expanding knowledge on human CNVs we suggest, in clinical reports, to describe the private rearrangements identified and to include a statement indicating that the identified rearrangements cannot currently be excluded as possible cofactors for the phenotype.

2715/F

Early serotonin depletion and decreased dopamine release are associated with a chronic brain energy deficit in Huntington disease mice. *F. Mochel[1,2,3], B. Durant[3], R. Schiffmann[3], A. Durr[1,2].* 1) INSERM UMRS975, Hosp La Salpetriere, Paris, France; 2) Department of Genetics, Hosp La Salpetriere, Paris, France; 3) Institute of Metabolic Disease, Baylor Research Institute, Dallas, TX, USA.

Background: Dopaminergic and serotoninergic alterations may account for motor and non-motor manifestations of Huntington disease (HD). Cerebral energy deficit also plays an important role in HD. Objectives: To reliably characterize the locoregional and temporal brain neurotransmitter and energy profile in a HD mouse model, we used a microwave fixation system that instantly inactivates brain enzymes and preserves in vivo concentrations of adenine nucleotides. Methods: We used an automated high-resolution behavioral analysis in R6/2 mice at ages 4 weeks (presymptomatic), 8 and 12 weeks (symptomatic). Mice were then sacrificed by microwave fixation. Dopamine (DA), serotonin (5-HT) and their metabolites (3-MT, HVA and 5-HIAA), as well as high-energy phosphates (ATP, ADP, AMP and phosphocreatine) and creatine were measured by HPLC in striatum, hippocampus and frontal cortex. Results: Compared to WT mice, we found significantly decreased DA in the striatum of R6/2 mice at 8 and 12 weeks while DA release was already significantly reduced in 4 weeks old R6/2 mice. 5-HT and 5-HIAA were significantly decreased in the striatum, hippocampus and frontal cortex of R6/2 mice at 4, 8 and 12 weeks, associated with a decreased turnover of 5-HT. Increased stress-like behaviors - jumping and grooming - and altered spatial learning in R6/2 mice were identified at 4 and 6 weeks respectively. In addition, we found a significantly decrease of ATP in the striatum, hippocampus and frontal cortex of 8 and 12 weeks old R6/2 mice associated with significantly increased phosphocreatine and creatine. There was no difference in ATP levels at 4 weeks of age between R6/2 and WT littermates, yet phosphocreatine and creatine were already significantly elevated in the striatum and frontal cortex of R6/2 mice. Instead, after an intraperitoneal injection of 3-nitroproprionic acid in WT mice - an acute model of striatal energy deficit - we found a significant decrease of most high-energy phosphate metabolites (ADP, ATP and phosphocreatine). Conclusion: We identified the earliest alteration of DA and 5-HT metabolism in R6/2 mice associated with stress-like behaviors. These neurotransmitter abnormalities were coupled with compensatory processes - i.e. increased phosphocreatine and creatine - for the chronic brain energy deficit of HD and constitute targets for therapy. Our system can be used to characterize the brain neurotransmitter and energy profile of many neurogenetic disease models.

2716/F

Evaluating endophenotypes for schizophrenia with the Endophenotype Ranking Value (ERV) statistic. *M. Zlojutro[1], R.C. Gur[2], M. Pogue-Geile[3], E. Hare[4], M.E. Calkins[2], K. Prasad[5], D. Glahn[6], J. Blangero[7], V. Nimgaonkar[7], R.E. Gur[2], L. Almasy[1].* 1) Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX; 2) Department of Psychiatry, University of Pennsylvania School of Medicine, Philadelphia, PA; 3) Department of Psychology, University of Pittsburgh, Pittsburgh, PA; 4) Texas Tech Health Science Center, Paul L. Foster School of Medicine, El Paso, TX; 5) Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA; 6) Department of Psychiatry, Yale University, Hartford, CT; 7) Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA.

Deficits have been observed for various cognitive measures in individuals with schizophrenia and their clinically unaffected family members, indicating that the deficits contribute at varying degrees to the genetic liability of schizophrenia and thus represent intermediate phenotypes or endophenotypes that lie on the causal pathway from genotype to disease outcome. Although the genetic analysis of cognitive measures is likely to be a powerful approach for identifying susceptibility loci in schizophrenia, each candidate endophenotype has its own complex genetic architecture and determining which are the most informative to the genetic underpinnings of schizophrenia has involved several criteria, with selection based mostly on their heritability and association with the disease. Here we introduce a new, quantitative measure for evaluating the suitability of traits as endophenotypes based on their shared genetic component with a disease of interest: Endophenotype Ranking Values (ERVs). The ERV represents a straightforward product of three estimates from bivariate genetic analyses of family data: square root of the heritability of the disease of interest (hd); square root of the heritability of the candidate endophenotype (ht); and the genetic correlation between the disease and endophenotype (ρg). The heritabilities represent the strength of genetic effects on the two traits and ρg the extent of pleiotropy or overlap in genetic influences. The ERV can be used to identify endophenotypes, as well as rank them to reduce multiple testing by using only the most promising traits in analyses. For a sample of 816 individuals from 43 families, with 79 participants diagnosed with schizophrenia or schizoaffective disorder-depressed type, the ERV was estimated for a battery of computerized tests designed to assess the following cognitive domains: abstraction and mental flexibility; attention; language; spatial processing; face memory; spatial memory; verbal memory; and emotion processing. The top scoring cognitive endophenotypes are abstraction and mental flexibility (0.317; p=0.002), language (0.284; p=0.03), attention (0.271; p=0.008), and emotion processing (0.269; p=0.003). These ERV estimates identify cognitive domains with genetic components that are significantly shared with the genetic liability of schizophrenia and thus represent appropriate endophenotypes for investigation into the genetic basis of this brain disorder.

2717/F

Psychiatric Genetics Goes to the Dogs: A Canine Model of Compulsive Behaviour. *P.D. Arnold[1], K. Tiira[2], C. Escriou[3], A. Thomas[3], S. Renier[3], C. De Citres[3], L. Koskinen[2], B. Minassian[1], H. Lohi[2].* 1) Program in Genetics and Genomic Biology, Hospital for Sick Children, Toronto, ON, Canada; 2) Canine Genomics Research Group, Department of Veterinary Biosciences, Department of Medical Genetics, Program in Molecular Medicine, The Folkhälsan of Institute of Genetics, University of Helsinki; 3) Veterinary School of Lyon, France.

Purebred dogs provide excellent genetic models for the study of human behaviours, since the history of inbreeding means that far fewer dogs are needed to map a given trait compared with humans. In this study, we set out to systematically characterize clinical and genetic features of tail chasing (TC) in purebred bull terriers, which has been suggested to be a model for human obsessive-compulsive disorder (OCD). A web-based questionnaire was administered to owners to elicit clinical and environmental features, with direct examination by a veterinarian occurred in a subset of cases. TC cases and normal controls were compared using a 200K Illumina genome-wide array. Genetic analyses were performed using PLINK. To date we have collected 154 Standard Bull Terriers (92 cases) and 57 Miniature Bull Terriers (22 cases). In more severe cases additional behaviours were commonly observed including aggression, “freezing” and staring episodes. Early onset was associated with increased frequency of TC, whereas improved diet (use of supplements) was associated with decreased severity. Our preliminary genome-wide association study (GWAS) of 24 TC dogs and 24 controls did not result in any genome-wide significant findings. In conclusion, TC is a behavioural syndrome commonly seen in bull terriers with distinct environmental and clinical correlates. Larger samples will be needed to identify genetic risk factors using GWAS. Genetic findings in TC may provide important clues to the genetic basis of human disorders characterized by complex repetitive behaviours, such as OCD and autism.

2718/F

Typical schizophrenia MRI brain volumetric reductions in 22q.11DS adults. *A.S. Bassett[1, 2], A. Ho[2], A. Crawley[3], C. Wei[3], D.J. Mikulis[3], E. Chow[1,2].* 1) Dept of Psychiatry, University of Toronto, Toronto, ON, Canada; 2) Clinical Genetics Research Program, Centre for Addiction and Mental Health, Toronto, ON, Canada; 3) Dept of Medical Imaging, Toronto Western Hospital, University of Toronto, Toronto, ON, Canada.

Background: Individuals with 22q11.2 deletion syndrome (22q11DS) are at high risk of developing schizophrenia. Imaging studies are inconclusive as to whether specific regional brain volume reductions are associated with expression of schizophrenia in 22q11DS. Methods: Using the largest sample of adults with 22q11DS studied to date (n=63), magnetic resonance brain images from 29 subjects with schizophrenia and 34 with no history of psychosis (NP) were analyzed using a voxel-based morphometry (VBM) method that also yielded volumes for region of interest (ROI) analyses. We compared gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) measures between the groups using an analysis of covariance model with total intracranial volume, age, sex, IQ and history of congenital cardiac defects as covariates, and a false discovery rate threshold of $p<0.05$ for VBM results. Results: VBM analyses identified significant GM reductions in the left superior temporal gyrus in the 22q11DS-schizophrenia group ($p=0.029$). There were no significant between-group differences in WM or CSF. ROI analyses of temporal lobe GM showed significant volume reductions in bilateral temporal lobes and superior temporal gyri in the 22q11DS-schizophrenia group. Conclusions: These structural brain findings in a 22q11DS form of schizophrenia are consistent with those from previous studies involving schizophrenia in the general population and provide further support for 22q11DS as a genetic subtype and useful neurodevelopmental model for schizophrenia.

2719/F

Partial *SPAST* and *DPY30* deletions in autosomal dominant hereditary spastic paraplegia. *S. Miura[1, 2], H. Shibata[2], H. Kida[1], K. Noda[1], A. Iwaki[2], M. Ayabe[1], H. Aizawa[1], T. Taniwaki[1], Y. Fukumaki[2].* 1) Department of Respirology, Neurology and Rheumatology, Department of Medicine, Kurume University School of Medicine, Kurume City, Fukuoka Prefecture, Japan; 2) Division of Molecular Population Genetics, Department of Molecular Genetics, Medical Institute of Bioregulation, Kyushu University.

Hereditary spastic paraplegia (HSP) is a genetically heterogenous neurodegenerative disorder characterized by progressive weakness and spasticity in the lower extremities. Spastic paraplegia type 4 (SPG4) is the most common HSP caused by mutations in *SPAST*. We studied a four-generation Japanese pedigree with autosomal dominant HSP both clinically and genetically. Eleven available family members (10 affected; 1 non-affected) and three spouses participated in this study after providing informed consent. Patients in most cases showed hyperreflexia both upper and lower extremities, spasticity of the lower limbs. Four (40%) had urinary dysfunctions and eight (80%) showed a decrease in vibration sense in the lower limbs. In seven patients (70%), gait disturbance was noticed before age 2. All four female patients experienced multiple miscarriages. Nerve conduction studies were performed in three patients. Sural nerve action potentials were below the detectable level in two. In the Wechsler Adult Intelligence Scale-Third Edition (WAIS-III), impaired cognitive function was observed in all three studied patients (Full Scale IQ were 65, 75 and 77). Tau levels in cerebrospinal fluid were mildly increased in two of the three patients. Linkage analysis of chromosome 2 using 26 fluorescent-labeled microsatellite markers spaced at intervals of approximately 13 Mb with the GENEHUNTER program revealed the highest multipoint logarithm of odds (LOD) score of 2.643 at 2p23-p21 where *SPAST* is located. Although we extensively scanned the entire exonic sequences of SPAST by direct sequencing, there is no mutation detected. Real-time quantitative polymerase chain reaction of all exons in *SPAST* using SYBR Green I dye demonstrated a heterozygous deletion of exons 1 to 4 of *SPAST*. Breakpoint analysis revealed a large deletion with the size of 63,851 bp of which the telomeric breakpoint is located in intron 3 of *DPY30* resulting in a deletion of exons 1 to 3 of *DPY30*. To our knowledge, this is the first report on SPG4 associated with partial deletions of both *SPAST* and *DPY30*. Since *DPY30* is known to be an essential component of the dosage compensation machinery, the partial heterozygous deletion of *DPY30* might modify phenotypic expression of SPG4 in the patients of this pedigree. This study was approved by the Ethics Committees of Kurume University School of Medicine and Kyushu University, Faculty of Medicine.

2720/F

The effects of parental age on the risk for neuropsychiatric disorders. *J.E. Buizer-Voskamp[1,2], W. Laan[3], W.G. Staal[4], E.A.M. Hennekam[5], S.A.J. de With[4], M.F. Aukes[1,3], R.S. Kahn[1], M.P.M. Boks[1,3], R.A. Ophoff[2,6].* 1) Rudolf Magnus Institute of Neuroscience, Department of Psychiatry, University Medical Center Utrecht, Utrecht, The Netherlands; 2) Complex Genetics Section, Department of Medical Genetics, University Medical Center Utrecht, Utrecht, The Netherlands; 3) Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, The Netherlands; 4) Department of Child and Adolescent Psychiatry, University Medical Center Utrecht, Utrecht, The Netherlands; 5) Department of Clinical Genetics, University Medical Center Utrecht, Utrecht, The Netherlands; 6) UCLA Center for Neurobehavioral Genetics, University of California, Los Angeles, California, United States.

High parental age may be a risk factor for developing psychiatric disorders. In this study parental age and the risk for developing schizophrenia, autism spectrum disorder (ASD), depressive disorders, and bipolar disorder were measured in the Dutch population. Additionally, effects of parental age on type of DSM-IV(TR) classification (Autism, Asperger Syndrome, Pervasive Developmental Disorder, not otherwise specified (PDD-NOS)) were tested in two ASD samples. Patients were collected through a patient registry. Date of birth of their parents was retrieved from the database of Statistics Netherlands (CBS) together with a fourfold number of matched controls. The ASD patient samples were recruited from two Dutch University hospitals with high expertise in ASD. In total, data on 2,627 schizophrenic, 2,329 autistic, 8,634 depression, and 1,133 bipolar disorder patients was available from the population-based registry. When analyzed as a continuous measure, the paternal age of autistic individuals was significantly higher compared to controls ($p=0.001$). When analyzed as a categorical measure, the age effect becomes even more pronounced: fathers <20 years of age have the lowest risk of having a child with autism (OR=0.37, 95%CI: 0.11-1.19), fathers >40 years of age have a significantly higher risk (OR=1.23, 95%CI: 1.01-1.50). For the clinical samples, data on 461 autistic individuals was analyzed. A greater difference in age between the parents increased the likelihood of a diagnosis of autism instead of Asperger syndrome or PDD-NOS ($p=0.038$). For schizophrenia and depressive disorders, only the categorical analysis showed significant paternal age effects. For schizophrenia, fathers aged >35 years appear to have significantly higher risk (OR=1.27, 95%CI: 1.05-1.53). For depressive disorders, there is a U-shaped effect. For bipolar disorder no parental age effects were found. This study shows that higher paternal age is associated with higher risk for autism. Additionally, greater difference in age between parents increases the odds of a diagnosis of core autism and not PDD-NOS or Asperger syndrome. Secondly, parental age effects were found in schizophrenia and depressive disorders, but not in bipolar disorder. As far as we are aware, this is the first large-scale population-based study examining the effects of parental age in the four major psychiatric disorders simultaneously.

2721/F

Genomewide linkage scans for loci predisposing to dependence on multiple substances. *BZ. Yang[1,4], S. Han[1], HR. Kranzler[5], L. Farrer[6], J. Gelernter[1,2,3,4].* 1) Psychiatry, Yale Univ Sch Med, New Haven, CT; 2) Genetics, Yale Univ Sch Med, New Haven, CT; 3) Neurobiology, Yale Univ Sch Med, New Haven, CT; 4) VA CT Healthcare Center, West Haven, CT; 5) Univ CT Health Center, Farmington, CT; 6) Boston Univ School Med, Boston, MA.

Dependence on multiple substances (MSD), including alcohol and a variety of drugs of abuse is common. MSD patients are often severely affected clinically. Genetic risk factors may play an important role in the development of MSD. With increasing evidence of a role for rare variants and copy number variation (CNV) in complex diseases, genetic linkage studies are still recognized as useful tools for gene discovery. An adequately powered linkage study design has the advantage of detecting diverse genetic effects that segregate in families, including common variants, multiple rare variants within one locus, and CNV. In this study, we hypothesized that different substance dependence (SD) traits share genetic liability. Linkage analysis of MSD could increase power to detect shared risk loci due to severe affection compared to analysis of an individual SD disorder. We genotyped 6,008 single nucleotide polymorphism makers for 952 individuals from 384 African American (AA) families and 806 individuals from 355 European American (EA) families ascertained on the basis of affected sib-pairs with cocaine and/or opioid dependence. We conducted a fuzzy clustering analysis on the SD traits including alcohol, cannabis, cocaine, opioid, and nicotine dependence for AAs and EAs separately, and performed genomewide linkage scans for the resultant membership coefficients from the fuzzy clustering analysis for each individual using Merlin-regression. In AAs, two linkage peaks with suggestive linkage were observed on chromosome 10 with a peak LOD = 2.66 at 96.7 cM and a peak LOD = 3.02 at 147.6 cM; these surround our previous linkage peak near 117.2 cM for alcohol dependence in AA. In addition, two other suggestive linkage regions were identified on chromosome 3 with a peak LOD = 2.81 at 145.5 cM and on chromosome 9 with a peak LOD = 1.93 at 146.8 cM. In EAs, we detected a genome-wide significant linkage signal on chromosome 4 with a peak LOD = 3.31 at 68.3 cM (point-wise p = 0.00005, empirical genome-wide p = 0.038). A suggestive linkage signal on chromosome 21 was also observed with a peak LOD = 2.37 at 19.4 cM. This evidence of genomewide linkage for MSD in the two major US population groups based on a dense SNP map can provide useful information in the search for genes that influence this phenotype.

2722/F

Targeted Next Generation Sequencing for Rare Variant Detection in Patients with Early Onset Parkinson Disease. *G. Bademci[1], Y. Edwards[2], A. Mehta[2], S. Zuchner[2], A. Torres[2], W.K. Scott[1], D. Hedges[1], J.M. Vance[1].* 1) Department of Human Genetics, University of Miami Miller School of Medicine, Miami, FL; 2) Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, Fl.

Parkinson disease (PD) is a complex, progressive neurodegenerative disorder. Rare variants (RV) are variants with a population frequency usually less than 2% in the human genome. They have been postulated to be a potential source of genetic variation in which some RV contribute to increasing an individual's risk to complex diseases like PD. For the first time Next-generation sequencing (NGS) allows the search for RV to be performed in complex diseases. We recently reported a joint PD GWAS analysis (Edwards et al. 2010), and have also completed a pathway-based meta-analysis of PD GWAS and gene expression. The pathway analysis identified calcium signaling and focal adhesion as two of the top over-represented pathways. As part of an initial pilot project comparing 3 different capture technologies, we evaluated 13 early-onset patients (£30 years) diagnosed with PD for RV in known PD genes, genes within identified pathways and genes with strong PD-risk association. The following genes were targeted for capture: Calcium signaling pathway (DRD1, NOS1A, NOS2A, CALM1, CD38, RYR1, CACNA1E), Focal adhesion (CAV2), Tyrosine metabolism (TH, MAOB, COMT) and MAPK signaling (MAPT). We also captured the following known PD genes and risk-associated genes: PARKIN, SNCA, NPAS3, GFPT2, ATP13A2, DBC1, USP24, RIT2, DGKQ and ELAVL4. NGS was performed using the ABI SOLiD 3 and NGS reads were mapped to the human hg18 reference genome. Variants aligned and called using MAQ version 0.7.1. Novel variants were defined as those not found in currently available dbSNP, Human Genome Mutation Database (HGMD) as well as a literature search. SeattleSeq annotation and Polyphen prediction were used for in silico functional analyses. Two patients were discovered with a novel homozygous variation (R366Q) and a previously reported mutation (G430D) in Parkin. Further, novel variants predicted to negatively impact protein function included missense changes in MAPT (G213R), RIT2 (R182H), and ATP13A2 (A1144T), and calcium signaling pathway genes CD38 (L18R) and CACNA1E (R920C). All variants were found in all 3 captures and were confirmed with Sanger sequencing. The number of RV in this small sampling of early-onset patients support the potential of RV contributing to PD and the utility of using NGS to find RV in a large dataset. Furthermore, we provide evidence to support the utility of pathway analysis to prioritize genes for the identification of RV in complex disease.

2723/F

Quantitative genetics of language measures in families selected for both autism and specific language impairment. *L. Hou[1], J.F. Flax[2], S. Buyske[2,3], A. Hare[2], Z. Fermano[2], C.W. Bartlett[1], L.M. Brzustowicz[2].* 1) The Battelle Center for Mathematical Medicine, The Research Institute at Nationwide Children's Hospital and Department of Pediatrics, The Ohio State University, Columbus, OH; 2) Department of Genetics, Rutgers University, New Brunswick, NJ; 3) Department of Statistics, Rutgers University, New Brunswick, NJ.

The purpose of this study is to estimate heritability of language and reading abilities in nuclear and extended pedigrees ascertained for autism and specific language impairment (SLI). By estimating the heritability of oral and written language measures in these pedigrees, we explored the shared genetics of the two disorders (autism and SLI) under the hypothesis of such an effect. Heritability was calculated twice; once on the entire sample and once removing the individuals with autism who had language and were able to accept the language and reading measures. We found that removing persons with autism increased the size of the standard errors (often considerably) while heritability estimates were less correlated with the original estimates compared to conditioning on ASD status as a covariate (R^2 from 0.96 to 0.32); both lines of evidence are fully consistent with a reduction in sample size as the cause of the additional variability. Conditioning on ASD status as a covariate, 5 individual measures did show significant changes by likelihood ratio testing versus a model that did not include ASD as a covariate (P<.05). Four of these measures involved higher order or metalingusitc tasks from the Comprehensive Assessment of Spoken Language, showing significant changes in heritability when ASD status was explicitly modeled (from 37%, 58%, 38% and 38% to 46%, 70%, 45% and 43%, respectively). Additionally, global language ability (Clinical Evaluation of Language Fundamentals) significantly increased from 42% to 53%. Increasing heritability when statistically controlling for scores from at least one person per pedigree indicates heritability of these tasks and is not consistent with a simple genetic model of correlation between relatives. These results might suggest 1) these tasks have a genetic threshold that is not being appropriately modeled through standard techniques, 2) evidence of unmodeled environmental interactions or, 3) measurement error. The last interpretation is unlikely since higher order language tasks tap into manipulation, generalization, and abstraction of language; skills that are compromised to non-existent in individuals with language disorders depending on the degree of involvement. Furthermore, persons with autism did not display floor effects or show greater variance than when the sample excluded the individuals with autism.

2724/F

A genomewide ordered-subset linkage analysis for alcohol dependence in African-Americans. *S. Han[1], B.Z. Yang[1,4], H.R. Kranzler[5], J. Gelernter[1,2,3,4].* 1) Dept Psychiatry, Yale Univ, New Haven, CT; 2) Dept Genetics, Yale Univ, New Haven, CT; 3) Dept Neurobiology, Yale Univ, New Haven, CT; 4) VA CT Healthcare Center, West Haven, CT; 5) Univ CT Health Center, Farmington, CT.

Alcohol Dependence (AD) is extremely costly to individuals and to society throughout the world. The etiology of AD is complex; genetic factors are known to be important to its development. With increasing evidence of a role for rare variants and copy number variation (CNV) in complex diseases, linkage analysis remains a useful approach to gene discovery. An adequately powered linkage study can detect diverse genetic effects that segregate in families, including common variants, multiple rare variants within one locus and CNV. Previously, we performed a genomewide linkage scan for AD in African-Americans (AA) (Gelernter 2009). The power of that linkage analysis could theoretically have been reduced by the presence of genetic heterogeneity due to the variation of admixture proportions across families in AA. In the current study, we hypothesized that a sample whose genetic ancestry was more homogeneous would increase power to detect linkage. To test this hypothesis, we first completed a genomewide linkage scan with 6008 single nucleotide polymorphism markers to map loci linked with DSM-IV AD in 384 AA families ascertained on the basis of multiple individuals affected with cocaine and/or opioid dependence. Using the admixture proportion as a covariate, we then applied the ordered subset linkage analysis (OSA) technique to identify a more homogeneous subset of families to determine whether it increased evidence for linkage with AD. Statistically significant increases in Kong-Cox LOD scores were observed on chromosomes 4 (P= 0.0001), 12 (P=0.021), 15 (P=0.026) and 22 (P=0.0069). In a subset of 44 AA families (with African ancestry proportions ranging from 0.858 to0.996), we observed a genomewide significant linkage at 180 cM on chromosome 4 (Kong-Cox LOD=4.1, pointwise P=0.00001, empirical genomewide P= 0.008). *GLRA3*, the gene encoding a subunit of the glycine neurotransmitter receptor, is located in this peak region; thus, it deserves further research attention. A parametric linkage analysis confirmed the significant findings from the non-parametric linkage analysis. Our results show new evidence for linkage and demonstrate that the admixture proportion can be used effectively as a covariate to reduce genetic heterogeneity and enhance the detection of linkage signals in AA using the OSA technique. The current study provides important clues for candidate gene and deep sequencing studies to identity causal variants for AD.

2725/F

Genome-wide linkage analysis of flexibility/insistence-on-sameness in multiplex families with Autism spectrum disorders. *N. Chapman[1], A. Estes[1], J. Munson[1], R. Bernier[1], S.J. Webb[1], J. Rothstein[1], G. Schellenberg[2], G. Dawson[1,3], E. Wijsman[1].* 1) University of Washington, Seattle, WA; 2) University of Pennsylvania School of Medicine, Philadelphia PA; 3) Autism Speaks.

Autism spectrum disorders (ASDs)involve qualitative deficits in social interaction and communication, and restricted interests or repetitive behavior. Genetic factors play an important role in autism susceptibility, and both rare and common alleles have been implicated in autism risk. Linkage signals have proven hard to replicate, likely due to phenotypic or genotypic heterogeneity. In order to meet the challenge of phenotypic heterogeneity, we focus on quantitative traits related to autism.

We examine the restricted interests and repetitive behavior domain of the autism phenotype, using the Flexibility/Range of Interests scale of the Broader Phenotype Autism Symptom Scale (BPF). BPF is the average of three scales addressing flexibility in (i) schedule and routine; (ii) physical environment and (iii) interests. Each scale takes integer values of 1 - 5, with 1 indicating above average flexibility, and 5 indicating well below average flexibility.

We present genetic analyses of BPF in 1253 people in 266 families. Families were ascertained through the existence of at least two children affected with an ASD. The families are mostly nuclear and BPF scores are available on 90% of individuals. MCMC oligogenic segregation analysis (OSA), which allows for simultaneous modeling of multiple loci, was used to explore the evidence for genetic influence on BPF. Analysis of age-adjusted BPF gave strong evidence for one QTL, with recessive inheritance and a broad-sense heritability of 41%.

A genome-scan of 387 multiallelic STRs is available on 247 of these families (1168 individuals, 94% genotyped). Using the model obtained from the OSA, single-marker parametric lod scores were calculated. Chromosomes with interesting lod scores were followed up with a novel multipoint approach: using gl_auto from MORGAN, all markers per chromosome were used to generate samples of inheritance vectors (IVs) compatible with the available genotypes, with the program IBDgraph identifying equivalent IVs. Likelihoods were calculated for each unique IV using the parametric model, and then averaged, with weights, over the sampled IVs. This yields a lod score that uses marker information on the entire chromosome, but models the disease locus separately. Two regions achieved lod scores >2 and will be the subject of further study.

2726/F

Phenotyping for a behavior genetics study. *R. Plaetke, V. Stegall, F. Balbi.* Department of Anthropology, University of Alaska, Fairbanks, AK.

Since the building of genome maps for the domestic dog (*Canis familiaris*) and silver fox (*Vulpes vulpes*), animal models have been developed to investigate the genetics of behavior in these species and transfer results to humans (1-4).

These studies are based on the evolutionary aspect of behavior as already proposed by Darwin. In the same way, as he suggested continuity between humans and other species related to “physical” traits, he discussed this concept related to emotions in his book *“The Expression of the Emotions in Man and Animals.”* He proposed that many of the basic emotions and accompanying facial expressions exist in both, animals and humans.

We investigate an extraversion-temperament component in the Alaskan Husky (sled) dog to determine whether phenotypes (1) can be constructed for its characterization and (2) are heritable; and (3) the dogs can serve as a model to investigate the genetics of this component.

Behavior tests of 63 dogs have been performed and videotaped. Videotapes are analyzed under standardized conditions. To date, 28 characterizations of responses and their intensities have been determined; 6 of those are quantitative variables.

We will present:

(1) Details about the behavioral assay and how it is performed in the field.
(2) How we evaluate validity & reliability of the tests.
(3) Construction of phenotypes and their distributions among the dogs.
(4) Segregation of phenotypes in ascertained pedigrees.
(5) Efficient management of “upside down” pedigree structure consisting of each dog, 2 parents, 4 grandparents, and 8 great-grandparents; the usual format provided by breeders.

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2727/F

Hypoxia in Endothelin-2 (Edn2) null mice is associated with photoreceptor (PR) rescue in multiple models of inherited PR degeneration (IPD). *A.N. Bramall[1,2], M.J. Szego[1,2], L.R. Pacione[1,2], T. Van Veen[4], M. Yanagisawa[5], R.R. McInnes[1,2,3].* 1) Program in Stem Cell and Developmental Biology, Hospital for Sick Children Research Institute, Toronto, Ontario; 2) Department of Molecular Genetics, University of Toronto, Toronto, Ontario; 3) The Lady Davis Institute, Jewish General Hospital, McGill University, Montreal; 4) Department of Ophthalmology Research, Lund University Hospital, Lund, Sweden; 5) HHMI & the Department of Molecular Genetics, University of Texas Southwestern Medical Center, Dallas.

IPDs are among the leading causes of inherited blindness in humans, affecting about 1/3600 individuals. These disorders are characterized by remarkable genetic heterogeneity, but nevertheless most or all IPDs appear to share at least two common properties: exponential kinetics of PR death (Clarke et al. 2000), and a dramatic increase in the PR expression of the *Endothelin-2* mRNA (Rattner et al. 2005). We previously reported at this meeting that the *Edn2* mRNA was 70-fold ($p< 0.009$) and 72-fold ($p<0.0009$) up-regulated in *Tg(RHO P347S)* and *Rd1*$^{-/-}$ retinas, respectively, and that the increased retinal *Edn2* expression was restricted to the mutant PRs. To determine whether the increased *Edn2* mRNA expression was proapoptotic, we had generated two IPD mouse strains that lacked the *Edn2* gene. We found that the absence of Edn2 expression in both *Tg(RHO P347S);Edn2*$^{-/-}$ mice (at post-natal day 40 (PN40)), and *Rd1*$^{-/-}$*; Edn2*$^{-/-}$ mice (at PN15), led to a 41% and 49% increase in mutant PR survival, respectively. Here we provide evidence suggesting that much, if not all the rescue conferred by deleting the *Edn2* gene results from extraocular mechanisms. *First*, whereas *Rd1*$^{-/-}$ retinal explants displayed PR death at rates comparable to those seen *in vivo*, there was no rescue of PR death in *Edn2*$^{-/-}$*; Rd1*$^{-/-}$ retinal explants ($n= 5;p>0.05$), in contrast to the rescue observed *in vivo*. *Second*, the re-introduction of *Edn2* mRNA expression in *Edn2*$^{-/-}$*; Rd1*$^{-/-}$ retinas (but not in the whole animal) using a subretinally injected AAV5-CBA-*Edn2* vector did not restore PR death ($n=6;p>0.05$) *in vivo*. *Third*, *Edn2*$^{-/-}$ mice have very poor pulmonary alveolarization, leading to systemic hypoxia; EPO was 11-fold up-regulated in *Edn2*$^{-/-}$ animals by ELISA ($n=7;p<0.05$). We propose that the *in vivo* rescue of PR death in *Edn2*$^{-/-}$*; Rd1*$^{-/-}$ retinas is due at least partly to the systemic hypoxia, because i) *Edn2*$^{-/-}$ retinas are hypoxic *in vivo*: VEGF, a major hypoxia response gene, was 4-fold elevated by immunobloting in *Edn2*$^{-/-}$ retinas at PN21 ($n=4;p<0.05$); and ii) in *Rd1*$^{-/-}$ retinal explants cultured in 6% O2 from PN10 to PN17, PR death was reduced by 32% vs. *Rd1*$^{-/-}$ explants in normoxia (17% O2) ($n=5$; $p<0.05$). The protective effect of hypoxia on PR death suggests that elucidation of the molecular mechanisms by which hypoxia exerts its protective effect will identify novel neuroprotective pathways in the retina.

2728/F

Identifying Copy Number Variation Influencing Risk for Alzheimer Disease in the Amish. *O.J. Veatch[1], D.R. Velez Edwards[2,3], W.K. Scott[2], J.R. Gilbert[2], M.A. Pericak-Vance[2], J.L. Haines[1].* 1) Center for Human Genetic Research, Vanderbilt Med Ctr, Nashville, TN; 2) J.P. Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL; 3) Vanderbilt Epidemiology Center, Vanderbilt University Medical School, Nashville, TN.

Numerous studies have shown that Alzheimer disease (AD) has a high degree of heritability suggesting strong genetic components influencing risk for developing the disease. However, the heterogeneous nature of the disease has made it difficult to identify the underlying genetic loci for AD. Current studies of human Copy Number Variations (CNVs) indicate these structural variations contribute significantly to genetic and allelic heterogeneity. CNVs affect gene function in many ways including deletion or amplification of regions containing variable numbers of genes that could influence neuropsychiatric disease susceptibility. Large-scale CNV studies require stringent control for population stratification to avoid false positive associations. To avoid issues arising from population stratification we ascertained subjects from Amish communities located in Indiana and Ohio assuming similar genetic substructure. To assess possible CNVs influencing AD, a total of 903 individuals, age ≥ 65 years, from this population were genotyped on an Affymetrix® SNP 6.0 microarray chip. Samples with quality control issues were removed from analysis and the remaining 846 male and female individuals (113 with AD, 466 cognitively normal, 267 with other cognitive phenotypes) were assessed for CNVs using Partek® Genomics Suite software. Copy number intensities were normalized to a baseline created from the cognitively normal Amish individuals, and variations were detected using a hidden Markov Model. A total of 270 CNV regions across the genome were detected in at least 30 individuals. No statistically significant differences in frequency were observed between affected and unaffected individuals in these preliminary data suggesting no strong relationship of these common CNVs with AD. More detailed statistical analyses are ongoing. We also identified regions of allelic imbalance and loss of heterozygosity utilizing cognitively normal Amish individuals as the baseline for both analyses. We are currently examining putative regions of interest and identifying candidate disease-susceptibility genes located in these regions.

2729/F

Expression QTL analysis of top loci from Psychiatric GWAS Consortium meta analysis reveal additional schizophrenia candidate genes. *S. de Jong[1], M.P.M. Boks[2,3], D.L.W.H. Zeegers[1], K. van Eijk[1], E. Janson[1], E. Strengman[1], J.H. Veldink[4], L.H. van den Berg[4], W. Cahn[2], R.S. Kahn[2], R.A. Ophoff[1,2,5], Psychiatric GWAS Consortium.* 1) Department of Medical Genetics, University Medical Center, Utrecht, Netherlands; 2) Department of Psychiatry, Rudolf Magnus Institute of Neuroscience, University Medical Center, Utrecht, Netherlands; 3) Julius Centre for Health Sciences and Primary Care, University Medical Center, Utrecht, Netherlands; 4) Department of Neurology, Rudolf Magnus Institute of Neuroscience, University Medical Center, Utrecht, Netherlands; 5) Department of Human Genetics, Neuropsychiatric Institute, University of California, Los Angeles, USA.

Schizophrenia is a severe psychiatric disorder characterized by delusions and hallucinations, in addition to negative and cognitive symptoms. The disorder affects up to 1% of the population and the genetic contribution is estimated to be around 80%. Thus far, genome-wide association studies (GWASs) of schizophrenia have had limited success, with the best finding at the HLA locus at chromosome 6p. While only a very small number of loci yield genome-wide significance for association, the vast majority of the genetic contribution to disease susceptibility remains uncovered. It is likely to be overrepresented in non-significant top SNPs of large-scale GWAS results. In order to uncover potential interesting candidates, we selected the top 6,000 single nucleotide polymorphisms (SNPs) with significance threshold at p<0.001 from the international PGC meta-analysis consisting of some 9,000 cases and 12,000 controls and examined their possible involvement in schizophrenia using gene expression data from whole blood. Expression QTLs (eQTLs) were calculated for this selection of SNPs in a large set of healthy controls (n=437). The transcripts significantly regulated by the top SNPs from the GWAS meta-analysis schizophrenia-related SNPs were subsequently tested for differential expression in an independent set of schizophrenia cases and controls (n=200). After correction for multiple testing, eQTL analysis yielded significant cis-acting effects, whereas no trans effects were observed. For seven of these transcripts we observed significant differential expression between cases and controls. Results include target genes of interest both in- and outside the HLA region and contain genes that are known to be expressed in brain. These genes are strong candidates for schizophrenia for which further genetic analysis is warranted.

2730/F

Huntington's disease in Peru:Spread of cases and Analysis of CAG triplet distribution in Peruvian population. *R. Lovaton Espadin, C. Timana Chavez, G. Torres Alva, M. Cornejo Olivas, M. Marca Ysabel, O. Ortega Davila, C. Cosentino Esquerre, L. Torres Ramirez, P. Mazzetti Soler.* Neurogenetics Laboratory,National Institute of Neurological Sciences (Lima-Peru).

Introduction: Huntington's disease is an autosomal dominant disorder characterized by involuntary movements, cognitive and psychiatric disorders. This neurodegenerative disease is caused by the expansion of a CAG triplet repeat in the first exon of the HD gene on chromosome 4p16.3. Huntington's disease cases have been reported in Peru; however, there is little information about the epidemiological characteristics of this population. The purpose of this work is to show the distribution of Huntington's disease cases and a possible founder's effect in Peru. **Materials and Methods:** The National Institute of Neurological Sciences (Lima-Peru) is the national reference center for neurological and neurogenetical diseases in our country, receiving most of the population with Huntington's disease in our country. From our database, 225 persons with more than 36 CAG repeats, independent from their clinical status, were chosen. Their birth place was obtained from their medical records. Graphics and maps were done in order to show how this population has spread inside our country during the last decades. **Results:** Up to 2010, our distribution of cases in relation to place of birth is as follows: most of them came from the Department of Lima (85%) and Cañete, a province from the Department of Lima, accounts for 27 % of cases. 48% of cases were female and 52 % were male. The age at onset of symptoms was 39.8±10 years. The maximum number of CAG triplet repeats that we found was 70.A map of Peru built with the data available shows cases distributed in 12 departments from Peru. **Discussion and Conclusions:** Huntington's disease population in Peru shows some special characteristics. The maximum number of CAG triplet repeats found is less than other Huntington's disease populations (Venezuela, Europe). Probably our Huntington's disease population is younger, the disease probably arrived to our country recently compared to other locations or there is a genetic determinant that prevents expansion of CAG triplets. Distribution of cases in Peru shows an spread of this disease from a probable origin located in the Department of Lima (Cañete) to other departments. This distribution of cases is not related with the population density in Perú. These findings reveal the importance of genetic counseling in patient's relatives to prevent further expansion of this disease.

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Association between Tryptophan Hydroxylase II gene and late onset depression. *D. Miranda[1], P. Pereira[1], M. Bicalho[1,2], L. De Marco[1], H. Correa[1], S. Campos[1], E. Moraes[2], K. Torres[1], B. Souza[1], M. Romano-Silva[1].* 1) Mental Heathy, UFMG, Belo Horizonte, Brazil; 2) Centro de Referência do Idoso, Hospital das Clínicas, Universidade Federal de Minas Gerais (UFMG), Brazil.

The aim of this study was to examine the association between polymorphisms in the TPH2 gene and late-onset depression in the Brazilian population. Serotonin is a major neurotransmitter in the central nervous system (CNS), and its role in psychiatric disorders is well documented. Tryptophan hydroxylase 2 (TPH2) catalyzes the rate-limiting step in the synthetic pathway for brain serotonin and is considered key for maintaining normal serotonin transmission in the CNS. TPH2 polymorphisms are associated with major depression and suicide, attention-deficit hyperactivity disorder, and repetitive behaviors in autism, which suggest that the TPH2 gene is involved in the development of many psychiatric disorders. We genotyped 8 tagSNPs in the TPH2 gene in 84 outpatients with diagnosis of late-onset depression and 79 individuals belonging to the comparison group to investigate an association between the TPH2 gene and late-onset depression. Our findings suggested an association between tagSNP rs4565946 heterozygous C/T (p= 0,009; χ2= 6,7) and decreased risk of late-onset depression. The tagSNP rs11179000 ancestral homozygous A/A (p = 0,01, χ2= 7,3) and increase risk of late-onset depression. Allelic association of ancestral allele A and raise risk of late-onset depression was demonstrated (p-value= 0,005, χ2= 7,8). We found the statistically significant association between two tagSNPs and late-onset depression. The limitation of our study includes the small sample size, the population stratification and no information about current or past stress. Our results support the hypothesis that the TPH2 gene is associated with late-onset depression.

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Targeted proteomic study of serum from children with autism showing differential expression of complement proteins. *H. Li[1], J.A. Caruso[6], D.C. Chugani[2,3,4,5], P.M. Stemmer[6].* 1) Division of Genetic and Metabolic Disorders, Children's Hospital of Michigan, Detroit, MI. 3901 Beaubien Blvd. Detroit, MI 48201; 2) Autism Center, Children's Hospital of Michigan 3901 Beaubien Blvd. Detroit, MI 48201; 3) Division of Clinical Pharmacology and Toxicology, Children's Hospital of Michigan 3901 Beaubien Blvd. Detroit, MI 48201; 4) Pediatrics Dept, Wayne State University School of Medicine 3901 Beaubien Blvd. Detroit, MI 48201; 5) Radiology Dept, Wayne State University School of Medicine 3901 Beaubien Blvd. Detroit, MI 48201; 6) Institute of Environmental Health Sciences Wayne State University 2105 Scott Hall, 540 E. Canfield, Detroit, MI 48201.

Objectives: To identify proteins that are differentially expressed in young children with autism compared to age matched healthy children for use as candidate plasma biomarkers of autism. Methods: Plasma samples were obtained from children with autism having elevated serotonin 2-6 years of age (n=10) and age and gender matched typically developing children (n= 10). Plasma samples were depleted using an IgY-12LC10 column and then labeled with TMT isobaric tags. TMT-labeled plasmas were pooled in sets of four with each set containing a standard sample to enable normalization of all data. TMT-labeled pooled plasma samples were separated on 8-16% SDS-PAGE gels then each lane was divided into 24 slices that were digested with trypsin. Peptides in the tryptic digests were separated on C-18 reversed phase nano-HPLC columns and analyzed using an LTQ MS system using ETD fragmentation. Protein identification was achieved using the Mascot algorithm and isobaric tags were quantified using Scaffold-Q software. Nineteen proteins selected in this discovery phase were then quantified in all 20 samples using a Selected Reaction Monitoring (SRM) strategy on a TSQ Vantage Triple quadrupole MS system. Experimental design and analysis for the SRM work were accomplished using SkyLine and LCQuan software. Results: We identified 85 proteins in the plasma samples that were quantified using the TMT reporter ions. 19 of those 85 proteins were analyzed in all 20 samples using the SRM strategy. The SRM analysis identified two proteins with significantly higher concentrations in the plasma from the children with autism: Complement factor H related protein (FHR1) and Complement C1q subcomponent subunit C. Conclusion: The requirement for large group sizes in experiments designed to identify plasma biomarkers made analysis using the isobaric tag labeling strategy unnecessarily complex and it was found to lack sensitivity to small changes in protein concentration. The targeted proteomic study using a SRM strategy approved to be more sensitive and to be applicable to large group sizes. Our data replicate a previous study showing higher complement factors in blood samples from individuals with autism. The complement system is involved in the lysis and removal of infectious organisms in blood and may be involved in cellular apoptosis in brain. These results may provide a clue to the etiology of autism and provide support for SRM as a useful strategy for biomarker development.

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Differences in telomere lengths between identical co-twins who are discordant for a history of childhood sexual abuse. *J. Brumelle[1], T. York[2], C. Jackson-Cook[1,2].* 1) Department of Pathology, Virginia Commonwealth University, Richmond, VA; 2) Human & Molecular Genetics, Virginia Commonwealth University, Richmond, VA, USA.

Chronic stress in adulthood has been associated with telomeric attrition and age-related diseases, but little is known about the long-term, cumulative effects of childhood stress on adults. To address this question we initiated a study of identical (MZ) twins who are discordant for a history of childhood sexual abuse (CSA). Because the DNA of discordant MZ twins differs only for environmentally induced changes, they provide a unique opportunity to study the impact of CSA. To date, lymphocytes have been collected from 36 females (12 complete pairs) ages 36 to 69, who were ascertained because they participated in previous studies that characterized their CSA history and behavioral phenotypes. The biological endpoints that were compared between the MZ co-twins included the: 1) frequency (quantified using a micronucleus [MN] assay) and chromosome-specific pattern (using SKY on MN) of acquired chromosomal instability; and 2) chromosome-specific telomere length (assessed using a Q-FISH assay). MN frequencies (average per 1000 binucleates) ranged from a low of 6.5 (52 yo) to a high of 31.5 (57 yo). While not significantly different, individuals with intercourse exposure (IE) tended to have higher frequencies of MN (20.1 ± 3.5 s.e.) than their unaffected co-twins (17.9 ± 2.4). Overall, a non-random pattern of chromatin was present in MN ($p<0.0001$), with chromosomes X (27.3% of MN) and 12 (7%) observed most often and chromosomes 17 (0.35%) and 21 (0.35%) least often in both co-twin groups. Telomere lengths of females having IE were significantly shorter than their unaffected co-twins ($p<0.001$ to 0.015), with the shortening observed for all telomeres except 4p. Telomere lengths between co-twins discordant for non-IE forms of CSA were not significantly different. In CSA and non-CSA twins, similarity in chromosome-specific length patterns were noted (9q, 2q, 22q and 17p, 1p, 4p had the shortest telomeres in both groups), suggesting that the attrition occurred in a consistent, near-linear manner. In summary, this is the first report of telomeric shortening in adult females having CSA with IE when compared to their unaffected identical co-twins. Given the morbidity and mortality of diseases with a stress-related etiology, this study could provide a foundation for development of a biomarker for stress, which could play an important role in preventative medicine.

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Genome wide analysis in twins discordant for bipolar disorder reveals altered DNA methylation of inflammatory and stress response genes. *M.P.M Boks[1], R.S Kahn[2], E.M. Derks[2], F. Colas[3], S. Jong de[4], K. Eijk de[4], R.A. Ophoff[3,4].* 1) Psychiatry and Epidemiology, University Medical Center, Utrecht, Utrecht, Netherlands; 2) Rudolf Magnus Institute of Neuroscience, department psychiatry, University Medical Center Utrecht, Utrecht, The Netherlands; 3) UCLA Center for Neurobehavioral Genetics, Semel Institute for Neuroscience and Human Behavior, Los Angeles, CA, USA; 4) Department of Medical Genetics, University Medical Center Utrecht, Utrecht, The Netherlands.

Epigenetic silencing by methylation of specific DNA loci throughout the genome is likely to be associated with the vulnerability to bipolar disorder. Studies investigating this are however vulnerable to confounding by genetic variation and medication use of bipolar patients. We therefore investigated DNA methylation differences between affected and non affected members of monozygous twin pairs (N=34) and subsequently compared the unaffected members from the discordant twin pairs to a sample of healthy MZ and DZ twins (N=96). Using DNA extracted from whole blood, we analyzed DNA methylation of over 27,000 loci simultaneous using array based technology. We found significant associations of disease status with DNA methylation levels within the discordant pairs, and identified differences between unaffected twin members and healthy twin pairs. DNA methylation loci associated with bipolar disorder highlighted corticoid pathway genes and include the genes B-cell CLL/lymphoma 2 (BCL2) and guanine nucleotide binding protein (GNAL) previously linked to bipolar disorders. Differential methylation in unaffected twin members was enriched for genes involved in inflammatory response. These findings provide support for altered inflammatory response associated with bipolar disorder susceptibility and fit previous experimental data suggesting that the stress response can be modulation by DNA methylation. This study of DNA methylation profiles in whole blood opens new avenues for genetic studies of neuropsychiatric disorders.

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Abnormally low serum acylcarnitine levels in narcolepsy patients. *T. Miyagawa[1], H. Miyadera[1], S. Tanaka[2], M. Kawashima[1], M. Shimada[1], Y. Honda[3], K. Tokunaga[1], M. Honda[2,3].* 1) Department of Human Genetics, Graduate School of Medicine, The University of Tokyo; 2) The Sleep Disorders Research Project, Tokyo Institute of Psychiatry; 3) Japan Somnology Center, Neuropsychiatric Research Institute.

Genome-wide association study identified a novel narcolepsy related SNP rs5770917, which affects expression level of adjacent *CPT1B* gene. CPT1B conjugates carnitine to long-chain fatty acyl coenzyme A and allows the transport of long-chain fatty acid into mitochondrial matrix for subsequent β-oxidation. We hypothesize the dysregulation of fatty acid β-oxidation in narcolepsy. We measured *CPT1B* gene expression in white blood cell by quantitative RT-PCR and serum carnitine fractions (total, free, and acylcarnitine) by enzymatic cycling method. Blood samples from 38 patients with narcolepsy and 56 healthy control subjects were used for *CPT1B* gene expression analysis, and the same 38 patients and 30 control subjects selected from the above 56 controls were used for carnitine fraction measurement. Stepwise multi-regression analysis showed that the risk allele (C) of SNP rs5770917 was associated with decreased *CPT1B* expression ($P = 1.0 \times 10^{-9}$), and the level of *CPT1B* expression was higher in narcolepsy patients than in control subjects ($P = 0.005$). Although the distribution of serum acylcarnitine level did not show significant difference, acylcarnitine levels in 21% (eight of 38) narcolepsy patients were abnormally low below the established normal range, while those of 30 control subjects were all within normal range, regardless of the SNP rs5570917 genotype. Stepwise multi-regression analysis using the dichotomous variable of acylcarnitine (normal or abnormal) as an objective variable revealed that the diagnosis of narcolepsy was associated with abnormally-low acylcarnitine level ($P = 0.006$) while other variables such as *CPT1B* expression level and BMI were not. Our results indicate the involvement of multiple factors in the regulation of serum acylcarnitine levels. Abnormally-low levels of acylcarnitine observed in narcolepsy suggest the dysfunction in the fatty acid β-oxidation pathway in narcolepsy.

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MicroRNA expression profiling in the prefrontal cortex of individuals affected with schizophrenia and bipolar disorders. *A. Kim[1], M. Reimers[2], B. Mahor[1,3], V. Williamson[1,4], O. McMichael[1], J. McClay[5], E. Van den Oord[1,5], B. Riley[1,3], K. Kendler[1,3], V. Vladimirov[1,5].* 1) Virginia Institute for Psychiatric and Behavioral Genetics, Department of Psychiatry, Medical College of Virginia of Virginia Commonwealth University, Richmond, VA; 2) Department of Biostatistics, Virginia Commonwealth Univ, Richmond, VA; 3) Department of Human and Molecular Genetics, Virginia Commonwealth University, Richmond, VA; 4) Department of Integrative Life Sciences, Virginia Commonwealth University, Richmond, VA; 5) Center for Biomarker Research and Personalized Medicine, School of Pharmacy, Medical College of Virginia of Virginia Commonwealth University, Richmond, VA.

MicroRNAs (miRNAs) are a class of small non-coding RNA molecules (~22nt) that primarily function to negatively regulate gene expression. The number of miRNAs identified is growing rapidly and approximately one-third are expressed in the brain where they have been shown to affect neuronal differentiation, synaptosomal complex localization and synapse plasticity, all processes thought to be disrupted in schizophrenia. Here we investigated the expression of 667 miRNAs (miRBase v.13) in the prefrontal cortex of individuals with schizophrenia (SZ, N = 35) and bipolar disorder (BP, N = 35) using a real-time PCR-based Taqman Low Density Array (TLDA). After extensive QC steps, 441 miRNAs were included in the final analyses. At a FDR of 10%, 22 miRNAs were identified as being differentially expressed between cases and controls, 7 dysregulated in SZ and 15 in BP. Using in silico target gene prediction programs, the 22 miRNAs were found to target brain specific genes contained within networks overrepresented for neurodevelopment, behavior, and SZ and BP disease development. Further testing of targets via real-time PCR revealed negative correlations of expression between miRNAs and their predicted gene targets, providing additional support for the in silico predictions.

2737/F

Affect of elevated reactive oxygen species and decreased antioxidants on the DNA quality of sperms in couples experiencing recurrent spontaneous abortions. *R. Dada[1], D. Pathak[1], K. Kumar[1], M.B. Shamsi[1], S. Venkatesh[1], D. Deka[2].* 1) Laboratory for Molecular Reproduction and Genetics, Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India; 2) Department of Obstetrics and Gynaecology, All India Institute of Medical Sciences, New Delhi, India.

Introduction: Sperm has dynamic and critical role in embryogenesis that extends beyond fertilization. Reactive Oxygen Species (ROS) in pathological concentrations challenge the antioxidant and DNA repair machinery and causes errors in genomic factors contributed to embryo by the sperm by inducing mutations in mitochondrial genome and also by inducing nicks in the nuclear genome. During embryonic development, nucleotide alterations if unrepaired are lethal to the accurate transmission of genetic information and affect the embryonic viability and post natal health. The current study was designed to understand the association of antioxidant and ROS with the mitochondrial mutations and nuclear DNA damage in male partners of women experiencing recurrent spontaneous abortions. **Material and Methods:** Forty seven male partners of women experiencing recurrent spontaneous abortions and 29 fertile controls were included in this study. DNA damage was assessed by Comet assay, ROS by luminol induced chemiluminescence and seminal antioxidants (Superoxide Dismutase- SOD, Catalase- Cat) by biochemical methods. Mitochondrial mutations were analyzed by PCR-sequencing. Semen analysis was done according to WHO 1999 guidelines. Statistical analysis was done using Mann-Whittney test. **Results:** In infertile men with normal sperm parameters (category A; n=19) mean ROS in neat semen was 13 410.76 as compared to infertile men with pathological sperm parameters (category B; n=28) where the mean ROS was 15 219.49 RLU/min/20 million sperms. In category A the mean ROS of washed sperms was 17 416.58 as compared to 20 014.87 RLU/min/20 million sperms in category B. ROS was significantly higher for both the washed and neat semen as compared to fertile controls ($P<0.05$). In category A the mean % DNA in comet head was 27.31 , in category B it was 18.54 as compared to 65.98 in fertile controls. The catalase and SOD levels in both category A and B was significantly lower as compared to controls ($P<0.05$). Novel, synonymous mitochondrial mutations observed in 4 patients are g8011A>G in COII gene, g9030C>T in ATP 6 gene, g10127A>T in ND3 gene and g13407A>G in ND5 gene. **Discussion:** Balance between ROS and antioxidants is critical for fidelity of sperm genome. ROS and DNA integrity are better markers of sperm fertilizing potential as compared to conventional semen analysis so their assessment should be included in the diagnostic workup of men in couples experiencing recurrent spontaneous abortion.

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Cytogenetic and Yq deletion molecular screening in infertile men opting for IVF/ICSI. *S. Dudeja[1], M.B. Shamsi[1], S. Venkatesh[1], R. Kumar[2], N. Malhotra[3], S. Mittal[3], P. Talwar[4], R. Dada[1].* 1) Laboratory for Molecular Reproduction and Genetics, Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India; 2) Department of Urology, All India Institute of Medical Sciences, New Delhi, India; 3) Department of Obstetrics and Gynaecology, All India Institute of Medical Sciences, New Delhi, India; 4) ART centre, Army Research and Refferal Hospital, New Delhi.

Introduction: Infertilty affects 15 % of couples in the reproductive age group and in 40 % of the cases the male factor is involved. The normal spermatogenesis requires the complete functional integrity of the factors mainly present on the Y chromosome. Due to the haploid nature of the Y chromosome genes and its inability to deploy recombination repair in retrieving lost genetic information, the Y chromosome is particularly susceptible to gene deletions. Assisted conception bypasses the natural mechanisms of selection so there is a high probability of transferring the Yq deletions to the offspring which may be particularly lethal if the conceived child is a male. Aberrant recombination, defective chromatin packaging and oxidative stress (OS) are also involved in the aetiology of DNA damage in the germ line. In this study the large base pair chromosomal aberrations and the Yq microdeletions were analyzed in the male partners of couples opting for IVF/ICSI. **Material and Method:** Forty nine male partners of couples opting for IVF/ICSI and 27 healthy fertile males (controls) were included in the study. Cytogenetic analysis was done using GTG banding of cultured lymphocytes. Microdeletions on long arm of Y chromosome were screened by standard PCR-sequencing technique. **Results:** Out of 49 infertile men screened in this study one had 47, XXY chromosomal complement. 45,XY rob (13q:15q) and 45,XY rob (13q:14q) robertsonian translocation was reported in 2 men. One autosomal translocation was reported 46,XY(70 %) / 46,XY del 3 (q24 ® q 25.2) (30 %). Deletion of AZF a (sY 84) and AZF b (sY 127) regions was reported in 2 different men. Two patients had AZF c (sY 254) deletion. **Discussion:** The correlation between Y-chromosome deletions and infertility, and the relative absence of such deletions in fertile men, suggest a cause-and-effect relation between the deletions and infertility. The clinical findings or even the semen analysis can not predict the Y-chromosome microdeletions. With the increase in assisted conceptions, the chances for passing on these defects to offspring are high and should be considered when infertile couples are counseled about this procedure.

2739/F

Trends of the incidence of twin births in Japan. *K. Kurosawa[1], K. Enomoto[1], N. Furuya[1], M. Masuno[2], Y. Kuroki[2].* 1) Kanagawa Children's Med Ctr, Yokohama, Japan; 2) Kawasaki University of Medical Welfare, Kurashiki, Okayama, Japan.

Assisted reproductive technology (ART) is more likely to provide multiple births. Multiple births are associated with increased risk for mothers and infants, including higher rates of caesarean deliveries, prematurity, low birth weight, birth defects, and infant disability. About 30% of deliveries following ART in USA and Europe are twins, compared with approximately 1% following spontaneous conceptions. The increased rate of multiple births from the age of 1980s in many Western countries is clearly contributed by advance and prevalence of ART. However, recent data from population-based epidemiological study presented the decreased number of multiple pregnancies in Japan. To elucidate the causes and the ratios of mono- vs di-zygosity of twin births, we analyzed the incidence of twin births from the data of population-based birth defects motoring system in Kanagawa Prefecture (KAMP), Japan. KAMP has been operated since 1981 as the first population-based monitoring system in Japan, which covers about half of total births in Kanagawa Prefecture, that is, 40,000 births annually. All malformed cases as well as multiple births are registered with two consecutive normal control infants. The zygosity of twins was estimated according to Weinberg's differential rule zygosity estimation methods. From the start of KAMP at 1981, the total births of twins increased consistently from 60 to 100 per 10,000 deliveries, which is consistent with those of Western countries. However, after the peak at 2003, the dramatic decrease in the rate of twin births was observed in KAMP. During the last 20 years, though the incidence of monozygotic twins is stable as 40 per 10,000 deliveries, the incidence of dizigotic twins presented dramatic variation. To our knowledge, this is the first epidemiological report on the trends of decreased rate of twin births. We discussed the causes and backgrounds of the trends observed in KAMP, Japan.

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Compromised DNA integrity in spermatozoa of infertile men - assessed by sperm chromatin structure assay (SCSA). *S. Venkatesh[1], A. Singh[2], MB. Shamsi[1], R. Kumar[3], NP. Gupta[3], DN. Mitra[2], R. Dada[1].* 1) Anatomy, Lab Molec Reproduction & Gen, New Delhi, India; 2) Dept. of Urology, AIIMS; 3) Dept. of Transplant Immunology.

Introduction:Spermatozoa with good chromatin is prerequisite for not only its physiological function but also for embryogenesis and fetal development. However, conventional semen analysis provides no clue about sperm chromatin structure, which is essential in such men opting for assisted procreation. Aim:To analyze 42 primary infertile men and 18 fertile controls for sperm DNA integrity by sperm chromatin structure assay (SCSA) and to find out the cut-off value to discriminate infertile and fertile men based on their sperm DNA fragmentation index (DFI) in Indian population Methods:Semen analysis were performed as per WHO, 1999 guidelines. Sperm DNA fragmentation index (DFI) was calculated by SCSA. Receiver operating curve (ROC) analysis was performed to find the cut-off value to predict the diseased subject. Mann-Whitney test was used compare semen parameters between infertile and control men. Results:Out of 42 infertile men evaluated for SCSA, the average mean DFI in infertile men was found to be 40.85±37; which is 1.59 fold higher than of control men (25.62±37;). DFI median (interquartile range) of infertile men was significantly ($p<0.0001$) higher compared to control population [(41.5 (34.28, 46.96) Vs 25.16 (22.93, 28.65)]. A cut off value of 31.42 with 83.33% sensitivity and 100 percent specificity (95 percent; CI 0.828 to 0.978) was found by ROC analysis. A strong negative correlation was observed between DFI and sperm count ($p<0.001$), sperm motility ($p<0.0001$), and normal sperm morphology ($p<0.05$) when in the study population, however no such correlation was found in the infertile men. However, men with longer duration of infertility (DI) were found to have increased DFI ($r=-0.330$, $p<0.05$) Conclusion:Impaired sperm DNA quality is the rationale behind male infertility in most of the idiopathic cases. Couples delaying in achieving parenthood have increased risk for getting pregnancy. DFI=31.42 is a valuable cut off point in our population, nearly similar to other studies above which couple may find difficulties in achieving pregnancy. SCSA is a reliable tool applicable beside conventional semen analysis and may be very useful in selecting couples for different ART procedures.

2741/F

Seminal oxidative stress, mitochondrial DNA mutations and nuclear DNA damage in men experiencing recurrent IVF/ICSI failure. *M.B. Shamsi[1], V. Sundararjan[1], R. Kumar[2], N. Malhotra[3], S. Mittal[3], P. Talwar[4], R. Dada[1].* 1) Laboratory for Molecular Reproduction and Genetics, Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India; 2) Department of Urology, All India Institute of Medical Sciences, New Delhi, India; 3) Department of Obstetrics and Gynaecology, All India Institute of Medical Sciences, New Delhi, India; 4) ART centre, Army Research and Refferal Hospital, New Delhi.

Background: Oxidative stress (OS) occurs due to excessive reactive oxygen species (ROS)generation beyond the scavenging capacity of antioxidant defenses. OS damages sperm DNA, thus may transfer defective paternal DNA to conceptus. The damage may be in mitochondrial (mt) genome or nuclear genome. Basic repair mechanism, absence of introns, and vicinity to site of ROS production increases the susceptibility for mt mutation. Presence of unrepaired single or double stranded DNA breaks in nuclear genome of sperm impairs the early and late post fertilization events which influence the pregnancy outcome. **Material and Method:** Thirty two idiopathic infertile men experiencing recurrent IVF/ICSI failure and 21 proven fertile controls were included. ROS in PBS washed sperm was quantified by chemiluminescence. Seminal total antioxidant capacity (TAC) was assayed by commercially available kit. Mt DNA and all exons of p53 gene were analyzed by PCR-sequencing and nuclear DNA damage was assessed by comet assay. Mann-whitney test applied for statistics and was $p<0.05$ considered significant. **Result:** The mean of ROS in infertile men was 1 75 000 as compared to 987 RLU/min/20 million in controls. The mean TAC was 2.78 as compared to 4.55 mM in controls. The DNA damage in infertile men was higher in infertile men as evident from low percent of DNA in sperm comet head in infertile men 18.43 as compared to 54.97 in controls.ROS and DNA damage was significantly higher while TAC was significantly lower in infertile men Two nucleotide variations in ATPase 6, four in ATPase 8 and 1 in COX II gene were observed in patients. Hetroplasmy at g.7572518C>A, g.7572540C>A were observed in P53 gene in 2 patients. **Discussion:** Association of sperm DNA damage and sperm-derived ROS suggests that DNA damage is due to defect in DNA repair mechanism and the antioxidant machinery. Mutations of P53 and an array of other DNA repair genes involved in repair may be responsible for DNA damaged sperms in the ejaculate. The inefficient antioxidant mechanism or the excessive generation of ROS produces mt mutations which manifests as abnormal sperm parameters affecting the fertilizing capacity of the sperm. Considering the spectrum of factors associated with OS, it is essential that males who are opting for assisted conception and experiencing recurrent IVF/ICSI failure be screened at the molecular level and a defined protocol for such assessment be included in the diagnostic workup of such men.

2742/F

Screening for microdeletions on the Y chromosome using array CGH in 72 Japanese patients with non-obstructive azoospermia. *T. Cui, H. Nakaoka, K. Akiyama, A. Tajima, I. Inoue.* School of Medicine, Tokai University, Isehara, Kanagawa, Japan.

Microdeletions of azoospermia factor (AZF) locus are considered to be the main genetic cause of infertility in men with idiopathic azoospermia and sever oligozoospermia. However, it is difficult to identify the infertility casing gene owing to the lack of multiple genes in the observed deletion region. By using array CGH, technology it becomes possible to increase the resolution in a target genomic region of interest, and to detect gene-specific small deletions that may contribute to the identification of a disease-causing gene. Here we have developed a high-resolution array CGH (Agilent, 44k) for Y chromosome, and carried out microdeletion screening in 72 Japanese patients with non-obstructive azoospermia (NOA). Microdeletions at different AZF regions were found in 8/72 patients with NOA (11.11%): one case in AZFa, two cases in AZFb, three cases in AZFa+b+c and two cases in AZFa+b+c+Yp. In addition, four small deletions (SDs) ranging from 5 kb to 125 kb in size were identified in AZF region and their interval. Interestingly, two SDs are located in region involving in intron 26 of ubiquitously transcribed tetratricopeptide repeat gene on Y chromosome (UTY) and BCL6 co-repressor-like 2 (Bcorl2), a noncoding gene respectively. We determined the mRNA expressions by real-time quantitative reverse transcription (RT) polymerase chain reaction (PCR) and found the enhanced testicular expression level of UTY in patient with SD compared to patients without SD of UTY, suggesting the putative association in the spermatogenesis. We showed for the first time the AZF deletion pattern using array CGH, and represented UTY and Bcorl2 gene-specific deletion.

2743/F

Micronuclei frequency and sperm DNA damage In Male Infertility. *V. DURGA RAO[1,3], A. RADHA RAMA DEVI[2,3].* 1) SCHOOL OF BIOTECHNOLOGY, MGNIRSA , HYDERABAD, Andhra Pradesh, India; 2) SANDOR PROTEOMICS, HYDERABAD, INDIA; 3) MOLECULAR DIAGNOSTICS,Center for DNA Fingerprinting and Diagnostics (CDFD). Hyderabad , Andhra Pradesh,India.

Presence of micronuclei is a marker for chromosomal damage and higher frequency of micronuclei is an indicator of chromosome instability causing male infertility. The presence of chromosome instability was studied through micronucleus assay in peripheral blood lymphocytes. Sperm DNA damage was studied in the ejaculated sperm of infertile men and controls by Single Cell Gel Electrophoresis (Comet assay). Micronuclei (MN) assay was carried out in 44 male infertile patients (mean age of 31.23 ± 6.08) and a control group of 28 men (mean age of 31.14± 2.59) that revealed a high frequency of MN in patients (28.62 ± 10.26) compared to controls (13.29 ± 4.02). No effect of age was found on the micronuclei frequencies in both the groups. . The mean comet tail length for the DNA damage in infertile patients was found to be 71.48± 5.1μm, while the controls showed a mean tail length of 54.18± 7.22 μm. In conclusion, the present study demonstrates that there is significant sperm DNA damage in male infertility.

2744/F

Detection of a New Unbalanced Rearrangement leading to a partial Monosomy Xq and a partial 18q Trisomy associated with Diminished Ovarian Reserve (DOR). *M. Paciolla[1,2], F. Fusco[1], M.B. Lioi[2], X. Li[3], R. Genesio[4], A. Conti[4], L. Poeta[1,2], D. Drongitis[1], E. Chen[5], M.V. Ursini[1], M.G. Miano[1].* 1) Institute of Genetics and Biophysics “Adriano Buzzati Traverso” CNR, Naples, Italy; 2) University of Basilicata, Potenza, Italy; 3) Department of Genetics, Kaiser Permanent Medical Center, San Jose, CA, USA; 4) Dipartiment of Biology and Cellular and Molecular Pathology, University of Naples “Federico II”, Naples, Italy; 5) Department of Genetics, Kaiser Permanente Medical Center, San Francisco, CA, USA.

Diminished Ovarian Reserve (DOR) is a heterogeneous disorder belonging to the gonad failure spectrum, characterized by low numbers of remaining oocytes in the ovaries, usually accompanied by high follicle stimulating hormone (FSH) level. Such diminished ovarian reserve is thought to reflect both a decreased number of eggs and a decrease in egg quality. DOR aetiology factors are multiple and different, such as genetic factors, aging, autoimmune disorders, adrenal gland impairment, iatrogenic, e.g., due to radiation or chemotherapy. At present the exact genetic cause of DOR is still unknown, although DOR with a severe reduction of reproductive performance was reported with a significant high incidence (44.4%) in females carrying X chromosome mosaicisms (45,X/46,XX; 46,XX/47,XXX) without balanced autosomal rearrangements. We report the first unbalanced translocation with segmental loss and gain of genomic fragments associated with DOR. Array CGH and FISH studies revealed a partial Xq monosomy and a partial 18q trisomy resulting from an unbalanced t(X;18) translocation. Real time experiments showed a Xq27 monosomy starting at position 141,2 Mb and a 18q22 duplication starting at position 64,5 Mb. Sequence inspection revealed the presence of a genomic context of LINE-1 (L1) clusters, at both the Xq27 and the 18q22 breakpoints. This structure might increase the likelihood of mispairing, unusual crossover and therefore, gain or loss of genomic materials. To further characterize the outcome of DOR rearrangement, the status of X chromosome inactivation was investigated. Androgen Receptor assay and late-replication DNA analysis revealed a preferential inactivation of rearranged X:18 chromosome, therefore completely skewed inactivation and late replication might mask the outcome of the 18 trisomy as well as of the X monosomy. Our patient has only DOR and no other unusual clinical features. This finding is in agreement with other reports that demonstrated that in X:autosome unbalanced translocation, inactivation signals of X-inactivation centre spread across the X:autosome boundary therefore, resulting in various attenuated phenotypes. Our data include DOR condition among the gonad failure spectrum of diseases linked to X-chromosome abnormalities, and confirm the feature of the Xq27-28 as a region of high genome instability. The hypothesis that the DOR phenotype occurs as a result of faulty meiotic pairing and recombination will be discussed.

2745/F

Incidence of Yq deletion in infertile males of Gujarat state. *S.S. Chettiar[1], S. Trivedi[2], D.D. Jhala[1,2], D. Chandel[1,2], M.V. Rao[1,2].* 1) GenDiCe, Zoology Dept, Gujarat Univ, Ahmedabad, India; 2) Human Genetics Unit, Department of Zoology, Gujarat University, Ahmedabad, India.

Infertility affects around 2-15% couples attempting for pregnancy, with 50% of cases due to male infertility and the causes are due to identifiable severe defects in sperm production, which are classified as azoospermia, oligospermia and abnormal motility/morphology. Genes on Y-chromosome (Yq11) (AZFa, AZFb, AZFc) are vital for spermatogenesis and Y-chromosome micordeletions are found exclusively in males with azoospermia, severe oligospermia or other spermatogenic defects. In order to define recurrently deleted regions of Yq, (II) determine the incidence of microdeletions among azoo-and oligozoospermic men; (iii) correlate the size and position of the deletions with the infertile phenotype, We have studied 58 infertile men from the Gujarat State, in the Western part of India with azoospermic, oligospermic, teratospermic and asthenospermic. All patients were excluded for cytogenetically detectable microdeletions of the euchromatic region of the Y-chromosome. DNA samples were PCR amplified using different sets of STS markers to assay for deletions in AZFa, AZFb and AZFc regions. Three infertile men (5.17%) were found with microdeletions of Y-chromosome. Fourteen had azoospermia or severe oligospermia, 21 had oligospermia, 18 of the incorporated patients were reported to be teratospermic and 5 had asthenospermia. The present data indicates that a small proportion of men with infertility had Y-chromosome microdeletions, but the size and position of the deletions correlate poorly with the severity of spermatogenic failure, and the deletion does not preclude the presence of viable sperm and possible conception.

2746/F

Cytogenetic and Molecular Causes of Male Infertility. *S.E. Hofherr[1,2], A.E. Wiktor[1,3], B.R. Kipp[1,4], D.B. Dawson[1,4], D.L. Van Dyke[1,3].* 1) Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN; 2) Clinical Biochemical Genetics; 3) Clinical Cytogenetics; 4) Clinical Molecular Genetics.

10-15% of couples attempting to conceive are infertile. According to the WHO, male infertility accounts for ≥50% of infertility among couples. Up to 25% of males with non-obstructive infertility have chromosomal abnormalities and/or microdeletions of the long arm of the Y-chromosome. These are detected by conventional chromosome and Y-microdeletion analysis. Following IRB approval, we reviewed the results of testing performed in the Mayo Clinic Cytogenetics and Molecular Genetics Laboratories and compared our findings with previously published reports. This study includes 2288 chromosome studies from males ≥18 years of age referred for infertility between 1989 and 2000 and 2749 Y-deletion molecular studies performed between 2002 and 2009. This study did not take into account the cause (obstructive, non-obstructive), or severity (azoospermia, oligospermia) of the infertility. A chromosome abnormality was detected in 333/2288 (15%) males. Of those, 294 (88%) were sex chromosome abnormalities. A 47,XXY or variant karyotype consistent with Klinefelter Syndrome (KS) was observed in 261 males (11%), and accounted for 78% of all abnormal results. This is consistent with previous studies suggesting that KS is the most common chromosome abnormality in the general male population (0.15%) and is associated with infertility. The remaining abnormalities were less common and included 12 Y-rearrangements (inversion, deletion, ring/deletion), ten 47,XYY, seven 46,XX males, two 48,XXYY, and two 48,XXXY. Balanced rearrangements were detected in 32 patients, 7 of which were Robertsonian translocations. An additional 7 specimens represented unbalanced rearrangements. Of the 2749 males tested for Y-microdeletion, 100 (4%) were identified with a deletion of at least one of the azoospermia factor regions (AZFa, AZFb, AZFc). We detected 64 patients with AZFc deletions, 5 with deletion of AZFa and 5 with deletion of the AZFb. Larger deletions encompassing both AZFb and AZFc regions were identified in 17 patients and 9 had deletion of all three AZF regions (AZFa,AZFb,AZFc). The most frequent Y-deletion spanned the AZFc region including DAZ (deleted in azoospermia), which is hypothesized as the cause of infertility in males with a Y-microdeletion. However, 10 patients had deletions of AZFa or AZFb but retained the entire AZFc region including DAZ. These results underscore the value of both chromosome studies and Y microdeletion studies in males with non-obstructive infertility.

2747/F

A retrospective study of preterm birth rate in China: 90,359 (5.4%) preterm births were identified from 1,686,224 newborns. *X.L. Zhao[1], N. Zhong[1,2].* 1) Peking University Center of Medical Genetics, Beijing, China; 2) New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY.

Preterm birth (PTB) indicates the delivery occurs before 37 weeks of gestation. The PTB has been widely recognized as one of the leading impact in the neonatal health. In the recent twenty years, a trend of increase in the PTB has been reported. In order to determine the rate of PTB in China and to initiate a campaign of prevention and intervention of preterm births we have conducted a retrospective hospital-based survey on the PTB. One hundreds and eleven hospitals in 23 provinces of China mainland were included in our survey. Hospitals' newborn registry from year 2005-2009 was retrieved. The week of gestation at birth was reviewed. A total of 1,686,224 newborns was included in our data, in which, 90,359 preterm births were recorded, which gave rise to an overall 5.36% of national rate of preterm births. The annual national rate grew from 4.86% of year 2005 to 5.90% of year 2009. The provincial rates are highly varied in the range between 2% to 10%, although most provinces had rates around the overall national rate. When considering the level of hospitals, Tier-1 (the primary healthcare) and Tier-3 (the tertiary healthcare) hospitals showed higher preterm birth rates of 11.98% and 8%. In comparison, the rate of Tier-2 hospitals is 3.97%. The result clearly showed that the preterm rate of costal region (5.02%) is lower than it of inland region (6.35%). The difference is statistically significant ($p<0.01$). This difference in rates of costal and inland regions was correlated with average income of each region. With our knowledge, this survey is the first study to investigate the national PTB rate of China, which may contribute to the international epidemiology of PTB.

2748/F

Genetic Variation in NAT1 Interacts with Cadmium Exposure to Influence Pregnancy Outcomes in non-Hispanic Black (NHB) Women. *A.E. Ashley-Koch[1], G.K. Swamy[2], M.E. Garrett[1], K.S. Quinn[1], A. Buskwofie[1], M.L. Miranda[3].* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) Department of Obstetrics and Gynecology, Duke University Medical Center, Durham, NC; 3) Nicholas School of the Environment, Duke University, Durham NC.

OBJECTIVE: To examine the contribution of the N-acetyltransferase genes (NAT1, NAT2) and cadmium exposure to the occurrence of maternal and infant pregnancy outcomes. **METHODS:** Healthy Pregnancy, Healthy Baby is a prospective cohort of pregnant women aimed at identifying genetic, social, and environmental contributors to racial disparities in pregnancy outcomes. English-literate women >18 yrs with a normal singleton pregnancy residing within Durham County, NC were enrolled prior to 28 weeks gestation. 594 NHB women were examined for the outcomes of maternal preeclampsia (BP>140/90 and proteinuria) and infant birthweight (BWT) in this analysis. Maternal samples collected during inpatient admission for delivery were analyzed for cotinine and cadmium. Haplotype tagging single nucleotide polymorphisms (htSNPs) were genotyped for NAT1 (n=19) and NAT2 (n=13) using Taqman assays. None of the htSNPs were in linkage disequilibrium. Logistic regression was used to examine the relationship between htSNPs and maternal preeclampsia, adjusting for maternal chronic hypertension, parity, infant sex, age, education, insurance, and cotinine exposure. Linear regression was used to examine the relationship between htSNPs and infant BWT, using the same covariates except chronic hypertension. We also examined potential interactions between NAT htSNPs and cadmium exposure. **RESULTS:** 121 participants (20%) were diagnosed with preeclampsia. Mean infant BWT was 3018 g (sd=663 g). Of the 32 htSNPs examined, 3 in NAT1 were nominally associated with preeclampsia (rs2410545, p=0.02; rs13278990, p=0.006; rs11777998, p=0.007). Three different NAT1 htSNPs were nominally associated with BWT (rs10107390, p=0.01; rs9325827, p=0.02; rs4921880, p=0.001 and met multiple testing correction). Two NAT1 htSNPs provided evidence for interactions with cadmium exposure predicting preeclampsia (rs7003890, p=0.03; rs8190845, p=0.009). Two htSNPs in NAT1 (rs17126345, p=0.01; rs4921879, p=0.01) and one in NAT2 (rs1799930, p=0.04) provided evidence for interactions with cadmium exposure predicting BWT. **CONCLUSION:** NAT1 may influence development of preeclampsia and infant BWT among NHB women, particularly in the context of cadmium exposure. Although evidence for association with NAT2 was limited, rs1799930 represents a slow acetylator form of NAT2 (Vatsis et al, 1991). NAT1 and NAT2 are involved in the metabolism of xenobiotics and are excellent candidates for gene*environment interactions.

2749/F

Genome-wide study of age at menarche in 1,704 US Latinas. *L. Fejerman[1], S. Huntsman[1], C. Eng[1], E.M. John[2,4], M.C. Stern[3], S.A. Ingles[3], E. Gonzalez Burchard[1,6], E. Ziv[1,5].* 1) Dept Med, Univ California, San Francisco, San Francisco, CA; 2) Cancer Prevention Institute of California, Fremont, CA; 3) Keck School of Medicine, University of Southern California, Los Angeles, CA; 4) Stanford Cancer Center, Palo Alto, CA; 5) Helen Diller Family Comprehensive Cancer Center, Univ California, San Francisco, CA; 6) Dept of Biopharmaceutical Sciences, Univ California, San Francisco, CA.

Age at menarche is a complex genetic trait related to breast cancer risk and osteoporosis. Several loci associated with age at menarche have been identified from genome-wide association studies in women of European and East Asian ancestry. Here we report results from the first genome-wide study of age at menarche in a sample of 1,704 US women of Latin American origin. We identified two new loci that showed a suggestive association with age at menarche. The US Latina samples are from the San Francisco Bay Area Breast Cancer Study and the Breast Cancer Family Registry and include both breast cancer cases and healthy controls ages 35 to 79. Genotypes were obtained using the Affymetrix 6.0 platform (~900,000 SNPs). We used imputation to define genotypes for SNPs not included in the Affymetrix chip, using HapMap phase 2 samples (Chinese, Japanese, Africans and Europeans) as references. Our final analysis included ~2,700,000 SNPs. SNPs with low MAF (<1%) were excluded from analysis. We used linear regression models to test the association between each of the SNPs (coded as counts of minor alleles) and age at menarche (defined as a continuous variable). To correct for potential confounding by population stratification, we adjusted for the top principal component of genetic variation which was a proxy for the European/Native American ancestry proportions in the study samples. The strongest associated SNPs were in chromosome 18. One is within the growth regulation by estrogen in breast cancer-like gene (GREB1L, rs9964746, p=1.6e-07) and the other is on 18q22 (rs4891982, p=3.4e-07). These loci are different from those described in East Asians and Europeans. Further investigation of the possible causative variants will shed light into the biological mechanisms that determine age at menarche and that differ between populations.

2750/F

Genome-wide association study of endometriosis in a Japanese population. *S. Adachi[1], A. Tajima[2], K. Yoshihara[1], T. Yahata[1], I. Inoue[2], K. Tanaka[1].* 1) Niigata University Graduate School of Medical Sciences, Niigata, Japan; 2) Tokai University School of Medicine, Isehara, Japan.

Endometriosis is a complex disease characterized by dysmenorrhea, dyspareunia, noncyclic pelvic pain, and infertility. To identify novel genetic contributors associated with endometriosis in Japanese women, we conducted genome-wide association (GWA) studies using two Japanese cohorts genotyped with the Affymetrix Mapping 500K Array or Genome-Wide Human SNP Array 6.0, respectively. A total of 696 Japanese endometriosis patients and 825 controls were passed our quality-control (QC) criteria based on genotyping quality, cryptic relatedness and population outliers. After applying SNP QC filters to individual chip-cohorts separately, we selected 330,753 SNPs (500K array) and 557,299 SNPs (6.0 array) for the GWA analysis. We performed a meta-analysis of the two GWA studies using the DerSimonian-Laird random-effects model to test for association between each QC-passed SNP and endometriosis. As a result, we identified five SNPs with p-value < 10^{-5}, 22 SNPs with p-value < 10^{-4} and 246 SNPs with p-value < 10^{-3} in the combined dataset. Four of the five SNPs with p-value < 10^{-5} were in high linkage disequilibrium each other and located in and around *IL1A* (interleukin 1 alpha) on 2q13. Further studies with large, independent samples are required to verify the association results.

2751/F

Ultrastructural analysis of spermatozoa in asthenozoospermic men. *S.Sh. Hayat[1], E.E. Bragina[1,2], L.F. Kurilo[1].* 1) Laboratory of Genetics of Reproductive Disorders, Research Centre for Medical Genetics, Russian Academy of Medical Sciences (RAMS) 115478, Moscow, Moskvorechie str. 1, Russian Federation; 2) Belozersky Institute, Moscow State University,Leninskie Gory 1,Bldg. 40, Russian Federation.

Introduction: Sperm motility is one of the major determinants of male fertility. Semen samples from 8150 men of different fertility status were obtained in Research Centre for Medical Genetics, RAMS. Cohort of asthenozoospermic men were examined for ultrastructural changes. Materials and Methods: 8150 semen samples were analyzed according to World Health Organization criteria. Electron microscopic studies in cohort of 98 infertile men with asthenozoospermia (a<25% or a+ b<50% (WHO 2010) were carried out. Results: Semen analysis showed that reduced sperm motility is the most common alteration in male infertility (88%). In 70% of cases low sperm motility is associated with abnormal sperm morphology. Electron microscopic studies revealed acrosome abnormalities (knobbed, ruffled and incomplete), head defects (abnormal chromatin condensation, decapitated), tail structural defects (peri-axonemal and axonemal abnormalities, lacking dynein arms), etc. Conclusion: Our study results suggest that reduced sperm motility concerns more complicated interactions involved in control of spermatogenesis, either directly or indirectly. The approach will next be identified by further investigations.

2752/F

Effect of smoking, alcohol, BMI and ROS on sperm chromatin structure in infertile men. *K. Kumar[1], J. Thilagavathi[1], S. Venkatesh[1], MB. Shamsi[1], R. Kumar[2], NP. Gupta[2], R. Dada[1].* 1) Lab for Molecular Reproduction and Genetcis, Dept. of Anatomy, AIIMS, New Delhi, India; 2) Dept. of Urology, AIIMS, New Delhi-29.

Introduction: Nicotine and alcohol are the most addictive substance widely used by human population all over the world, especially in large numbers of men at reproductive age. Body mass index (BMI) is an important criterion for any human to be fit and alteration in BMI may affect the quality of life. Effect of reactive oxygen species (ROS) and the above factors are suspected are important factors that affect men at reproductive age **Aim**: The current study was aimed to find the effect of smoking, alcohol, BMI and ROS levels on sperm DNA integrity by sperm chromatin structure assay (SCSA) in infertile men. **Methods**: 40 infertile men and 20 controls were included in the study. Semen analysis was performed as per WHO, 1999 guidelines. Flow cytometry based sperm chromatin structure assay using acridine orange dye was used to find sperm DNA fragmentation index (DFI). Smoking and alcohol consumption were ranked according to self designed scale. Height and weight were recorded and BMI was calculated. ROS level in the raw semen was measured by luminol-chemiluminescence assay. **Results**: Median (interquartile range) DFI was significantly (P<0.001) higher in infertile men compared to controls. Similarly, sperm count, motility and normal morphology were significantly lower in infertile men compared to controls. No correlation between smoking (r=0.031), alcohol consumption (r=-0.019), BMI (r=0.063) was observed. However ROS level in the neat semen showed a weak negative correlation(r= -0.125) with DFI in the study population. **Conclusion**: Various endogenous and exogenous factors affect sperm DNA integrity. However, suspected factors such as smoking, alcohol consumption, body mass index and seminal ROS levels have no profound effect on sperm DNA integrity. Therefore it is necessary to elucidate the unknown factors associated with the impaired sperm DNA integrity in this population.

2753/F

Nucleotide variations in mitochondrial DNA and supra-physiological ROS levels in cytogenetically normal cases of premature ovarian insufficiency. *M. Kumar[1], D. Pathak[1], A. Kriplani[2], A.C. Ammini[3], P. Talwar[4], R. Dada[1].* 1) Laboratory for Molecular Reproduction and Genetics, Department of Anatomy, All India Institute of Medical Sciences; 2) Department of Obstetrics and Gynaecology, All India Institute of Medical Sciences; 3) Department of Endocrinology & Metabolism, All India Institute of Medical Sciences; 4) Assisted Reproduction Technology Centre, Army Hospital Research & Referral, Delhi Cantonment, Delhi, INDIA.

Premature Ovarian insufficiency (POI) is defined as the cessation of ovarian function under the age of 40 years and is characterized by amenorrhea, hypoestrogenism and elevated serum gonadotrophin concentration (FSH). It is a heterogeneous disorder with a multicausal pathogenesis, however majority of cases are idiopathic. In idiopathic POI, involvement of unknown mechanisms may increase rate of oocyte apoptosis. Studies have shown that elevated oxidative stress (Reactive Oxygen Species) affects the quality of gametes. Mitochondrial mutations in different complexes of electron transport chain have been reported to disrupt the electron flow which leads to formation of more superoxide ions or increased levels of ROS. This study was aimed to screen the mitochondrial genome for variations in idiopathic POI (n=25) and occult ovarian insufficiency (OI) (n=5) patients. 30 diagnosed patients with POI and occult OI were enrolled in this study. Blood samples were collected from the patients and controls. DNA was extracted using phenol chloroform method. A total of 102 nucleotide variations were observed in patients as compared to 58 nucleotide variations in controls. 24% variations were found to be non-synonymous and 76% were synonymous. It was found that 48% variations were in complex I, 8% in complex III, 24% in complex IV and 20% were in complex V of electron transport chain. We found most of the non-synonymous mitochondrial variations in complex I (48%) of the respiratory chain which is the largest of enzyme complex and is associated with oxidative stress. Some non-synonymous pathogenic alterations (p.M31T, p.W239C, p.L128Q) and non pathogenic alterations (ATPase6:p.T53I, ATPase6:p.L190F, ATPase6:p.L199L) were found to be significantly higher in cases as compared to controls. The preliminary data suggest that the mitochondrial mutations and subsequent decline in ATP levels may accelerate follicular atresia and lead to premature ovarian insufficiency. The results of this preliminary study highlight the need to extend this study by analyzing large number of samples in different ethnic populations and analyze for ROS levels and mitochondrial mutations in oocytes as they are of different embryonic origin and develop in a different microenvironment.

2754/F

Genetic Variation in the Inflammatory Pathway Contributes to Preeclampsia in non-Hispanic Black (NHB) Women. *A. Buskwofie[1], G.K. Swamy[2], M.E. Garrett[1], K.S. Quinn[1], M.L. Miranda[3], A.E. Ashley-Koch[1].* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) Department of Obstetrics and Gynecology, Duke University Medical Center, Durham, NC; 3) Nicholas School of the Environment, Duke University, Durham NC.

OBJECTIVE: To examine the contribution of genetic variation in the inflammatory pathway to the occurrence of preeclampsia. **METHODS:** Healthy Pregnancy, Healthy Baby is a prospective cohort of pregnant women aimed at identifying genetic, social, and environmental contributors to disparities in pregnancy outcomes. English-literate women >18 yrs with a normal singleton pregnancy <28 wks, residing within Durham County, NC were enrolled. Data were available for 594 NHB women. Preeclampsia was defined as BP>140/90 with proteinuria and chronic hypertension (CHTN) was defined as BP>140/90 before 20 wks. A total of 73 haplotype tagging single nucleotide polymorphisms (htSNPs) were examined in the following genes: IL1A (n=7), IL1B (n=3), IL1RN (n=9), IL2 (n=3), IL4 (n=8), IL5 (n=1), IL6 (n=4), IL8 (n=2), IL10 (n=5), IL12A (n=3), L12B (n=13), IL13 (n=5), CRP (n=3), IFNG (n=2), and TNFA/LTA cluster (n=5). Genotyping was performed using Taqman assays from Applied Biosystems Incorporated. Logistic regression was used to examine the relationship between maternal genotype and preeclampsia, adjusting for CHTN, parity, infant sex, age, education, insurance, and tobacco use (as measured by cotinine levels in maternal blood). **RESULTS:** 121 participants (20.4%) were diagnosed with preeclampsia. Two htSNPs in IL1A (rs2856838, p=0.01 and rs1894399, p=0.02) and one htSNP each in IL1RN (rs2071459, p=0.04), IL12B (rs1003199, p=0.008) and LTA (rs2229094, p=0.002) were nominally significant. The two htSNPs in IL1A which were associated with preeclampsia were not in linkage disequilibrium. **CONCLUSION:** The association of IL1A and IL1RN with preeclampsia is interesting as these genes regulate opposing inflammatory effects and are located in a cluster on chromosome 2 with IL1B which was not associated in our data set. These genes have been previously implicated in the occurrence of preeclampsia (Goddard et al., 2007), suggesting that homeostasis of this part of the inflammatory cascade is important in the development of preeclampsia. The association with rs2229094 in LTA is also interesting, as this SNP is a missense mutation and LTA has been implicated in maternal-fetal genotype incompatibility as it relates to risk for preeclampsia (Parimi et al., 2008). These data add to the body of literature implicating the inflammatory pathway as being an important contributor to preeclampsia.

2755/F

Preimplantation Genetic Diagnosis (PGD) for germ line mosaicism. *G. Altarescu, T. Eldar-Geva, B. Brooks, E.J. Margalioth, E. Levy-Lahad, P. Renbaum.* Zohar PGD Lab & IVF Unit, Shaare Zedek Medical Ctr, Jerusalem, Israel.

Introduction: Single cell diagnosis for PGD requires simultaneous analysis of multiple linked polymorphic markers (in addition to the mutation) and building family haplotypes spanning at least two generations. In cases of germline mosaicism the wild type haplotype may be observed in both affected and unaffected children making the diagnosis more complex. Aims: To discriminate between wild type and mutant alleles in two families: family #1 has two children affected with severe myoclonic epilepsy (SCNA1A del exons1-22); and family #2 with 2 children with Tuberous Sclerosis (TSC1 C1327T) along with two healthy children. In both families, analysis of DNA extracted from peripheral blood lymphocytes and buccal cells was negative for the mutation, in both parents. Material and Methods: Twelve informative microsatellite markers flanking the SCNA1A gene and 12 markers flanking the TSC1 gene along with the identified mutations were used to construct haplotypes using genomic DNA. Single sperm analysis was performed using a multiplex assay that included the 12 markers and the TSC1 C1327T mutation. Results: Neither mutation was detected in genomic DNA derived from blood or buccal cells from either parent. In family #1 the deletion was demonstrated to be of maternal origin. In family #2 both affected children shared the same paternal allele but had different maternal alleles. However, one of the two healthy children also shared this same paternal allele. I In order to confirm paternal transmission we performed single sperm analysis for the mutation along with the 12 informative markers. Of 44 single sperm analyzed, 4 bared the mutant T allele, allowing linkage between the mutation and the genetic markers. Conclusions: Germline mosaicism complicates allele assignment when constructing haplotypes for PGD. Sperm or polar body analyses are useful tools for verifying allelic linkage.

2756/F

Clinical results on single cells from 470 embryos using 23-chromosome single nucleotide polymorphism (SNP) microarray preimplantation genetic screening (PGS) from 45 patients. *A. Benner[1], A.S. Gabriel[2], C. Chipko[1], D.K. Griffin[2], W.G. Kearns[1,3].* 1) Shady Grove Center for Preimplantation Genetics, Rockville, MD; 2) Dept of Biosciences, University of Kent, Canterbury, UK; 3) Dept of Gynecology/Obstetrics, The Johns Hopkins University School of Medicine, Baltimore, MD.

Objective: To amplify DNA from single blastomeres or trophectoderm cells and perform SNP microarray genetic analyses for the incidence and origin of aneuploidy. **Design:** Prospective study **Materials and Methods:** PGS patients between 12/1/09 and 4/25/10 were analyzed. 45 patients underwent embryo biopsy by laser and SNP microarray PGS primarily due to > 2 spontaneous miscarriages. 3 of these patients were tested for translocation abnormalities and aneuploidy. The maternal ages ranged from 34 to 44yrs. 398 embryos from 37 patients underwent day-3 biopsies, whereas 72 embryos from 8 patients underwent blastocyst trophectoderm biopsies. We amplified the DNA using a modified whole genome amplification (WGA) protocol. We used the Illumina Cyto-12 microarray to determine chromosome aberrations and to obtain genotype data for 300K SNPs. A high-resolution copy-number profile was used to identify copy number variations (CNVs). Parental DNA was simultaneously analyzed with embryo DNA for CNVs and heritability correlations. All aneuploidies will be classified as meiotic I, meiotic II or mitotic errors. Data was analyzed with Illumina GenomeStudio software and Bespoke software to examine informative SNPs and corresponding heterozygosity in and around the centromeric regions to determine the parent and phase of aneuploidy. **Results:** 45 families and 470 embryos were tested. 37 patients undergoing a day-3 biopsy were considered for embryo transfer along with 1 patient undergoing a blastocyst biopsy. All embryos from an additional 7 patients undergoing blastocyst biopsies and PGS were frozen. 59% (277/470) of the embryos were abnormal. Many embryos with CNVs were considered normal because of parental CNV analysis. 3.1 euploid embryos were identified per patient/cycle. The overall clinical pregnancy rate was 62%. The clinical pregnancy rate for women < 35 was 56% (5/9), for women 35-37 it was 63% (5/8), for women 38-40 the clinical pregnancy rate was 75% (9/12) and for women > 40 it was 44% (4/9). The blastocyst biopsy transferred patient is pregnant. All families are currently undergoing analysis for meiotic I, meiotic II or mitotic errors. **Conclusions:** 23-chromosome SNP microarray PGS is a valuable technology in an effort to optimize the transfer of euploid embryos and to increase the delivery rates of couples undergoing in vitro fertilization (IVF) due to repeat miscarriages.

2757/F

Validation and Clinical Application of Karyomapping for PGD Combined with 24 Chromosome Aneuploidy Screening. *G. Harton[1], A. Handyside[2], A. Gabriel[3], D. Griffin[3], R. Prates[1], S. Tormasi[1], D. Wells[4], S. Munne[1].* 1) Reprogenetics LLC, Livingston, NJ, USA; 2) London Bridge Fertility, Gynaecology and Genetics Centre, UK; 3) School of Biosciences, University of Kent, Canterbury, UK; 4) Institute of Reproductive Sciences, Oxford, UK.

Aim: To validate genome-wide SNP genotyping followed by analysis using Karyomapping and apply clinically to test embryos for a single gene defect and screen for 24 chromosome aneuploidy simultaneously. Method: Extensive in-house validation using cell lines known to be affected with, or carriers of, a specific monogenic disorder and/or known to be aneuploid was undertaken using the Illumina HumanCytoSNP-12 beadchip and Karyomapping (Handyside et al. 2009). Following these validation experiments, clinical PGD for single gene defects was performed to diagnose embryos from in vitro fertilization (IVF) cycles from fertility centers around the United States. Prior to clinical testing, DNA from various family members was collected and tested using the same SNP chip. Patients then underwent ovarian stimulation at the ART center, followed by egg collection, insemination, embryo growth and biopsy. The individual biopsied cell or trophectoderm samples were washed and placed into sterile tubes in lysis buffer for shipment to the reference PGD laboratory. Upon receipt of the sample, the embryo sample was lysed, subjected to whole genome amplification (SurePlex or REPLI-g) and finally analysed on the Illumina genotyping platform. Following SNP analysis, Karyomapping software was used to analyze the inheritance patterns of each embryo as compared to the family members. Simultaneous detection of single gene defect status was carried out along with aneuploidy screening prior to decision making on individual embryo transfer. Results: Karyomap analysis was able to detect genetic disease (carrier, affected and unaffected embryos were diagnosed) and aneuploidy status as expected. Multiple chromosome aneuploidies were detected in the embryos including both maternal (trisomy 15, monosomy 10, 20, 21 and 22) and paternal aneuploidies (monosomy 2 and 16). All Karyomap results were confirmed with arrayCGH and direct mutation testing by polymerase chain reaction (PCR). Discussion: Karyomapping combines accurate analysis of single gene defects as well as the incidence and origin of chromosome abnormalities. Partial deletions or duplications can be detected as well as whole chromosome aneuploidies and their parental and phase of origin revealed. The prevalence of chromosomal abnormalities in the patient's embryos underlines the importance of combined gene and chromosome analysis for PGD.

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Evaluation of early embryonic development and gene expression in a mouse model for Down syndrome. *M. Johnson[1], A. Pennington[1], L. Reinholdt[2], M. Davisson[2], G. Smith[1].* 1) Obstetrics and Gynecology, University of Michigan, Ann Arbor, MI; 2) The Jackson Laboratory, Bar Harbor, ME.

Despite more than 75% of human preimplantation embryos containing cells with aneuploidies and/or segmental aneusomies, little is understood about the biological effects of genomic imbalances at this early developmental stage. This study evaluated how a complex segmental trisomy affects development and gene expression of preimplantation mouse embryos. Embryos were produced in vitro using oocytes from females carrying the Rb(12.Ts171665Dn)2Cje derivative chromosome that results in triplication of ~7 mb of MMU17 and ~13 mb of MMU16, a segment syntenic with a region of HSA21 that includes the Down syndrome critical region. Embryos were monitored for development up to blastocyst stage (120 hours postfertilization) and genotyped by performing FISH on trophectodermal biopsies. The remainder of the blastocyst was lysed and frozen. Genotypic analysis of 140 blastocysts revealed 37% trisomic, 42% disomic, 12% trisomic/disomic mosaic and 2 % triploid, consistent with 50% transmission of the derivative chromosome. Review of developmental progression found no differences between genotypic classes. Lysates from 10 blastocysts of the same genotype were pooled (3 pools each, trisomic and disomic), total RNA was isolated, transcripts were amplified and gene expression was assessed using Illumina MouseWG-6 v2.0 expression beadchips. Expression data, analyzed with Illumina's BeadStudio software and the R Lumi and Limma packages, showed similar numbers of genes to be expressed in all 6 data sets (12,419 ± 752 with detection p-value £ 0.05). Within the triplicated regions, MMU16qC3.3:term and MMU17cen:qA1, respectively, 44 ± 6 of 86 and 19 ± 3 of 48 genes were expressed. Overall, gene expression profiles were very similar, with r values between 0.97 and 0.98 for each array pair. While 185 genes showed ≥1.5-fold differential expression between disomic and trisomic data sets, after FDR correction for multiple hypothesis testing, no gene was significantly differentially expressed. Of note, none of the ≥ 1.5-fold changes mapped to the triplicated regions. Studies are ongoing to validate expression of select genes from disomic and trisomic regions. These preliminary data suggest that genomic imbalances may not lead to gross disturbances in developmental progression or gene expression profiles during the preimplantation period. These findings have implications for gene expression or phenotype-based approaches for identifying preimplantation embryos with genomic imbalances.

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Single-cell, genome-wide aneuploidy, CNV, SNP and mutation profiling using microarrays and NGS for pre-implantation genetic screening, and cancer research and diagnostics. *J. Langmore, V. Makarov, E. Kamberov, T. Kurihara, J. M'Mwirichia.* Rubicon Genomics, Inc, Ann Arbor, MI.

Accurate single-cell analysis (SCA) of mutations and aneuploidies of polar bodies and blastomeres are critical for successful pre-implantation genetic diagnosis and screening (PGD/PGS). Previously, PCR and FISH SCA have been used for PGD/PGS, however advances in whole genome amplification (WGA) might enable array and NGS SCA. However WGA has never been proven to have low enough background and high enough reproducibility for array or NGS-based embryo selection. We present qPCR, array and NGS results showing that PicoPlex™ WGA enables reproducible profiling of single-gene disorders and mutations, as well as genotyping and copy number variation in single human cells. These results are also applicable to analysis of lineage of cancer tissue and circulating tumor cells (CTC).

Single RWPE cells (SV40-transformed normal prostate epithelial cells) in PBS were flow-sorted, lysed and amplified 1 million fold using PicoPlex WGA according to directions. 50 ng aliquots of amplified tumor DNA were evaluated to quantify the stochastic and systematic bias of PicoPlex. The representation and repeatability of PicoPlex WGA were tested using 48 human qPCR assays representing a large range of GC-content. Agarose gels showed a broad peak from 250 to 850 bp. About 50% of the loci were as accurately represented in aDNA as in unamplified DNA. The remaining loci were reproducibly under- or over-represented. Non-human aDNA was less than 30% of the total aDNA.

In testing by third-party reference labs, single-cell qPCR and microarray analysis was possible with >95% of the single embryo and cancer cells tested using BlueGnome, Perkin Elmer, Agilent, OGT, NimbleGen, and Illumina aCGH and SNP arrays, as well as NGS. Aneuploidy of blastomeres, polar bodies, sperm, and cancer cells were accurately tested. Deletions £75 kb were reproducibly detected. Although Illumina SNP call rates were only 50-60%, LOH was less than 10%, showing that the SNPs were accurately called. PCR-based SNP and mutation analyses were 95% accurate.

These results translate into increased accuracy and reproducibility of single-cell PGD/PGS testing using PCR, microarrays and NGS, and open the doors to successful genetic profiling of cancer and stem cells for research and diagnostics.

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Preimplantation Genetic Diagnosis for GM1-Gangliosidosis, Type I with Linked Polymorphic Markers. *E. Pomerantseva, D. Pauling, O. Verlinsky, S. Rechitsky.* RGI, Chicago, IL.

GM1-Gangliosidosis (MIM #230500) is an autosomal recessive lysosomal storage disease characterized by the accumulation of ganglioside substrates in lysosomes. GM1-Gangliosidosis is caused by the deficiency of beta-galactosidase-1, which is coded for by the GLB1 gene located in gene map locus 3p21.33. Clinically, patients show variable degrees of neurodegeneration and skeletal abnormalities. There are 3 main clinical variants categorized by severity and variable residual beta-galactosidase activity. Type I, or infantile form, shows rapid psychomotor deterioration beginning within 6 months of birth, generalized central nervous system involvement, hepatosplenomegaly, facial dysmorphism, macular cherry-red spots, skeletal dysplasia, and results in early death. A preimplantation genetic diagnosis (PGD) cycle was performed for a family which lost one child to GM1-Gangliosidosis and had to terminate another pregnancy after a CVS diagnosis revealed the fetus to be affected with the same disease. There are two different mutations of GLB1 gene in this family. The partner is heterozygous for H281Y and the patient is heterozygous for c.1310delA. Their only surviving child carries the paternal mutation. Linkage was established by analysis of family members including DNA from the recent affected pregnancy. Nested and hemi-nested multiplex PCR protocol was developed for both mutations and tightly linked Short Tandem Repeats (STRs). The final protocol included the following polymorphic markers: STR1, STR2, STR3, D3S4525, D3S1619, D3S3512, D3S1768, D3S1277, along with the mutations. The mutations were studied by restriction endonuclease digestion followed by gel electrophoresis, while polymorphic markers were analyzed by fluorescent fragment analysis. The family underwent one cycle of IVF. PGD was performed on single blastomeres. Out of 19 embryos 5 were found to be normal non-carriers, 6 were normal carriers, 6 were predicted to be affected and 2 embryos were abnormal due to aneuploidy for chromosome 3. Overall 11 embryos were cleared for transfer, of which 6 were frozen and 2 transferred, resulting in a singleton pregnancy. Amniocentesis, performed by an independent laboratory, confirmed our PGD results, and a healthy non-carrier female child was born.

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First experience of preimplantation genetic diagnosis (PGD) for de-novo mutations. *S. Rechitsky, K. Pamerantseva, T. Pakchalchuk, D. Pauling, O. Verlinsky, A. Kuliev.* Reproductive Genetics Inst, Chicago, IL.

PGD is currently performed for inherited conditions with known parents' mutations, detected by direct mutation analysis or haplotyping in oocytes or embryos. However, this cannot be applied when parent(s) or affected children have a de-novo mutation, as neither origin, nor relevant haplotypes are available for testing in single cells. We developed the PGD strategy for a total of 102 families with 38 different genetic disorders, determined by 33 dominant, 3 recessive and 2 X-linked de novo mutations. 97 families were with dominant mutations, of which 40 were of paternal origin, including 2 cases of gonadal mosaicism, 50 of maternal origin, including 1 with gonadal mosaicism, and 7 detected for the first time only in the affected child. PGD design for the following dominant de-novo mutations were developed: atypical Rett syndrome, brachidactily, brain tumor SMARC B1, corneal dystrophy, Darier disease, Diamond-Blackfan anemia, Emery-Dreifus disease, facioscapulohumeral muscular dystrophy, hereditary multiple exostoses, familial adematous polyposis, incontinentia pigmenti, osteogenesis imperfecta 1, retinoblastoma, spinocerebral ataxia 6, metaphyseal dysplasia, MEN 1, MEN 2B, NF1, NF2, optical atriophy 1, tuberous sclerosis type 1 and 2, Marfan, Gorlin, Kalman, Crouson, Peutz-Jeghers, Pfieffer, Loes-Dietz, Treacher Collins, Sotos, Strickler and Von Hippel-Lindau syndromes. All 3 PGD cases for de-novo recessive disorders were of paternal origin, including cystic fibrosis, SMA and Fanconi anemia. PGD for 2 X-linked de-novo mutations included PGD for chronic granulomatosys and incontinentia pigmenti. The development of specific PGD strategy for each of the couples involved an extensive DNA analysis of the parents and affected children prior to PGD, including the mutation verification, polymorphic marker evaluation in blood, whole and single sperm, and polar body analysis in order to establish the normal and mutant haplotypes. Overall, 152 PGD cycles have been performed for 82 of 102 families under study, for which a specific PGD design has been established, resulting in preselection and transfer of 240 (1.83 per cycle) mutation free embryos in 131 (86%) cycles, yielding 62 (54.1%) unaffected pregnancies and birth of 55 healthy children, confirmed to be free of the de-novo mutations tested. The data show feasibility of PGD for de-novo mutations, which may now be routinely performed with the accuracy of over 99%, using the established PGD strategy.

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Direct evaluation of CVS: Impact of switching from karyotypes analysis to FISH. *R. Schreck, L. Freilich.* Medical Genetics Institute & Pathology & Lab Med, Cedars-Sinai Med Ctr, Los Angeles, CA.

The purpose of chromosome analysis in prenatal diagnosis is to detect chromosome abnormalities responsible for abnormal development in time for intervention, if desired, and to relieve parental anxiety. Thus both accuracy and timeliness are key components of the process. To address the time factor, many laboratories are offering a rapid, preliminary screen of amniocytes, either by FISH or PCR. Chorionic villus sampling (CVS) which occurs during the first trimester and addresses the time issue, is complicated by the presence of chromosome abnormalities that are restricted to the extra-embryonic tissues or confined placental mosaicism (CPM) in about 1-2% of studies. In addition, the not infrequent discrepancies between results obtained from "direct" preparations and cultured cells, often lead to additional prenatal testing by amniocentesis. We have looked at the use of FISH with probes for chromosomes 13, 18, 21, X and Y and its effects on the levels of accuracy and identification of CPM in CVS studies, both by retrospective analysis of studies using chromosome analysis of spontaneously dividing cells, and by comparing the abnormality rates in this population with a matched population in which only FISH was employed for direct analysis. Evaluation of 8739 CVS analyses in which direct chromosome analysis was done yielded 441 abnormal results (5%) of which 51 (0.58% of all studies) would have been missed by FISH. However, of the "missed" abnormalities where follow-up studies were available less than half (40%) were confirmed by amniocentesis or termination tissue. This suggests that the majority of abnormalities missed by the FISH approach to rapid CVS results represent CPM which raises parental anxiety but is not indicative of an actual chromosome abnormality in the fetus. The rare true abnormalities (2/1000 procedures) missed by the FISH approach were all detected by analysis of the CVS culture. Thus FISH is actually preferable to chromosome analysis as a rapid screen in CVS, as it can reduce the need for second invasive procedures in normal pregnancies.

2763/F

Genetic factors in the spermatogenesis of infertile males. *M.S. Juchniuk de Vozzi[1], C.S. Pereira[1], S.A. Santos[1], L. Pola[1], P.A. Vozzi[1], M.A.C. Vasconcelos[2], A.C.J.S. Rosa-e-Silva[2], P.A. Navarro[2], L. Martelli[1].* 1) Dept of Genetics, School of Medicine of Ribeirão Preto, University of São Paulo, Brazil; 2) Dept of Gynecology and Obstetrics, School of Medicine of Ribeirão Preto, University of São Paulo, Brazil.

Male infertility is responsible for 50% of in vitro fertilization procedures. Semen analysis to assess the male factor is the first step for infertility investigation. Additionally, genetic analysis of sperm provides information about numerical and structural chromosomal aberrations which were able to finish meiosis without being detected by meiotic checkpoints. Apoptosis is a type of cell death involved in different steps of spermatogenesis, first at puberty, at the beginning of spermatogenesis and subsequently in adult gonads, controlling the normal spermatogenesis. Several studies have shown that the deregulation of apoptosis in germ cells can result in male infertility. The objective of this study is to evaluate the meiotic segregation of gametes and the frequence of apoptotic germ cells of oligozoospermic men with a diagnosis of infertility. We have studied semen samples of fifteen infertile men (group I) and seven male normal controls with proven fertility (group II). Cytogenetic studies of peripheral blood were also carried out for both groups. The fluorescent in situ hybridization (FISH) technique was used for evaluation of the meiotic segregation of chromosomes 3, 13, 21, 22, X and Y in the gametes. The presence of apoptosis was detected by externalization of phosphatidylserine using annexin V-labeled fluorescein. We have detected heteromorphism of chromosome 9 in 13.3% of the infertile man karyotypes and one patient had a marker chromosome. The frequency of total aneuploidies of chromosomes was 6, 88% in infertile men and 3.98% in the control group. The frequencies of disomies of chromosome 3, 13, 21, 22, XY, XX and YY respectively was 0.34%, 0.51%, 0.77%, 0.65%, 0.68%, 0, 19% and 0.20% in infertile patients. Infertile men and controls showed significant differences ($p<0.05$) in the frequencies of aneuploidies of chromosomes 13, 21, 22 and XY disomy. The differences between the groups could be explained by failure in meiotic recombination, interfering with the correct process of segregation. Our results revealed a significant difference between the incidence of apoptotic cells in infertile men and controls ($p<0.05$). The increased frequencies of apoptotic gametes in infertile men could be explained by the process called abortive apoptosis. The possible relationship between total disomies and sperm morphology suggests that the selection of gametes would be strictly controlled in spermatogenesis, being unregulated in oligozoospermic patients.

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BCL2 Anti-apoptotic Gene Alterations in Repeated Pregnancy Loss. *S.M. Seyedhassani[1,2], M. Houshmand[1], A. Aflatoonian[2], S.M. Kalantar[2], G. Modabber[1].* 1) Med Gen, National Inst Gen, Tehran, Iran; 2) Research and Clinical center for Infertility, Yazd, Iran.

Introduction: Recurrent pregnancy loss (RPL) is a critical medical problem in about 0.5-2% of women. Molecular genetics background for spontaneous abortion is being understood more and more, and some polymorphisms have been reported so far. This study investigates the alterations of BCL2 gene as an anti-apoptotic gene in women with idiopathic RPL. Material and methods: The frequency of mutations in BCL2 gene of 96 idiopathic RPL women was studied here in comparison to a sample of 96 healthy women. The promoter and the entire coding regions were amplified using a polymerase chain reaction (PCR). The PCR products were documented by 2% agarose gel electrophoresis, and were verified by an automated DNA sequencing method. The result of DNA sequence analysis was compared with the NCBI database using the Clustal X program. Results: The mean age of the women with RPL at the time of blood sampling has been 28.73 and the mean gestational age at the time of miscarriages has been 10.35. The mean age of control group has been 30.20 while they had 2- 4 children. Sequencing of BCL2 gene showed G66C variation in the exon 1 of all RPL and control women and A735G variation in exon 2 of both groups. In A735G variation, there were AA, AG and GG genotypes in 41(42.7%), 40(41.7%) and 15(15.6%) of the RPL and 31(31.3%), 46(47.9%) and 19(19.8%) of the control women respectively. This difference was not significant (P= 0.32) and both of them encode amino acid threonine. There was not a significant association between A and G alleles at nucleotide 735 and the occurrence of RPL (P= 0.14). Discussion: Our result does not indicate any significant variant in BCL2 gene which affects apoptotic process in RPL women. However, the decreased expression of BCL2 is reported in aborted chorionic villi and the importance of BCL2/BAX ratio is shown in different stages of fetal growth and development. The study of post transcriptional and post translational modifications is recommended to clarify the possible role of BCL2 gene in RPL especially on aborted material.

2765/F

RNA-based genetic study of teratozoospermia; *ZPBP1* mutations associated with abnormal semen morphology in infertile men. *A. Yatsenko[1,2], D. O'Neil[1], A. Roy[1], P. Arias-Mendoza[1], R. Chen[1], L.J. Murthy[3], D.J. Lamb[3,4], M.M. Matzuk[1,2,4].* 1) Pathology, Baylor College of Medicine, Houston, TX; 2) MH Genetics, Baylor College of Medicine, Houston, TX; 3) Urology, Baylor College of Medicine, Houston, TX; 4) Molecular and Cell Biology, Baylor College of Medicine, Houston, TX.

Male infertility is a global health problem of multifactorial etiology, affecting nearly 7% men. It is estimated that genetic factors account for at least 50% of cases of male infertility. Teratozoospermia is a common semen abnormality condition associated with male infertility. It is defined as an abnormal morphology with less than 4% of the normal spermatozoal content. However, genetic defects that cause teratozoospermia are mainly unknown in the majority of patients. In the present study, we investigated the role of zona pellucida binding protein, ZPBP1, in teratozoospermia in humans. Previous studies demonstrated that male mice with the disrupted ZPBP protein were unable to fertilize oocytes due to abnormal spermatozoal morphology and forward sperm motility. Electronic microscopy of spermatozoa showed abnormal ultrastructure, round headed, globozoospermia-like morphology. The ZPBP is a structural protein, predominantly expressed in spermatozoal acrosome, and plays important role in acrosome reaction, binding to oocyte zona pellucida, and ultimately in oocyte penetration. Therefore, it was suggested that abnormal morphology affects acrosome compaction and oocyte penetration that results in inability to fertilize oocytes. To study association between *ZPBP1* mutations and teratozoospermia, we selected 192 cDNA samples from infertile males with abnormal semen morphology and 100 normozoospermic controls. Following RT-PCR, DNA and cDNA sequencing have been performed with patients and control samples. Preliminary, we identified several novel heterozygous missense and splicing alterations in mRNA from teratozoospermic semen samples. The observed cDNA defects were confirmed in the genomic DNA. Analysis of 100 controls did not identify these alterations. In addition, none of these nucleotide changes are listed as known polymorphisms in the dbSNP database. Our preliminary data suggest that mutations in *ZPBP1* may contribute to a significant fraction of teratozoospermia in infertile men. Further genetic studies are in progress. This study was supported in part by the National Institutes of Health Infertility Center (P01HD36289) and U54 the Specialized Cooperative Centers Program in Reproduction and Infertility Research to DJL and MMM.

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Rare allele tagged SNPs can identify the Tay Sachs 1278insTATC allele for a universal Preimplantation Genetic Diagnostic assay. *P. Renbaum[1], S. Katz[1,3], S. Zeligson[1], S. Perlberg[1], T. Eldar-Geva[2,3], E.J. Margalioth[2,3], E. Levy-Lahad[1,3], G. Altarescu[1,3].* 1) ZOHAR PGD Unit and Genetics Institute, Shaare Zedek Medical Center, Jerusalem, Israel; 2) IVF Unit, Shaare Zedek Medical Center, Jerusalem, Israel; 3) Hebrew University Medical School, Jerusalem, Israel.

Background: Preimplantation Genetic Diagnosis (PGD) relies on genetic information obtained from a single cell. In addition to mutation detection, accurate diagnosis includes identification of the affected allele by linkage using multiple informative markers. Routinely in PGD, families are first haplotyped using fluorescently labeled Short Tandem Repeat (STR) markers, and a specific multiplex assay is constructed for each case and then used on single cells (polar bodies or blastomeres) for PGD. While STRs are highly variable, they are limited in number, and in some instances it is difficult to find sufficient informative assays flanking the mutation. SNP markers have only two variants (A and B) and as such make the distinction of specific alleles in blastomeres difficult; however, they are present at a much higher frequency throughout the genome. Aims: To examine the use of SNP markers as a tool for PGD diagnosis, and the identification of a universal set of SNP tags which specifically and differentially detect the Tay Sachs (TS) HEXA 1278insTATC Ashkenazi Jewish (AJ) founder allele. Materials and Methods: We genotyped sixteen unrelated carriers of the TS 1278insTATC mutation using Affymetrix 250k Nsp arrays. A region of +/- 2Mb, including 221 SNPs surrounding the HEXA gene, was compared to 40 non-Tay Sachs, unrelated, AJ controls. Results: Heterozygote frequencies of eleven specific SNPs were 5-8 times higher in TS carriers than in AJ controls, suggesting that the 1278insTATC founder mutation occurred on a rare allele. This set of 11 SNPs, represent a rare allele tag and can be used to identify the TS 1278insTATC allele in any carrier. Conclusions: Since this TS allele is very rare, the corresponding wild type allele from either parent can be easily distinguished in blastomeres using a TS universal 11 SNP assay in any carrier couple, without the need to prepare family specific assays. When founder mutations occur on uncommon alleles, a similar strategy can be used to identify a set of rare allele tagged SNPs (RATS) for the establishment of specific universal PGD assays for other disorders.

2767/W

Aggregating information across loci when testing the genome for associations. *N. Cardin, J. Witte.* Human genetics, Epidemiology and Biostatistics, UCSF, San Francisco, CA.

There is much current interest in elucidating the role of rare genetic variation in common human disease. However the analysis of rare variants is complicated by the inherent lack of power that one can achieve with sparse data. In order to address this challenge it has become common to combine the effects of multiple rare SNPs [1,2,3], for example within regions or pathways. In effect this approach assumes that all combined variants have the same effect and tests the genes or pathways themselves for an association with disease. However, there is no agreement on how to aggregate these variants and most current approaches fail to jointly utilize the evidence from common variants. A common approach taken [1,2] is to choose a frequency threshold, below which variants are aggregated whilst above this threshold variants are analyzed individually. However there is no optimal cut off for such a procedure and in principle we can gain power by combining signals of association across common and rare variants. One approach to this is to use a multi-SNP regression model, under which each variant has its own effect size. However, because of sparse data and correlations induced by LD, this can lead to instability in results and very large confidence intervals for effect size estimates. We propose to take a fully Bayesian approach where effects are sampled from the joint posterior allowing the evidence for association across a set of SNPs (eg. within a gene) to be more robustly examined. It is not yet known to what extent causative variants cluster, eg. within genes or pathways, or to what degree certain classes of SNPs may be enriched amongst causative loci, eg. non synonymous SNPs or variants which interrupt protein function. Even if estimates are taken from the literature these effects may vary from disease to disease. We place a hierarchical prior on the distribution of disease effects which allows the data to inform the degree of clustering of causative loci within a variety of structures. If the literature does provide relevant information then this is also a natural structure within which to make use of it. To demonstrate the utility of the method we provide results from simulated data where we investigate the power of the method under various scenarios and the stability of the results compared to maximum likelihood inference. [1] Morgenthaler and Thilly, Mutat Res, 2007 [2] Madsen and Browning, PLoS Genet, 2009 [3] Li and Leal, Am J Hum Genet, 2008.

2768/W

Using treelets to smooth complicated pedigrees. *A. Crossett[1], N. Melhem[2], FA. Middleton[6], L. Klei[2], S. Tiobech[3], C. Otto[3], AB. Lee[1], B. Devlin[2], W. Byerley[5], M. Myles-Worsley[4], K. Roeder[1].* 1) Department of Statistics, Carnegie Mellon University, Pittsburgh, PA; 2) Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, PA; 3) Belau National Hospital, Korror, Palau; 4) Department of Psychiatry, University of Utah, Salt Lake City, UT; 5) Department of Psychiatry, University of California, Irvine, CA; 6) Department of Neuroscience and Physiology, College of Medicine, State University of New York, Syracuse, NY.

Beyond a few degrees of relationship pedigrees are rarely known with absolute certainty. This uncertainty is often elevated in population isolates, in which all extant individuals trace their ancestry to a limited number of founders. As noted by Choi et al. (Genet Epidemiol, 33:668-78, 2009) this cryptic relatedness can have a detrimental impact on nominal false positive rates for genetic association tests. Choi and colleagues develop an algorithm overcoming this problem: first they estimate the relatedness of all pairs of individuals assessed for association; then they adjust a chi-square test for association on the basis of relatedness. It is important to recognize that using genotype information to estimate relationships between pairs of individuals can be very noisy.

Treelets are an adaptive approach to dealing with noisy, high-dimensional and unordered data. Treelets simultaneously construct a hierarchical tree and an orthonormal basis that represent the internal structure of the data. We propose to use treelets on estimated relationship data by examining each individual's relationship to everyone else. Noise is removed by identifying the most important features of the basis and then reconstructing the data. We apply these techniques to data from Palau, an Oceanic nation of relatively recent origin in human history. These data are part of an ongoing project to understand the genetic basis of schizophrenia.

2769/W

Mixed Model Coexpression (MMC): calculating gene coexpression under the presence of systematic confounding. *N. Furlotte[1], H.M. Kang[4], C. Ye[3], E. Eskin[1,2].* 1) Department of Computer Science University of California Los Angeles, CA; 2) Department of Human Genetics University of California Los Angeles, CA; 3) Department of Computer Science University of California San Diego, CA; 4) Department of Biostatistics, University of Michigan, Ann Arbor, Michigan.

The analysis of gene coexpression patterns is at the core of many types of genetic analysis. The coexpression between two genes can be easily calculated using a simple Pearson's correlation coefficient. However, technical confounding, such as batch effects, may cause inflation of the Pearson's correlation so that uncorrelated genes appear to be highly correlated. Many methods have been suggested which aim to correct gene expression data for confounding effects. Methods such as COMBAT are applied in order to correct gene expression data for known batch effects. Another method, Surrogate Variable Analysis (SVA), does not depend on a priori knowledge of confounding effects, but rather incorporates the observed correlation structure of the gene expression data into the correction procedure. SVA uses the observed inter-array correlation in order to estimate a discrete number of "surrogate variables", which represent the contribution to the measured gene expression by a set of unknown confounding factors. These factors can be regressed out in order to obtain a corrected dataset. Both COMBAT and SVA have their drawbacks. Namely, they both rely on the assumption that the effects of confounding can be estimated with a discrete number of factors and it is unlikely that this assumption will hold as the patterns of confounding increase in complexity. In this paper, we present a statistical model for calculating gene coexpression called Mixed Model Coexpression (MMC). Our MMC method models coexpression using a mixed model framework, in which technical confounding effects are represented as a random variable in a statistical model for coexpression. Our method assumes that technical confounding effects can be modeled with a random component and that this random component has variance proportional to the inter-array correlation matrix. The key intuition is that technical confounding effects cause the overall array expression patterns to be similar, causing arrays to appear highly correlated. This increased inter-array correlation induces correlation between genes. By utilizing the observed inter-array correlations, we are able to inform our calculation of coexpression, and effectively remove many of the spurious gene correlations. We apply our method to human and yeast datasets and show it is better able to effectively prioritize strong coexpressions when compared with a standard Pearson's correlation and a Pearson's correlation applied to data corrected with SVA.

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Composite likelihood-based meta-analysis of breast cancer genome-wide association data. *I. Politopoulos[1], W. Tapper[1], J. Gibson[1], S. Ennis[1], D. Eccles[1,2], A. Collins[1].* 1) Genetic Epidemiology & Bioinformatics Group, Human Genetics Research Division, School of Medicine, University of Southampton, Southampton, Hampshire, United Kingdom; 2) Cancer Sciences Division, School of Medicine, University of Southampton, Southampton, Hampshire, United Kingdom.

We have implemented a model which tests association with disease in genomic regions defined on a linkage disequilibrium unit (LDU) map. This approach has the advantages of evaluating the evidence for association in fixed regions describing comparable levels of linkage disequilibrium, combining the evidence for association from multiple single nucleotide polymorphisms (SNPs) and requiring a substantially reduced correction for the number of statistical tests, compared to single SNP-based analysis. The composite likelihood model provides a point estimate of location which becomes more precise as sample sizes are increased through meta-analysis and information weights facilitate combination of evidence and increase power (*Tapper et al, BMC proceedings 2007 1:S18*). Power is further enhanced by imputation of missing genotypes to increase SNP coverage in each region. P-values from each sample are combined through a weighted Z-transform test (*Whitlock, J Evol Biol 18, 2005, 1368-1373*). As a demonstration of the approach we combined association evidence from 1,143 breast cancer cases and 1,139 controls from the Cancer Genetic Markers of Susceptibility (CGEMS) study (with 498,786 SNPs genotyped and 488,991 imputed) and 280 early onset breast cancer cases from the Prospective Study of Outcomes in Sporadic versus Hereditary breast cancer (POSH) and 5,200 Wellcome Trust Case Control Consortium (WTCCC) Phase 2 controls (with 506,610 SNPs genotyped and 472,799 imputed). Evidence was combined across ~14,000 genomic regions each spanning four LDUs. The point estimate with the strongest association signal in the combined sample is within FGFR2 on chromosome 10 (chi-square of 24.51). Other high ranking signals were identified in the FBN1 gene (chromosome 15, chi-square 14.47) and NTSR1 (chromosome 20, chi-square 12.93). We anticipate extension to include additional breast cancer genome-wide association data sets, including further samples from the POSH consortium, which will increase the understanding of underlying genetic risk factors involved in early onset breast cancer.

2771/W

A gene-based test of association. *C.S. Tang, M.A. Ferreira.* Genetic Epidemiology, Queensland Institute of Medical Research, Brisbane, Australia.

Results from recent genome-wide association studies indicate that multiple independent risk loci may be present in the same region, suggesting that association methods that can test multiple SNPs simultaneously may have improved power to identify genes with weak effects on disease risk. To address this possibility, we extended our recently developed multivariate test of association that is based on canonical correlation, to test the strength of association between a set of SNPs and a phenotype. This approach is applicable to both continuous or discrete traits measured in unrelated individuals. Simulations show that this test has improved power when compared to standard single-locus analyses when there are multiple independent risk loci in a region. We illustrate the applicability of this novel approach by analysing data from a genome-wide association study of asthma.

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A novel genome-information content-based statistic for genome-wide gene-gene co-association analysis designed for next-generation sequencing data. *M. Xiong, L. Luo, Y. Zhu.* Dept Biostatistics, Univ Texas Hlth Sci, Houston, TX.

The current popular methods for testing association of multiple loci are designed for common alleles. The critical barrier in multi-locus analysis for rare variants is as follows. First, multiple rare variants that jointly have significant risk effects, but individually make only a small contribution. In the presence of allelic heterogeneity, the power of current variant-by-variant tests for interaction between rare variants will vanish. Second, new sequence technologies are highly error prone. Due to their large genotyping errors and low frequencies, the signal of rare alleles may be compatible with genotyping errors. Unless genotyping errors are properly taken into account, many multi-locus tests will be invalidated. The great challenges for successful association studies incorporating interaction are (1) lack of deep understanding measure of interaction and statistics with high power to detect interaction or to test for the association taking interaction into account , (2) lack of concepts, methods and tools for detection of interactions for rare variants, (3) severe multiple testing problems, and (4) heavy computations. To meet these challenges, we propose a novel concept of gene-gene co-association in which a gene is taken as a unit of association analysis and shift the paradigm of association studies from discrete loci to continuous genome regions, which allows us to extend association test from common alleles to both common and rare alleles and provide a unified framework for association analysis of both common and rare alleles. We validated the null distribution and the type I error rates of the new statistic through intensive simulations. To evaluate its performance for gene-gene co-association analysis , the proposed test statistic was applied to two independent GWAS datasets of psoriasis. The first dataset included 955 individuals with psoriasis and 693 controls with typed 443,018 SNPs, and the second dataset included 466 individuals with psoriasis and 732 controls with typed 439,201 SNPs. We found that In total, 20 pairs of genes showed significant evidence of co-association in two independent studies. These 20 pairs of genes included 27 distinct genes located in immunology, inflammatory, cytokines-chemokines and cell signaling pathways.

2773/W

Detection of Disease-related recent common ancestral haplotypes. *D.G. Ying[1], W.L. Yang[2], L. Zhang[2], P.S. Sham[1], Y.L. Lau[2].* 1) Department of Psychiatry, The University of Hong Kong, Hong Kong; 2) Department of Paediatrics, The University of Hong Kong, Hong Kong.

Genome-wide association studies (GWAS) are usually followed by selection a number of SNPs with significant disease association P value. Genetic variants with high penetrance can be effectively detected by GWAS but remains the variants with low penetrance. Haplotype allele with these variants can be inherited from a recent disease ancestor and shared by a small portion of disease population. We have developed a program that can detect the haplotype allele shared by several case individuals with unknown relationship out of disease population using normal Plink format genotype data. Thousands of simulated cases with different haplotype age and number of shared individual are performed for the testing while The results shows well for detecting the age of the haplotype from 10 to 50 generations for at least 4 individuals. Those haplotype alleles with high rank usually have rare block combinations and are highly suspicious to have disease related variants and worth to do further analysis. The time and space requirement for the program is linearly related to number of overall SNPs and square of the number of population size.

2774/W

Genome-Wide Association Study of Specific Language Impairment using a gene-based approach. *P. Evans[1], E. Gamazon[1], K. Kelsey[2], J. Tomblin[2], D. Nicolae[1], N. Cox[1].* 1) Gen Med, Univ Chicago, Chicago, IL; 2) Speech Pathology and Audiology, Iowa City, IA.

Specific language impairment is a highly heritable disorder characterized by deficits in language development in a child despite normal overall intelligence. We performed a genome-wide association study on 429 genetically European individuals on the Affymetrix 6.0 genotyping chip for two different phenotypes: lcompf (change these phenotype names) and ldiagf. A third phenotype ppcompf, was analyzed in 258 genetically European individuals for which we had data on this phenotype. LCOMPF is a measure of language ability. LDIAGF is a dichotomous trait based on LCOMPF, with LCOMPF scores less than -1.1 (roughly 10%) leading to an affected status for LDIAGF. The PPCOMPF phenotype represents a measure of phonological memory and awareness thought to be impaired in individuals with SLI. The genome-wide association results using the HapMap imputed SNPs for each phenotype were used to perform a functional genome-wide association study. Nonsynonymous SNPs and eQTLs are included in a gene-based analysis. This allows us to look at the functional SNPs for each gene and combine p-values of those SNPs to give an overall gene-based score. The gene NRN1, is the top gene for the lcompf phenotype with and without the parental education as a covariate. NRN1 is involved in postmitotic-differentiating neurons of the developing nervous system and is also involved in the development of neuronal structures involved in plasticity in adults. In vitro assays have shown that this gene promotes neurite outgrowth. The function of the most significant genes for other phenotypes have functions that are not as clear for how they would cause SLI. The top genes using the ldiagf phenotype are OCIAD1, C6orf108, ALDH3B2, DPY19L2P1, LIPI, and IFNGR2. OCIAD1 is thought to be a cell adhesion protein overexpressed in ovarian cancer. ALDH3B2 is involved in alchohol metabolism and lipid peroxidation. DPY19L2P1 is a pseudogene of a membrane protein. LIPI is a membrane associate phospholipidase. and IFNGR2 is interferon gamma receptor and is involved in susceptibility to mycobacterial infection. The top genes for ppcompf are PXMP4, and ALDH3B2. PXMP4 is a peroxisomal membrane protein, and ALDH3B2 is also seen in LDIAGF.

2775/W

A framework for assessing association between gene and disease by evaluating component SNPs. *B.A. Goldstein[1], A.E. Hubbard[1], E.C. Polley[1], L.F. Barcellos[2], IMSGC.* 1) Division of Biostatistics, Univ California, Berkeley, Berkeley, CA; 2) Division of Epidemiology, Univ California, Berkeley, Berkeley, CA.

The primary goal of large SNP association studies is to identify genes that are associated with disease. However, most methods involve analyzing variation only at the level of the SNP. This approach ignores composite properties of variation within a gene that may be more explanatory of function. Currently available methods that attempt to examine a particular gene as a unit (e.g. haplotype analysis, rare variant analysis) are limited by their assumptions and applications. We propose a method to define a functional form for the effect of multiple SNPs within a gene on an outcome, providing a means to better understand the gene-disease relationship. The function for each gene is derived using the Super Learner algorithm, an ensemble learner that makes no assumptions about the underlying causal mechanism. A novel wald-like test of association is performed between the gene's derived function and the chosen outcome, to detect genes of interest. A summary measure is also reported based on the area under the curve (AUC). Theoretic and simulation results show this method maintains perfect Type I error control when the null is true. Moreover, it is able to detect true associations that would otherwise be missed based on marginal testing. We apply this method to a large candidate gene study in multiple sclerosis, comprised of more than 2,000 genes with 27,000 SNPs and ~3,000 individuals. Using FDR for Type 1 error control, our procedure identifies 76 genes across 10 chromosomes (10 located outside the MHC on chromosome 6). Typical univariate testing identifies 70 genes across 7 chromosomes (8 located outside the MHC). This procedure provides a new approach for considering SNP data, with an emphasis on the gene, rather than individual polymorphism(s).

2776/W

Association between SLC2A9 transporter gene variants and the serum uric acid phenotype reveals within-gene differences between persons of European and African ancestry. *S.T. Turner[1], A.D. Rule[1], M.E. Matsumoto[2], T.H. Mosley[3], S.L.R. Kardia[4], M. de Andrade[2].* 1) Div Nephrology & Hypertension, Mayo Clinic, Rochester, MN; 2) Div Biomedical Statistics and Informatics, Mayo Clinic, Rochester, MN; 3) Dept Geriatric Medicine, University of Mississippi Medical Center, Jackson, MS; 4) Dept Epidemiology, University of Michigan, Ann Arbor, MI.

It is well known that increased uric acid levels lead to gout, kidney stones, hypertension, and cardiovascular disease. Uric acid is filtered, reabsorbed, and secreted in the kidney under regulation by the SLC2A9 transporter in renal tubules. Genetic variation in the SLC2A9 gene has an established association with serum uric acid in European ancestry populations. The Genetic Epidemiology Network of Arteriopathy family-based cohorts underwent study visits between 2001 to 2005 that included a blood draw and a spot urine collection. Genotype was obtained using the Affymetrix Genome-Wide Human SNP Array 6.0. The associations between SNPs in the SLC2A9 transporter gene and each phenotype were assessed using a linear mixed effects model taking into account the family relationship. Analyses were adjusted for age, sex, diuretic use, body mass index, homocysteine, and triglycerides in a parsimonious model after considering 30 covariates with known associations with uric acid. A gene-level Bonferroni corrected p-value <0.01 was considered statistically significant. We identified SLC2A9 gene variants showing association with serum uric acid in 1155 subjects of African ancestry (53 SNPs) and 1132 subjects of European ancestry (63 SNPs). SNPs in the first half of the gene showed weakly significant association in subjects of African ancestry but not in subjects of European ancestry. SNPs in the latter half of the gene were the most statistically significant SNPs for both cohorts, and generally were more significant in subjects of European ancestry than African ancestry. The most statistically significant SNP was in the latter half of the gene and explained 2.77% and 2.71% of the variation in serum uric acid in subjects of European and African ancestry, respectively. After adjustment for the most statistically significant SNP, 0.86% of the variation in serum uric acid in subjects of African ancestry was explained by a SNP in the first half of the gene. These SNPs were not associated with differences in fractional excretion of urate or urine urate to creatinine ratio. These findings show that SLC2A9 transporter variants associate with serum uric acid levels in subjects of African and European ancestry, but there are race-differences in the specific gene regions that most affect serum uric acid levels.

2777/W

A Genome-wide Association and Imputation Software Pipeline for The Identification of Shared Susceptibility Loci. *D. Ellinghaus[1], M. Nothnagel[2], S. Schreiber[1,3], A. Franke[1].* 1) Institute of Clinical Molecular Biology, Christian-Albrechts-University, Schittenhelmstr. 12, 24105 Kiel, Germany; 2) PopGen Biobank, Christian-Albrechts-University, 24105 Kiel, Germany; 3) First Medical Department, University Clinic S.-H., Campus Kiel, Germany.

Genome-wide association studies (GWAS) and subsequent meta-analysis projects have been successful in identifying novel genetic susceptibility factors for several autoimmune and inflammatory diseases. Although many examples exist that different disorders share common genetic risk loci (e.g. IL23R in Crohn's disease, ulcerative colitis and psoriasis; NOD2 in Crohn's disease and sarcoidosis), systematic and genome-wide approaches are scarce. We therefore developed a fast and flexible genome wide association and imputation software pipeline for an automated processing of genome-wide association data. Different and complementary analysis strategies are currently developed to identify common disease loci and to prioritize associated loci for follow-up studies: GWAS datasets will be combined via confirmed-marker-, joint-, ranking-, meta- and difference-analyses. The software pipeline is implemented in Python and R in an object-oriented style, makes use of the open source PLINK library and the BEAGLE software package, and runs on a Linux compute cluster with a batch processing system. Nearly all parameters, options and datasets can be combined in a flexible way and can be read from configuration files. An attached comprehensive and fully automated plotting pipeline facilitates a fast evaluation of candidate loci (regional association plots) or summary statistics (IBS-, Manhattan-, and QQ-plots). We have recently completed GWAS for various complex diseases. More than 8,000 controls and 16,000 cases for 15 distinct diseases were genotyped on Affymetrix or Illumina SNP arrays and were prepared for combined analyses. The project takes a systematic, genome-wide approach by studying an overlay of GWAS data sets from different diseases in clinically relevant combinations. A large-scale replication of the identified loci as well as subsequent resequencing of replicated disease loci will identify the broad spectrum of variation and will yield lists of potentially causative variants that are relevant for more than one disease. Association analysis results for both the individual analyses and the combined analyses will be made publicly available through a UCSC custom track. The current status of the project will be presented at the conference and the software will be publicly released.

2778/W

Increasing power for family-based association in the presence of locus heterogeneity using a mixture TDT. *D. Gordon, D. Londono, S. Buyske.* Dept Gen, Rutgers, State Univ NJ, Piscataway, NJ.

Background: Locus heterogeneity can significantly reduce statistical power to detect association in both case-control and trio designs. Recent work by Zhou and Pan uses a mixture model to allow for locus heterogeneity in a case-control design.

Methods: We extend the work of Zhou and Pan to the TDT statistic for trios. We specify that in the presence of locus heterogeneity, each set of trios is a mixture of associated and non-associated trios. We perform simulations to determine the empirical type I error rate and power of the mixture TDT in the presence of locus heterogeneity.

Results: Our simulations suggest that the mixture TDT maitains the correct empirical type I error rate when there is no disease locus. Also, power gains in the presence of locus heterogeneity may be substantial.

Conclusions: It is possible to address locus heterogeneity in association testing as well as linkage analysis. We have software to perform our analyses.

2779/W

A simple method for tightening the confidence limits reported in a genetic epidemiological study. *W.-Y. Lin, W.-C. Lee.* Graduate Institute of Epidemiology, National Taiwan University, Taipei, Taiwan.

In this paper, we develop a simple method to improve the efficiency of a genetic epidemiological study. The effects of single-nucleotide polymorphisms (SNPs) in a study are assumed to arise from an unspecified prior distribution with an unknown mean and an unknown variance. We use the observed log odds ratios (logORs) and their variances to estimate the prior mean and the prior variance. And the posterior distribution will be asymptotically normally distributed regardless of the prior distribution of these effects. Based on these, we proposed a simple formula to tighten the confidence limits of the many logORs reported in a study. We evaluate the performances of our method by simulation studies. We found that the proposed method can indeed booster efficiency while maintaining the average coverage probability at a desired level. The larger the ratio of the prior variance to the average variances of logORs, the smaller the total number of logORs is needed to guarantee the coverage probability. For example, when the ratio of the prior variance to the average variances of logORs is 2, the efficiency of our method relative to the traditional confidence interval method is 1.5, and roughly 50 logORs (or 50 SNPs) can guarantee the coverage probability. We further apply our method to a genetic epidemiological study on age-related macular degeneration to demonstrate the tightening of the confidence limits. Our method is easy to implement and is to be recommended for improving the efficiency of a genetic epidemiological study.

2780/W

Genetic variants within chromosome 4q28.3 are not reproducibly associated with Age-related Macular Degeneration (AMD). *J. Gibson[1], H. Griffiths[2], A. Collins[1], JRW. Yates[3], JC. Folk[4], JS. East[4], AJ. Lotery[2,5], S. Ennis[1].* 1) Human Genetics Division, School of Medicine, University of Southampton, UK; 2) Clinical Neurosciences Division, School of Medicine, University of Southampton, UK; 3) Institute of Ophthalmology, University College London, UK; 4) Department of Ophthalmology and Visual SciencesThe University of Iowa Carver College of Medicine, Iowa City, Iowa, USA; 5) Southampton Eye Unit, Southampton General Hospital, Southampton, UK.

Age-related Macular Degeneration (AMD) is a major cause of vision loss and several genes have been associated with AMD susceptibility. A meta-analysis combining data from six linkage genome scans identified regions of interest on seven chromosomes(1). The most significant regions were subsequently found to harbour the susceptibility loci, LOC387715/ARMS2, HTRA1 and CFH. The aim of this study was to assess next most significant region on chromosome 4q28.2-4q32.3 for association with AMD in a UK sample (470cases,470controls). Following an initial scan of 501 SNPs we found one significant SNP (rs4863760,p=6.96E-05) and carried out higher density screening of 51 SNPs around this SNP (138.6-138.8Mb,UCSC Mar06). Standard quality control filters were applied. A further two SNPs were associated, rs4391086 and rs4554127 (corrected p=0.003,0.007). LD is almost complete between these three SNPs; allele frequencies and odds ratios are also very similar suggesting these SNPs represent the same association signal. Haplotypes were inferred across seven haplotype blocks. The haplotype CGC (rs12645301,rs10021969,rs9996605) was the most significant (permutation-corrected p=0.002) and marginally more significant than any of the three significant SNPs. The haplotype GTTGAAATCGGTTC-GAA in the adjacent block, containing all three significant SNPs, is also significant (corrected p=0.014). It is possible that these blocks represent a larger block, in which haplotypes are frequently inherited together. Significant results were interrogated in two further independent samples. In a US sample, of 331 cases and 332 controls, two of the three most significant SNPs (rs4391086,rs4863760) were genotyped along with the two haplotype tagging SNPs (rs12645301,rs9996605). To test a possible population-specific effect, two SNPs were analysed in a second UK sample of 969 cases and 5038 controls. No association was detected. To-date one gene has been identified in this region, although complement factor I is located distantly (27.8Mb) from our significant SNP. This study reports three nominally significant SNPs, but which were not replicated in two independent samples suggesting a false positive result. An alternative explanation is that the power to replicate is limited due to reliance on a broad phenotype. Analyses in larger samples, with careful characterisation of sub-phenotypes, may further elucidate the role of this region in AMD. (1)Fisher,S.A.et al.(2005) Hum.Mol.Genet.14,2257-2264.

2781/W

Using publically available control data for GWAS - a simple strategy for genetic matching. *C.C. Teerlink[1], J.M. Farnham[1], K. Allen-Brady[1], B.D. Horne[2], N.J. Camp[1], L.A. Cannon-Albright[1,3].* 1) Dept of Internal Medicine, University of Utah, Salt Lake City, UT; 2) Dept of Cardiology, Intermountain Healthcare, Salt Lake City, UT; 3) George E. Wallen Department of Veterans Affairs Medical Center, Salt Lake City, UT.

Publicly available genotyped control subjects are a valuable contribution to genetics in the form of providing control genotype data at little to no cost. They may also increase statistical power for genomewide association studies (GWAS) through many-to-one matching to cases. However, to appropriately utilize these controls care must be taken to remove population stratification due to admixture that may still be present even after matching cases and controls on stated ethnicity. We present a method to identify a set of genetically matched controls from within a set of self-identified ethnically matched controls from a publicly available genotype dataset. The method uses multi-dimensional scaling (MDS) to select controls based on their genetic distance from the case set. We demonstrate the fitness of controls selected by this method through a small GWAS case-study for a common cancer (n = 70 subjects). We compare the Genomic Inflation Factor (λ) for the controls selected by our method to that obtained from two sets of locally ascertained controls. In this case study, our proposed method was able to identify a large, genetically representative set of controls that provide sufficient power for genome-wide significance, despite the small number of cases analyzed. We recommend the use of this method applied to publicly available controls as a powerful and cost efficient method for GWAS of varying size.

2782/W

Effect Of Population Stratification On Quantitative Trait Association Analyses. *S. Ghosh, T. Haldar.* Human Genetics Unit, Indian Statistical Inst, Kolkata, India.

It is now well established that population stratification can result in spurious association findings in genetic case-control studies. However, very few studies have addressed similar issues for mapping quantitative traits. Since quantitative phenotypes are often precursors of clinical end-point traits and carry more information on within-genotype trait variability, it is being argued that studying these quantitative traits may be a more powerful strategy to map genes than the binary clinical end-points. Thus, it is of interest to evaluate the adverse effects of population stratification on the analyses of quantitative traits. The two popular statistical tests of association for quantitative traits using population level data are ANOVA and Kruskal-Wallis. We have theoretically shown that neither genetic heterogeneity nor phenotypic heterogeneity alone can affect the false positive rate of either of the tests. However, if the data comprise subpopulations with different allele frequencies at the marker locus of interest as well as different phenotypic means or distributions, the rate of false positives will be elevated. We have also carried out extensive simulations under different genetic models and probability distributions of quantitative traits to assess the extent of increase in the rate of false positives in the presence of population stratification. We find that the rate of false positives increases at a very fast rate with increase in differences in the standardized phenotypic means and marker allele frequencies in the subpopulations.

2783/W

A Simple Path Model for Assessmet of Pleiotropic Associations for a Dichotomous Trait and a Related Quantitative Trait. *R.L. Hanson, C.C. Mason, W.C. Knowler.* Diabetes Epidemiology Clin Res, NIDDK, Phoenix, AZ.

Genetic studies are often designed to examine determinants of a quantitative trait related to a disease in addition to the disease itself (e.g, global transcription studies). We have developed a simple path model to assess joint associations between genotype, disease and a quantitative trait. Logistic regression models are used to assess association between quantitative trait and disease, between genotype and quantitative trait and between genotype and disease (assuming an additive polytomous model). In path model analyses, the odds ratios from these models are transformed using Yule's Q method and analyzed as correlations. A "pleiotropic" path model is used to estimate the effect of genotype on disease (b_D), effect of genotype on the quantitative trait (b_Q) and the residual correlation. The pleiotropic path ($b_D b_Q$) quantifies the extent to which genotype may influence both traits and its standard error is calculated from the "correlation" matrix. We conducted a simulation study to assess the properties of this pleiotropic test statistic (χ^2_{PL}) and to compare it with tests for association of genotype with the quantitative trait (χ^2_Q), disease trait (χ^2_D) and the 2 df joint test for association with both phenotypes (χ^2_B). Simulations were performed for 1500 individuals with prevalence of disease 0.2. Different models were run with different amounts of variance explained by genotype for the quantitative trait and disease (h^2_Q, h^2_D). Area under the receiver operating characteristic curve (aROC) was used to examine the ability to distinguish data generated under the hypothesis of a pleiotropic association from that generated under the null. Three null hypotheses were assessed: 1.) no association with either trait; 2.) association with disease but not the quantitative trait; 3.): association with the quantitative trait but not disease. For replicates simulated under each of the three null hypotheses, the type I error of χ^2_{PL} was generally at or below nominal values. Power of χ^2_{PL} was generally similar to the less powerful of χ^2_D or χ^2_Q. The aROC was consistently higher for χ^2_{PL} than for χ^2_D, χ^2_Q or χ^2_B. For example, for distinguishing a model with h^2_Q=0.10 and h^2_D=0.01 from similar models in the absence of pleiotropy, the aROC was 0.96 for χ^2_{PL}, compared with 0.86 for χ^2_B, 0.84 for χ^2_Q and 0.79 for χ^2_D. The high aROC suggests that χ^2_{PL} may be useful in screening large numbers of genotypic-phenotypic associations with disease.

2784/W

Association mapping of quantitative traits in samples with related individuals. *J. Jakobsdottir[1], M.S. McPeek[1,2].* 1) Dept Statistics, Univ Chicago, Chicago, IL; 2) Dept Human Genetics, Univ Chicago, Chicago, IL.

Association studies commonly use samples of unrelated individuals with no known familial relationships. When data on related individuals are available, power can be gained by including all individuals in the analysis, while properly accounting for the dependence among them. We propose an association test for quantitative traits, the MQLSQ test, that is valid when some sampled individuals are related with known relationships. The MQLSQ is an extension of the MQLS case-control association testing method of Thornton and McPeek from binary to quantitative traits. We treat the genotype data as random, and condition on the phenotype data and any covariates. The analysis makes use of estimated polygenic and environmental components of variance of the phenotype. The MQLSQ test is valid even when the phenotype model is misspecified and with either random or phenotype-based ascertainment. The genotype of a given individual receives a weight that depends not only on the phenotype and covariate information of that individual but also on the phenotypes and covariate information of the relatives of the individual. As a result, individuals with missing genotype information but available phenotype and covariate information will contribute to the genotype-specific weight of their relatives and, thus, indirectly increase the effective sample size when testing for association of the marker. We show theoretically that this weighting scheme should have positive impact on power as it does in the case-control setting. The MQLSQ is a quasi-likelihood score test for association between marker and phenotype. Advantages of our quasi-likelihood score test approach include: (1) computational feasibility; (2) no need to specify the null joint distribution of phenotypes for related individuals; (3) asymptotic optimality properties; (4) robustness to misspecified trait model; (5) incorporation of covariates and polygenic components of variance; (6) applicability to completely general combinations of family and case-control designs. We perform simulation studies to confirm correct type I error of the method and to evaluate power and robustness of the MQLSQ in comparison to a previously proposed method, GTAM.

2785/W

Natural Selection and Efficient Phenotype Association in Exome Resequencing Studies. *C.R. King[1], P.J. Rathouz[1], D.L. Nicolae[2].* 1) Health Studies, Univeristy of Chicago, Chicago, IL; 2) Statistics, University of Chicago, Chicago, IL.

Projects to determine the impact of rare genetic variation on human disease with base-pair by base-pair resequencing of many participants are underway. Recent authors have recognized the importance of using population genetics to inform the hypothesized causal model, particularly with respect to the role of rare variation. We present a generalized linear mixed model which uses population genetic theory to suggest a pooling strategy for all information within a gene for efficient testing. We demonstrate analysis of whole exome sequencing of a case-control study. In simulation we show power improvements over alternative methods in scenarios of interest. We show how to incorporate additional prior information on SNP effects from mutation type, location and phylogenetics. Our method produces easily interpreted parameter estimates and model summaries.

2786/W

Association of MYH9 and lupus renal disease in multiple populations. *C.P. Lin[1], J.A. Kelly[1], P.M. Gaffney[1], K.M. Kaufman[1,2,3], B.I. Freedman[11], J. Anaya[4], M.E. Alarcon-Riquelme[1,6], J. Martin[5], S. Bae[7], S. Park[7], S.A. Boackle[8], E.E. Brown[9], L.A. Criswell[10], J.C. Edberg[9], P.K. Gregersen[12], D.L. Kamen[13], C.O. Jacob[14], J.A. James[1,2], R.P. Kimberly[9], J.T. Merrill[1], T.B. Niewold[15], R.H. Scofield[1,2,3], A.M. Stevens[16], B.P. Tsao[17], T.J. Vyse[18], C.D. Langefeld[19], K.L. Moser[1,2], J.B. Harley[2,3,20], C. Gray-McGuire[1].* 1) Oklahoma Medical Research Foundation, Oklahoma City, OK; 2) University of Oklahoma Health Sciences Center, Oklahoma City, OK; 3) Oklahoma City VA Medical Center, Oklahoma City, OK; 4) Universidad del Rosario, Colombia; 5) Instituto de Parasitología y Biomedicina, CSIC, Parque Tecnológico de Ciencias de la Salud, Avenida del Conocimiento s/n 18100-Armilla, Granada, Spain; 6) GENYO, Andalusian Center for Genetics and Oncological Research -Pfizer-Universidad de Granada-Junta de Andalucia, Granada, Spain; 7) Hanyang University, Seoul, Republic of Korea; 8) University of Colorado Denver, Aurora, CO; 9) University of Alabama at Birmingham, Birmingham, AL; 10) University of California, San Francisco, CA; 11) Wake Forest University School of Medicine, Winston-Salem, NC; 12) Robert S. Boas Center for Genomics and Human Genetics, The Feinstein Institute for Medical Research, North Shore Long Island Jewish Health System, Manhasset, NY; 13) Medical University of South Carolina, Charleston, SC; 14) University of Southern California Keck School of Medicine, Los Angeles, CA; 15) University of Chicago, Chicago IL; 16) Seattle Children's Hospital, Seattle, WA; 17) University of California-Los Angeles, Los Angeles, CA; 18) Imperial College London, London, UK; 19) Wake Forest University Health Sciences, Winston-Salem, NC; 20) Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

Lupus nephritis is among the most severe clinical manifestations of Systemic lupus erythematosus (SLE), a disease that affects African Americans (AA) three times more than European Americans (EA). Genetic determinants of SLE susceptibility and specific SLE manifestations are supported by high twin concordance rates, linkage and association study findings and the identification of variants associated with disease. Given a threefold increased risk of lupus nephritis in certain populations, we investigated a recently identified gene for non-diabetic end stage renal disease, MYH9 (Myosin, heavy Chain 9), shown to be associated in AA. Genetic association tests were performed within MYH9 region in 6 different populations of SLE cases shown to have American College of Rheumatology classification criterion for renal disease. We investigated MYH9 in independent cases and controls, respectively, from 6 population groups: AA (635/1734),EA (1119/3546), Asian (531/1270), Native American (111/193), Hispanic (548/619) and Gullah (70/122). Controls were defined as either SLE cases without renal involvement or unaffected individuals. Results were similar, thus only those using unaffected individuals were presented. Seventy-three single nucleotide polymorphisms (SNPs) in moderate linkage disequilibrium (LD) ($r^2 > 0.80$) were selected. We then performed single SNP tests for association with renal disease status using logistic regression adjusted for global African, European and Asian ancestry and sex assuming additive/dominant genetic model. In EA, the association peak centered around 35,040,000 base pairs with multiple SNPs showing association. We identified three SNPs rs5750250, rs2413396 and rs4820232 within MYH9 that were strongly associated with renal disease ($1 \times 10^{-4} \pounds p \pounds 9 \times 10^{-4}$) with high LD ($D' \geq 0.99$).The best associated SNP was rs5750250 ($P=2.32 \times 10^{-4}$). In the Gullah, significant association centered at 22q35.05. No statistically significant associations were detected in populations of other ancestral backgrounds. While we did not replicate previous findings in AA, a significant association in the Gullah population, despite a sample size of only 192, confirms the elevated risk of SLE renal disease in populations of African ancestry. The presence of our strongest effect in EA is a novel finding and suggests the role of MYH9 in lupus nephritis in diverse populations.

2787/W

Bayesian Variable Selection for Survival Regression in Genetics. *I. Tachmazidou[1], M. Johnson[2], M. De Iorio[3].* 1) MRC Biostatistics Unit, Cambridge, United Kingdom; 2) Division of Experimental Medicine, Imperial College London, United Kingdom; 3) Department of Epidemiology and Biostatistics, Imperial College London, United Kingdom.

Variable selection in regression with very big numbers of variables is challenging both in terms of model specification and computation. We focus on genetic studies in the field of survival, and we present a Bayesian-inspired penalized maximum likelihood approach appropriate for high-dimensional problems. In particular, we employ a simple, efficient algorithm that seeks maximum a posteriori (MAP) estimates of regression coefficients. The latter are assigned a Laplace prior with a sharp mode at zero, and non-zero posterior mode estimates correspond to significant single nucleotide polymorphisms (SNPs). Using the Laplace prior reflects a prior belief that only a small proportion of the SNPs significantly influence the response. The method is fast and can handle datasets arising from imputation or resequencing. We demonstrate the localization performance, power and false positive rates of our method in large simulation studies of dense-SNP datasets and sequence data, and we compare the performance of our method to the univariate Cox regression and to a recently proposed stochastic search approach. In general, we find that our approach improves localization and power slightly, while the biggest advantage is in false positive counts and computing times. We also apply our method to a real prospective study, and we observe potential association between candidate ABC transporter genes and epilepsy treatment outcomes.

2788/W

Genetic association test for a longitudinally-measured trait in samples with related individuals. *X. Wu[1], M.S. McPeek[1,2].* 1) Department of Statistics, University of Chicago, Chicago, IL; 2) Department of Human Genetics, University of Chicago, Chicago, IL.

Although there are many successful applications of GWAS in identifying complex disease susceptibility loci, few published studies have investigated the contribution of genetic variants to disease susceptibility over time. For analysis of a longitudinally-measured trait, it is important to account for the time-dependence among observations for a given individual. When the sample includes related individuals, there is dependence across individuals as well, due to the effects of genetics as well as possible environmental effects. We develop a statistical method for genetic analysis of a longitudinally-measured trait in samples with related individuals. We propose quasi-likelihood score tests for genotypes conditional on longitudinal phenotype measurements and covariates. The conditional model for genotypes is derived from a phenotype model that includes components of variance for polygenic effects, inter-individual variation caused by non-genetic effects, time-varying correlation of observations within individuals and measurement errors. An advantage of analyzing genotype conditional on phenotype is that one can still obtain a valid test even when some important aspects of the trait model are not correctly specified. We perform simulation studies to verify that the type 1 error of the proposed approach is correct, and to demonstrate the power advantage and robustness to model misspecification of the proposed approach. The method is applied to analysis of longitudinal blood pressure measurements from the Framingham SHARe data.

2789/W

Power of genome-wide search strategies for binary trait loci. *Z. Wu[1], H. Zhao[2].* 1) Mathematical Sciences, WPI, Worcester, MA; 2) Epidemiology and Public Health, Yale, New Haven, CT.

For more fruitful discoveries of genetic variants associated with diseases in the genome-wide association studies, it is important to know whether joint analysis of multiple markers is more powerful than the commonly used single-marker analysis. We provide analytical power calculations for various methods to detect binary trait loci: the marginal search, the exhaustive search, the forward search, and a two-stage screening search. In the context of binary traits, we define genetic models based on disease odds for joint genotypes and derive asymptotic distributions of score tests in logistic model fitting. Our statistical framework takes into account linkage disequilibrium, random genotypes, and correlations among test statistics. We derive the analytical results under two power definitions: the power of finding all the associated markers and the power of finding at least one associated marker, and two types of error controls: the discovery number control and the Bonferroni type I error rate control. After demonstrating the accuracy of our analytical results by simulations, we apply them in a broad genetic space to investigate the relative performances of different search methods under different power and error control definitions. The relative performances for binary trait have both similarities and differences from those in quantitative trait study. Our analytical study provides rapid computation as well as insights into the statistical mechanism of capturing genetic signals. We have implemented our method in the R package markerSearchPower which provides researchers a convenient tool to find proper sample size in experimental design, to decide suitable strategies in data analysis, and to increase chance for true findings. Our statistical approach can also be used to address model selection problems of binary responses with general random predictor settings in other application areas.

2790/W

Comparing methods for association test of low-frequency variants using GWAS data with family relatedness. *Q. Zhang, I. Borecki, M. Province.* Division of Statistical Genomics, Washington University School of Medicine, St. Louis, MO.

Due to sparse distribution plus genetic relatedness between samples, association test for low-frequency variants in GWAS data from many different pedigrees is more prone to produce biased p-values, which usually increases false positives. Under the generalized frame of linear model, we summarize (and propose) multiple possible methods that could be utilized to deal with this problem, and investigate the statistical performance of these methods by comparing p-value distributions under the null hypothesis. We include bootstrapping, permutation and mixed model based methods for comparison, and generate the data under the null by semi-simulation based on a real GWAS data set of 2700 subjects from 508 pedigrees. We focus on quantitative traits with different heritabilities and investigate the inflation rate of p-values for variants with different frequencies. Our findings and conclusions may provide a useful guide for association scan of low-frequency variants in GWAS.

2791/W

HIERARCHICAL GENERALIZED LINEAR MODELS FOR DETECTING RARE VARIANTS WITH DISPARATE EFFECTS IN ASSOCIATION STUDIES. *D. Zhi, N. Yi.* Department of Biostatistics, University of Alabama at Birmingham, Birmingham, AL.

Rare genetic variants are believed to be responsible for part of the missing heritability overlooked by common variants-based genome-wide association studies. However, existing association tests for common variants do not offer a sufficient power for rare variant association. Therefore, collapsing methods, which test the combined effects of multiple variants in a functional unit, typically a gene, have been proposed to test the combined effect of multiple rare variants. However, collapsing methods only achieve high power when the effects of individual variants in the combination are of the same direction (i.e., all disease-causing or all protective). In reality often variants with disparate effects coexist. In this work we developed hierarchical generalized linear models for the identification of multiple groups of rare variants. Our model allows that each group contains variants with similar effects while different groups having disparate effects. In simulation, we show that our method can detect associations missed by existing methods.

2792/W

Adjustment of Local Ancestry in Family-based Genetic Association Analysis of Admixed Populations. *X. Wang[1], X. Zhu[2], M. Li[1].* 1) Department of Biostatistics and Epidemiology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104; 2) Department of Epidemiology and Biostatistics, School of Medicine, Case Western Reserve University, Cleveland, Ohio 44106.

Family-based study design is commonly used in gene mapping studies of complex human diseases. Most family-based studies use the transmission of alleles to assess the evidence of genetic association. It is generally believed that the transmission disequilibrium test (TDT) is robust again spurious association due to population stratification or admixture. While this is true when population stratification is due to discrete population structure with non-admixed populations, one should use the TDT-type methods with caution when they are applied to admixed populations. As described in the original TDT paper (Spielman et al., 1993), the TDT statistic depends on the difference of transmitted and non-transmitted alleles, which is a function of the linkage disequilibrium (LD) coefficient between the marker and disease loci in the parental generation. Ewens and Spielman (1995) further showed that for a recently admixed population such as African American and Hispanic Americans, the LD coefficient between the marker and disease loci in the parental generation (which is also admixed) contains a spurious component from the admixture process. Due to this reason, for admixed populations, the TDT can only be used as a test of linkage, but it cannot distinguish between “spurious association” and “true association”. To our knowledge, currently there are no family-based testing procedures that distinguish between spurious and true associations for admixed populations. Here we propose a retrospective likelihood framework that solves this problem. Unlike the TDT and other family-based tests of association, our method does not rely on transmission disequilibrium. Indeed it models the conditional distribution of a test SNP given disease status of the child and genotypes of ancestry informative markers surrounding the test SNP in a parents-offspring trio. This retrospective likelihood allows us to explicitly model local ancestry difference between study trios and thus eliminates the effect of population stratification at the test SNP. Our preliminary simulation studies show that our method can control type I errors under a wide range of population stratification mechanisms. We will conduct thorough simulations to evaluate the power of our method.

2793/W

A sequential Bonferroni procedure using smoothed weights for genome-wide association studies. *G. Gao.* Dept Biostatistics, Virginia Commonwealth Univ, Richmoned, VA.

In genome-wide association studies (GWAS) with a case-control design, the Cochran-Armitage trend test (CATT) assuming the additive disease model and the two degree of freedom genotype association test (GAT) using a chi-square statistic have been widely used for detecting disease-susceptibility markers. The CATT usually has higher power than the GAT under additive and multiplicative disease models, but has lower power than GAT under dominant and recessive disease models. In addition, both tests have relatively low power for dominant and recessive disease models. To generate a method that is statistically powerful for the four commonly used disease models (additive, multiplicative, dominant, and recessive) in GWAS, we apply the generalized sequential Bonferroni (GSB) procedure of Holm to GWAS using the CATT. We estimate a weight for each marker as reciprocal of the p-value from a HWD test using cases only. The HWD test can provide useful information for detecting marker-disease association under dominant and recessive disease models, but have almost no power for additive and multiplicative disease models. Furthermore, we smooth the weights in the GSB procedure to decrease the negative influence of those weights which do not provide useful information. The GSB procedure using smoothed weights is referred to as smoothed-GSB procedure. Simulation studies showed that in GWAS the smoothed-GSB procedure has higher power than the CATT and GAT under the four models except that it has power comparable to the CATT under additive and multiplicative models. We have applied the smoothed-GSB procedure to GWAS of the coronary artery disease dataset from the Wellcome Trust Case-Control Consortium.

2794/W

A generalized Kruskal-Wallis test for association analysis of imputed SNPs. *E. Acar[1], L. Sun[1,2].* 1) Department of Statistics, University of Toronto, Toronto, Ontario, Canada; 2) Dalla Lana School of Public Health, University of Toronto, Toronto, Ontario, Canada.

Research on imputation-based genetic association studies has mostly focused on evaluation and improvement of genotype imputation accuracy. However, while many algorithms have been developed, few statistical testing strategies have been proposed in this domain. The popular dosage approach, using the expected number of copies of the risk allele to define the genotype variable, and the best-guess approach, identifying the genotype with the highest posterior imputation probability, both have drawbacks. The former imposes a restrictive additive model on the association analyses, and the latter fails to incorporate imputation uncertainty in the testing procedure.

A robust, powerful association test statistic is developed by generalizing the Kruskal-Wallis test to account for imputation uncertainty. Using the posterior probability of each genotype group, we construct a nonparametric test statistic and derive its asymptotic null distribution. The extended statistic uses all available information and requires no assumption on the data distribution.

Simulation and application studies show that the proposed test outperforms its parametric predecessors. Depending on the specific model assumptions, the gain of the power could be over 50%, while the loss of power is negligible when the simulated data are tailored for a best-guess or dosage model. With its ease of implementation, this generalized Kruskal-Wallis test could shed new light on current genetic association studies of imputed SNPs or other studies with group uncertainties.

2795/W

Meta-analysis vs GWAS in lung cancer studies. *K. Ahn, C.J. Gallagher, J.E. Muscat.* Dept Public Health Sciences, Penn State Col Med, Hershey, PA.

The sequencing of the human genome has made it possible to identify an informative set of more than one million single nucleotide polymorphisms (SNPs). Genome Wide Association Studies (GWAS) have now become a standard method for the discovery and replication of new candidate loci or genes. Since GWAS use tag SNPS, they cannot be directly compared to previous candidate genes. In addition, many associations with candidate genes have not been replicated. This study compared the results from a GWAS study of lung cancer to candidate genes that have been analyzed in meta-analysis. The goals were to determine the consistency of findings across all study designs. We studied the population of white Caucasian drawn from a European GWAS of lung cancer. We included SNPs in Linkage Disequilibrium with 10 candidate genes that were part of meta-analysis (MA). Finally, we have 14 SNPs in MA and 23 SNPs in the GWAS. We found that 5 SNPs from 4 genes in MA are significant statistically and 3 SNPs from one gene in the GWAS are. The mixed results indicate that GWAS findings are sometimes inconsistent with the results from MA. These differences may be due to the quality of studies, appropriate control of covariates and other factors. The findings indicate that for certain genes, their role in the development of lung cancer is unclear and that GWAS findings are possibly not the gold standard in etiologic research.

2796/W

A General Statistical Framework for Genome-wide Association Studies (GWAS) Based on Bayesian Graphical Modeling. *L. Briollais[1], H. Massam[2], A. Dobra[3], J. Liu[1].* 1) Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Canada; 2) Dept. of Stat. and Math., York University, Toronto, Canada; 3) Dept. of Stat., University of Washington, Seattle, USA.

The actual paradigm to analyze GWAS is to perform an exhausting testing of all single SNP associations with the response variable with the major drawback that the selected subset of SNPs has in general a very low predictive value. As a shift to the usual approach, we propose here a general statistical framework for GWAS based on Bayesian graphical modeling and able to: 1) Assess the joint effect of multiple SNPs (linked or unlinked); 2) Explore the model space efficiently using the Mode Oriented Stochastic Search (MOSS) algorithm of Dobra and Massam (2009); 3) Incorporate expert prior knowledge in the model search in particular to enhance the detection of rare functional genetic variants. We illustrate our new methodology through an application to the CGEM breast cancer data. Our algorithm selected several SNPs embedded in multi-locus models with high posterior probabilities. Most of the selected SNPs have a biological interest. Interestingly, several of them would not have been detected in the single-SNP testing approach. Finally, we discuss the impact of informative priors in this statistical framework through simulations.

2797/W

Population-based linkage in GWAS bipolar data. *S.R. Browning[1], B.L. Browning[2].* 1) Biostatistics, University of Washington, Seattle, WA; 2) Medical Genetics, University of Washington, Seattle, WA.

We performed population-based linkage analysis in the Wellcome Trust Case Control Consortium bipolar disorder study using a novel algorithm for detecting haplotype-cluster identity by state (HC-IBS). Our method detects "recent" identity by descent due to shared ancestry within the past 20 generations, while accounting for haplotype phase uncertainty and linkage disequilibrium. We found a significant excess of HC-IBS in case-case pairs compared to control-control and case-control pairs, indicating increased relatedness within cases. In contrast, the estimated kinship coefficients are similar between case-case pairs and control-control pairs, because the kinship coefficient in unrelated pairs of individuals is primarily influenced by ancient identity by descent which is more prevalent than recent identity by descent. Much of the increased HC-IBS in case-case pairs is due to pairs of individuals who share at least 0.1% of their genome in the HC-IBS analysis (no pair of individuals in this study shares more than 6%), suggesting that the excess in shared ancestry is primarily due to common ancestors within the past 10 generations. The excess HC-IBS in case-case pairs is distributed across the whole genome, which is consistent with a polygenic component to this disorder. Our HC-IBS analysis identified two loci with strong signals for population linkage after correcting for the overall increase in HC-IBS in case-case pairs. One of these loci is located in a region that has been repeatedly linked with bipolar disorder in family-based linkage studies. A replication analysis using GAIN bipolar disorder data is in progress.

2798/W

Haplotype and Dosage-based Tests for Rare Variant Association. *A. Byrnes[1], M. Li[2], Y. Li[1].* 1) Department of Biostatistics, Department of Genetics, University of North Carolina, Chapel Hill, NC 27599; 2) Department of Biostatistics and Epidemiology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Empirical evidence suggests that both common and rare variants contribute to complex disease etiology. Although the effects of common variants have been thoroughly assessed in the most recent wave of genome-wide association studies (GWAS), our knowledge on the impact of rare variants remains limited. A number of methods have been proposed to test for rare variants association in sequencing-based studies, a study design that may become predominant in the imminent future. On the contrary, few methods are available to detect rare variants in GWAS data we have in the current era. There are good reasons for the lack of methods targeting GWAS data: commercial panels for GWAS studies were designed to cover most of the common variants and have poor coverage of rare variants in our genome and tests proposed for common variants are underpowered for rare variants. Now that public sequencing data from the 1000 Genomes Project are being rapidly generated and released, an attempt to detect rare variants with GWAS data holds promise before large investment is made for resequencing. Here we propose two methods for rare variant analysis: a haplotype based approach and an imputation dosage based approach that maximally extract information for rare variants within a pre-specified region. Although directly applicable to resequencing data, our methods are designed for data in the current era and manifest their advantages most with GWAS or candidate gene genotyping data where more accurate information on a larger number of rare variants is inferred through haplotyping or imputation with the aid of external sequencing data. Using real GWAS data and a reference sample of 60 individuals, we showed that rare variants (allele frequency 1-5%) can be reasonably inferred with an overall accuracy of 99.2% . The accuracy increases to 99.8%, when we have a reference sample of 500 individuals, a sample size aimed in the 1000 Genomes Project for each major continental group. Given reasonably well-inferred less common variants, we have shown our methods improve power for rare variants association with GWAS data in extensive simulation studies as well as in the WTCCC data for type-1 diabetes in the IFIH1 region. Permutation p-values generated by our methods for WTCCC IFIH1 region are in the order of 10-3 while the best p-value using existing tests for rare variants is greater than 0.2.

2799/W

An efficient, hierarchical Bayes approach to simultaneous analysis of SNPs in genome-wide association studies. *P.S. Carbonetto, M. Stephens.* Dept. of Human Genetics University of Chicago Chicago, IL.

Simultaneous analysis of all SNPs in a genome-wide association study offers a number of advantages, in principle, over standard SNP-by-SNP analyses. Significantly, simultaneous analysis allows one to control for the most strongly associated variants when examining more borderline associations, and to improve power to distinguish false positive associations from true positives. Multi-SNP analyses also lend themselves more naturally to useful extensions, such as the discovery of interacting genetic risk factors, or gene-environment interactions. Two well-studied approaches to performing such genome-wide analyses are penalized regression (e.g. LASSO), and Bayesian variable selection regression (BVSR). A key advantage of the latter is that it reports levels of confidence in individual findings. However, the former is substantially more computationally tractable, since exact algorithms are available (BVSR relies on Markov chain Monte Carlo methods that can take weeks of computer time). We address this problem by developing a more efficient approach to inference for BVSR using variational approximation techniques. We demonstrate, through simulation, that our proposed method recovers results on par with those from extensive MCMC computation, and at a fraction of the computational expense. We illustrate the use of our method on data from the Pharmacogenomics and Risk of Cardiovascular Disease (PARC) study, a large, multiple-phase analysis with > 500,000 SNPs from Illumina SNP chips for ascertaining genetic factors relating to C-reactive protein. We contrast our results with standard single-SNP analyses, and illustrate how our method provides more easily interpretable measures of the strength of evidence for association at each SNP.

2800/W

A generalized estimating equations strategy for genome-wide association studies of dichotomous outcomes in general pedigrees. *MH. Chen[1,3], X. Liu[2], M.G. Larson[3,4], V. Ramachandran[5,3], Q. Yang[2,3].* 1) Department of Neurology, Boston University School of Medicine, Boston, MA; 2) Department of Biostatistics, Boston University School of Public Health, Boston, MA; 3) The NHLBI's Framingham Heart Study, Framingham, MA; 4) Department of Mathematics and Statistics, Boston University, Boston, MA; 5) Preventive Medicine and Epidemiology, Boston University School of Medicine, Boston, MA.

Genome-wide association studies (GWAS) have been frequently conducted on general or isolated populations with related individuals. However strategies for analyzing dichotomous phenotypes obtained on general pedigrees are not well developed. We propose a generalized estimating equations (GEE) strategy that uses a robust variance estimator with independence working correlation structure to account for multiple level of relatedness in a general pedigree. For low minor allele frequency (MAF) genetic variants, low prevalent disease, or small sample size, we propose using a one-step jackknife variance estimator that has better control of type I error than the robust variance estimator. We compare our proposed strategies with another popular method, generalized linear mixed model (GLMM), and with different working correlation structure choices in GEE through simulation studies. Our proposed strategy has better power than GLMM for low prevalence traits and is more computationally efficient. We also showed that for GEE, using independence structure performs similarly to using kinship coefficient matrix as working correlation structure in robust variance estimators. We apply our proposed strategy and alternative strategies to GWAS of gout in the Framingham Heart Study.

2801/W

Power and sample size calculations for case-control genome-wide association studies considering rare variants by high-throughput sequencing. *F. De La Vega[1], C. Barbacioru[1], D. Gordon[2].* 1) Genetic Systems R&D, Life Technologies, Foster City, CA; 2) Department of Genetics, Rutgers University, Piscataway, NJ.

Genome-wide association studies (GWAS) have been successful in identifying common genetic variation reproducibly associated with disease. However, most associated variants confer very small risk and after meta-analysis of large cohorts a large fraction of expected heritability still remains unexplained. A possible explanation is that rare variants currently undetected by GWAS with SNP arrays could contribute a large fraction of risk when present in cases. This concept has spurred great interest in exploring the role of rare variation in disease. As the cost of sequencing continue to plummet, it is becoming feasible to directly sequence case-control samples for testing disease association including rare variants. When designing a GWAS by sequencing trade-offs need to be made in terms of sample size and depth of sequence coverage to achieve a desired statistical power. We have developed a test statistic that allows for association testing among cases and controls using sequence reads. In addition, our method allows for random errors in sequence reads. We determine the probability of a true sequence read based on the observed sequence reads using the EM algorithm. Using classical statistical methodology, it is straightforward to create a statistic that tests for association among cases and controls. In addition, we derive the non-centrality parameter for this statistic, so that we can compute power and sample size calculations for any specified input parameters. We document the validity of our method through simulations. Under fixed cost, increasing sample size at the expense of sequence coverage depth improved poser. However, sequencing platform accuracy is a critical factor in achieving power with low pass sequencing coverage.

2802/W

Family Enrichment Improves Power for Finding the Missing Heritability. *P. Du[1], A. Rossi[1], H. Jafri[1], F. Kuo[2,3], C. Huang[2,3], L. Michelson[1], S. Diehl[2,3].* 1) High Performance and Research Computing, UMDNJ, Newark, NJ; 2) Center for Pharmacogenomics and Complex Disease Research, UMDNJ-New Jersey Dental School, Newark, NJ; 3) Health Informatics, UMDNJ-School of Health Related Professions, Newark, NJ.

The missing heritability problem refers to the fact that genome-wide association studies (GWAS) have failed to find most of the genes that underlie complex diseases. The common gene-common disease model has been questioned and an alternative based on many rare, higher risk genes has been proposed. Traditional case-control designs have very low power to detect rare genes. It has been suggested that using multiplex families may help to address this problem by enriching for rare higher risk susceptibility genes. However, no one has systematically evaluated this in models that include both common genes (detectable by GWAS) together with rare higher risk variants. In this study, we used high performance computing to assess power of 36,000 two gene models. Prevalence ranged from 10-35%, allele frequencies from 0.001-0.35, and genotype relative risks for the common gene ranged from 1-1.9 and 1-5.0 for the rare gene. SIMPED was used to simulate two (unlinked) disease genes and penetrance functions assigned affection status based on model parameters. Enrichment criteria focused exclusively on siblings. Only one case or one control was sampled from a family for association tests. Enriched cases were defined as coming from families with at least half of the siblings affected and enriched controls had no siblings affected. We also sampled cases and controls (ignoring family history) for tests of the traditional case control design. The Armitage trend test was used to calculate power for detecting the disease genes. We evaluated power for tests of enriched cases versus traditional controls and for enriched controls versus traditional cases. We found that power of these enrichment strategies often far exceeded that of the traditional case-control design as shown by the following example: 20% prevalence, minor allele frequencies of 0.3 and 0.01 for the common and rare disease genes, and relative risks of 1.45/1.9 and 2.0/3.0, respectively, and sample size 1,000 cases and controls. In the traditional case-control design, the common gene has genome wide significance of 2.37E-09 but the rare gene has expected P-value of 0.006. Using family enrichment, power is greatly improved for the rare gene, with an expected P-value of 5.99E-11, sufficient for genome-wide adjustment. These results demonstrated that family enrichment has the potential to very substantially increase power to detect rare disease-causing genes and help reveal at least part of the missing heritability.

2803/W

Runs Of Homozygozity In European Outbred Populations And Applications For Genome-Wide Association Analyzes. *T. Esko[1,2,3], R. Mägi[1], M. Nelis[1,2,3], P. Palta[1], F. Zimprich[4], D. Toncheva[5], M. Macek[6], L. Peltonen[7], M. Lathrop[8], M. Krawczak[9], T. Meitinger[10], B. Melegh[11], D. Toniolo[12], P. Gasparini[13], J. Klovins[14], V. Kucinskas[15], J. Lubinski[16], S. Limborska[17], S. Antonarakis[18], A. Franke[19], M. Remm[1], A. Metspalu[1,2,3].* 1) Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia; 2) Estonian Biocentre, Genotyping Core Facility, Tartu, Estonia; 3) Estonian Genome Center, University of Tartu, Tartu, Estonia; 4) Department of Clinical Neurology, Medical University of Vienna, Vienna, Austria; 5) Department of Medical Genetics, Medical University of Sofia, Sofia, Bulgaria; 6) Department of Biology and Medical Genetics, Cystic Fibrosis Centre, University Hospital Motol and 2nd School of Medicine, Charles University Prague, Prague, Czech Republic; 7) Wellcome Trust Sanger Institute, Cambridge, UK and the Institute of Molecular Medicine, Biomedicum Helsinki, Finland; 8) Commissariat à l'Energie Atomique, Institut Genomique, Centre National de Gènotypage, Evry, France; Fondation Jean Dausset-CEPH, Paris, France; 9) PopGen Biobank, University Hospital Schleswig-Holstein, Campus Kiel, German; 10) Institute of Human Genetics, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany; 11) Department of Medical Genetics and Child Development, University of Pécs, Pécs, Hungary; 12) Division of Genetics and Cell Biology, San Raffaele Research Institute, Milano, Italy; 13) Medical Genetics, Department of Reproductive Sciences and Development, IRCCS-Burlo Garofolo, University of Trieste, Italy; 14) Latvian Biomedical Research and Study Center, Riga, Latvia; 15) Department of Human and Medical Genetics, Vilnius University, Vilnius, Lithuania; 16) International Hereditary Cancer Center, Pomeranian Medical University, Szczecin, Poland; 17) Department of Molecular Genetics, Russian Academy of Science, Moscow, Russia; 18) Department of Genetic Medicine and Development, University of Geneva Medical School, Geneva, Switzerland; 19) Institute of Clinical Molecular Biology, Christian-Albrechts University, Kiel, Germany.

Human genome harbors different kind of structural variations and one of the recent ones are long streces of homozygoze SNP's, also known as Runs of Homozygosity (ROH). It have been shown that this structural variation is common in inbreed cohorts although it is also very common in outbreed populations. We have analyzed ROH patterns in about 5,500 individuals from 17 populations (21 cohorts) genotyped with Illumina high-density arrays for the ROH patterns and added the 11 HapMap3 populations to measure the patterns in different continents, giving total 6,800 samples. Our analyses reveal a huge variety of ROH alleles in every single population and covering the whole genome, although the mean number and length varies by continents. The transmission of ROH alleles was checked both in HapMap3 and Estonian Biobank trios. The Genome-wide ROH association analyzes (GWRA) method was applied on two large case-control cohort of Ulcerative Colitis and Chrohn's Disease to evaluate if ROHs are able to reveal the "missing heritability". We were able to pick up several already known UC and CD loci from the genome. To conclude in addition to SNP's and CNV's, ROH's can be used as genetic markers in WGA studies for finding disease genes.

2804/W

Controlling for undetected cofactor structure in genome-wide association analyses (GWAS). *C. Gao, J. Mezey.* Biological Statistics and Computational Biology, Bornell, ithaca, NY.

The problem of how to account for environmental or population structure in genome-wide association studies (GWAS) is well defined in cases where contributing co-factors are measured or can be inferred. However, it is also well appreciated that undetected co-factors can exist and produce false positives in GWAS and it has been unclear how to correct for such undetected effects. We hereby propose a novel Restricted Factor Analysis (RFA) approach to deal with this problem. Our approach is to include factors without pre-defined loading matrix L or factor effect vector F directly into a linear modeling framework used to analyze genetic marker associations in GWAS. While such models are unidentified with respect to L and F treated separately, these models are identified with respect to LF. We therefore treat this as a nuisance parameter for the purposes of identifying genetic marker associations. We analyze marker associations with our undetected factor model using an Expectation-Maximization (EM) algorithm. We demonstrate with a simulation analysis that for cases where a set of traits m are affected by both allelic variation at several loci and by a undetected factor F that our method is able to account for the effects of the factor. We find that our method outperforms approaches that ignore the possibility of undetected factors for identifying false positives, increasing power in the detection of associations, and in the estimation of effect sizes of associate markers. We also demonstrate that our method can account for the effects of cryptic population structure on the phenotypes, without directly inferring population structure from genotype data, and that when we account for structure that is not orthogonal to the associated markers, our method can reduce false positives significantly. We applied our method to analyze genome-wide gene expression data collected for the immortalized lymphoblastoid cell lines produced from the Hapmap phase II individuals. By accounting for hidden factor structure in these data when analyzing within each population, we are able to identify more eQTLs compared to standard methods that ignore the undetected structure.

2805/W

Multi-SNP Analysis of Lipid Traits in The Woman's Genome Health Study. *Y. Guan[1,2], M. Barber[1], R. Krauss[3], P. Ridker[4], D. Chasman[4,5], M. Stephens[1,2,5].* 1) Dept. of Human Genetics, Univ Chicago, Chicago, IL; 2) Dept. of Statistics, Univ Chicago, Chicago, IL; 3) Children's Hospital Oakland Research Institute, Oakland, CA; 4) Division of Preventive Medicine, Brigham and Women's Hospital, Boston, MA; 5) These authors jointly direct this project.

Almost all ongoing Genome-wide association studies have limited their initial analysis to simple single SNP tests. Once a genetic association has been identified in a region, attention naturally turns to better understanding and characterizing that association: for example, understanding which SNPs, and how many SNPs, may be independently influencing outcome. Here we use Bayesian multi-SNP analyses to examine this issue for lipid traits in a large study of approximately 23,000 individuals: the Women's Genome Health Study (WGHS). Our aim is to provide a comprehensive picture of the patterns of associations in regions previously identified through single-SNP meta-analysis, and to provide a global assessment of the extent to which such regions are likely to harbor multiple functional SNPs. We performed comprehensive multi-SNP association analysis in the WGHS samples, focusing on approximately 100 genomic regions. For each region we assess both the overall strength of evidence for an association in the WGHS data, and the evidence for multiple SNPs independently affecting phenotype. We also attempt to narrow down the most plausible functional candidate SNPs, among genotyped and imputed SNPs, based on relative strengths of association. We also use simulation to assess whether individual untyped functional SNPs may be partly responsible for apparent multi-SNP signals. Of the 49 regions showing moderate to strong overall evidence for association in the WGHS data, 12 showed strong evidence for multiple SNPs affecting phenotypes. Simulations suggest that the majority of these multi-SNP signals are due to the regions genuinely harboring multiple functional SNPs, rather than due to untyped variants that are correlated with combinations of typed SNPs. Furthermore, several of these regions (eg. APOB, APOC-APOE, ABCA1, and CETP) show strong evidence for three or more independent SNPs affecting phenotypes. For these regions, using multi-SNP analysis we estimate the proportion of variance in relevant phenotypes explained by each locus to be substantially greater than that explained by any individual SNP. We also attempt to identify the most plausible candidate functional SNPs in each region, and investigate possible interactions among independent functional SNPs. Our work illustrate the potential helpfulness of these kinds of analysis, both for improving power to detect associations and, more importantly, for providing fuller explanations for observed associations.

2806/W

Increasing power of meta-analysis when effects are present in a subset of the studies. *B. Han[1], E. Eskin[1,2].* 1) Computer Science, University of California, Los Angeles, Los Angeles, CA; 2) Human Genetics, University of California, Los Angeles, Los Angeles, CA.

In genetic association studies, meta-analysis is becoming a popular tool for pooling information from multiple studies. One important issue in meta-analyses is the heterogeneity between studies. We focus on a specific type of heterogeneity that the genetic effect of a variant is present in only a subset of the studies because of hidden factors masking the effect of the variant. In this case, the current approaches of fixed effects model (FE) and random effects model (RE) have relatively low power. We introduce a new model called randomly-present fixed effects model that models the possible absence of effects. We propose a statistical testing procedure under this model using an Expectation-Maximization algorithm and develop an importance sampling procedure that efficiently estimates p-values. In simulations, our method shows higher power than FE and RE if the effects are absent in one or more studies and comparable power if the effects are present in all studies. This shows that our method can bring the variants to attention as genome-wide significant that are only moderately significant in current approaches because of the masking of the effects. Besides introducing the new approach, we also show the relationship between the two widely used statistics based on FE: the weighted sum of z-scores and the inverse-variance-weighted effect size. It follows that the optimal weights of z-scores are function of allele frequency as well as sample size in contrast to the current weighting scheme which weights only by the square-root of the sample size.

2807/W

A powerful and efficient variable selection method for genome-wide association studies. *Q. He, D.Y. Lin.* Department of Biostatistics, University of North Carolina at Chapel Hill, Chapel hill, NC.

Genome-wide association studies (GWAS) involving half a million or more single nucleotide polymorphisms (SNPs) allow genetic dissection of complex diseases in a holistic manner. The common practice of analyzing one SNP at a time does not fully realize the potential of GWAS to identify multiple causal variants and to predict risk of disease. Existing methods for joint analysis of GWAS data tend to miss causal SNPs that are marginally uncorrelated with disease and have high false discovery rates (FDRs). We introduce GWASelect, a statistically powerful and computationally efficient variable selection method designed to tackle the unique challenges of GWAS data. This method searches iteratively over the potential SNPs conditional on previously selected SNPs and is thus capable of capturing causal SNPs that are marginally correlated with disease as well as those that are marginally uncorrelated with disease. A special resampling mechanism is built into the method to reduce false-positive findings. Simulation studies demonstrate that the GWASelect can be substantially more powerful than existing methods in capturing causal variants while having a lower FDR. In addition, the regression models based on the GWASelect tend to yield more accurate prediction of disease risk than existing methods. The advantages of the GWASelect are illustrated with the Wellcome Trust Case-Control Consortium (WTCCC) data.

2808/W

Analysis of Secondary Phenotypes in Case-Control Genetic Association Studies: A Gaussian Copula Approach. *J. He, H. Li, M. Li.* Dept Biostatistics, Univ Pennsylvania, Philadelphia, PA.

In many case-control genetic association studies, a set of correlated secondary phenotypes that may share common genetic factors with the disease status are collected. Examination of these secondary phenotypes can yield valuable insights about the disease etiology. The standard regression-based analysis by assessing the effect of a test SNP using cases only, controls only, or combined samples of cases and controls is appropriate only when the test SNP is not associated with the disease. Otherwise, the aforementioned methods will yield inflated type I errors and biased parameter estimates, due to the unequal sampling probabilities between cases and controls. To solve this issue, we developed a Gaussian copula based approach that efficiently combines information on disease status and correlated secondary phenotypes. Our method can incorporate a single secondary phenotype as well as several correlated secondary phenotypes. To evaluate the performance of our method, we conducted extensive simulations under various settings. We considered a single secondary phenotype that is correlated with the disease status. Our simulation results showed that the copula-based approach yields unbiased parameter estimates for the analysis of the secondary phenotype and the type I errors are well controlled at the 1% nominal level under all settings we considered. On the contrary, the regression-based analysis without adjustment of the disease status gives highly inflated type I error of 7% when the secondary phenotype and the disease status are correlated (correlation = 0.5) and the test SNP is moderately associated with the disease (OR = 1.2). We also applied our method to the analysis of LDL-C level in a genome-wide association study on high HDL, where “cases” are defined as those individuals with extremely high HDL-C level and “controls” are defined as those with extremely low HDL-C level. We treated LDL-C as a secondary phenotype and tested for association with SNPs located in CETP, a gene that is well-known to be associated with HDL-C but not LDL-C. Using the copula-based approach, we did not find any evidence of association, but the regression-based analysis without adjustment of “case-control” status identified 13 significantly associated SNPs ($P < 0.01$). Taken together, our results suggest that it is important to appropriately model secondary phenotypes in case-control genetic association studies.

2809/W

Scalable multiple-locus analysis algorithms with increased power to detect weak associations in genome-wide association studies (GWAS). *G.E. Hoffman[1], J.G Mezey[1,2].* 1) Department of Biological Statistics and Computational Biology, Cornell University, Ithaca, NY; 2) Department of Genetic Medicine, Weill Medical College of Cornell University, NY, NY.

All corroborated disease loci that have been discovered in genome-wide association studies (GWAS) were identified by independently analyzing each genetic marker in a study. While the success of individual marker analysis is unequivocal, it is well appreciated that this standard technique suffers from low statistical power to detect weak associations due to small locus effects, weak linkage, or low minor allele frequencies. With the goal of increasing power to detect these weak associations, we apply a class of multiple-locus methods that simultaneously assesses the associations of all genetic markers. We develop a class of algorithms that scale extremely well and overcomes previous computational limitations so that we can perform a GWAS analysis of a thousand samples and one-million markers in less than 24 hours on a standard desktop (with large memory capacity). The class of multiple-locus methods presented here leverages the framework of penalized regression and generalized linear models to provides great versatility for analyzing case/control and continuous phenotypes using a range of popular and theoretically justified penalties such as Lasso and MCP. We have undertaken a simulation study to assess performance and demonstrate that our multiple-locus methods have increased power over individual marker analysis to detect weak associations in large datasets. Through our analysis of available GWAS data from the Wellcome Trust Case Control Consortium, we demonstrate that our algorithms produce a qualitatively different ordering of the top ’hits’ and identifies novel associations and epistatic interactions between markers that were missed by individual marker analysis.

2810/W

Analysis of Untyped SNPs: Maximum Likelihood and Imputation Methods. *Y. Hu, D. Lin.* Biostatistics Dept, Univ North Carolina, Chapel Hill, NC.

Analysis of untyped SNPs can facilitate localization of disease-causing variants and permit meta-analysis of association studies with different genotyping platforms. We present two approaches for using the linkage disequilibrium structure of an external reference panel to infer the unknown value of an untyped SNP from the observed genotypes of typed SNPs. The maximum likelihood approach integrates prediction of untyped genotypes and estimation of association parameters into a single framework and yields consistent and efficient estimators of genetic effects and gene-environment interactions with proper variance estimators. The imputation approach is a two-stage strategy which first imputes the untyped genotypes by either the most likely genotypes or the expected genotype counts and then uses the imputed values in downstream association analysis. The latter approach has proper control of type I error in single-SNP tests with possible covariate adjustments even when the reference panel is misspecified; however, type I error may not be properly controlled in testing multiple-SNP effects or gene-environment interactions. In general, imputation yields biased estimators of genetic effects and gene-environment interactions, and the variances are underestimated. We conduct extensive simulation studies to compare the bias, type I error, power, and confidence interval coverage between the maximum likelihood and imputation approaches in the analysis of single-SNP effects, multiple-SNP effects and gene-environment interactions under cross-sectional and case-control designs. In addition, we provide an illustration with genomewide data from the Wellcome Trust Case-Control Consortium (WTCCC) [2007].

2811/W

The Use of Imputed Values in the Meta-Analysis of Genome-Wide Association Studies. *S. Jiao, L. Hsu, C.M. Hutter, U. Peters.* Fred Hutchinson Cancer Research Center, Seattle, WA.

Genome-wide association studies (GWAS) have been proven to be effective in studying the association between common single nucleotide polymorphisms (SNPs) and common complex traits and diseases. To add value from GWAS data, it is common practice to impute the genotypes of untyped SNPs by exploiting the linkage disequilibrium (LD) structure among SNPs. The imputed genotypes not only improve genome coverage, but also make it possible to combine studies which are genotyped on different platforms in meta-analyses. A popular way of employing imputed data is the “expectation-substitution” method, which treats the imputed dosage (expected number of minor alleles) as if it were the true genotype and performs an association test. However, two important questions remain unanswered: 1) Does the expectation-substitution method give unbiased estimates of the effect size (strictly speaking strength of the association)? 2) Does the imputation quality need to be incorporated into the weights in a meta-analysis? We show, both theoretically and numerically, that the estimates given by the expectation-substitution method are approximately unbiased under practical conditions. In addition, we show that the variances of the estimates are proportional to the imputation quality. As a result, the usual inverse variance weighting scheme automatically and adequately incorporates imputation quality in the meta-analysis, and there is no need to further incorporate imputation quality scores.

2812/W

Analyzing contiguous homozygosity to quantify haplotype structure differences between case and control samples. *T. Johnson[1], T. Tanaka[2], M. Kubo[3], Y. Nakamura[4], T. Tsunoda[1].* 1) Laboratory for Medical Informatics, Center for Genomic Medicine, RIKEN Yokohama Institute, Yokohama, Kanagawa-ken, JAPAN; 2) Laboratory for Cardiovascular Diseases, Center for Genomic Medicine, RIKEN Yokohama Institute, Yokohama, Kanagawa-ken, JAPAN; 3) Research Group for Genotyping, Center for Genomic Medicine, RIKEN Yokohama Institute, Yokohama, Kanagawa-ken, JAPAN; 4) Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo, Japan.

We describe a method that we term *agglomerative haplotype analysis* (AHA) to supplement single-locus tests of genome-wide association study (GWAS) data by comparing cases and controls for haplotype structure differences. Based on analysis of the structure and extent of contiguous homozygosity, segments of contiguous homozygous genotypes in each individual are first detected and the length of any segments intersecting a particular SNP *i* stored in an intersecting segment length vector (ISLV*i*). For each disease, we then create two ISLVs for each SNP that are specific to the disease's case and control samples and calculate their empirical cumulative distribution functions ECDF*i*:cases and ECDF*i*:controls. We quantify case-control differences using the Cramér-von Mises criterion ω^2 (the integral of the squared difference between the two ECDFs) and determine the statistical significance of each ω^2 value using an adaptive permutation test framework. Due to the high computational load for testing >400k SNPs, we first select a set of the most extreme ω^2 values by using a peak/peak-outlier detection algorithm and perform permutation testing for that reduced set of »2,000 positions. A genome-wide significance level (GWSL) is determined using a minimum p-value based analysis of simulated case-control datasets by creating 100 random sets of case samples and controls, performing AHA for each set, and specifying the GWSL as the 5th percentile of the set of lowest p-values across those analyses. We illustrate this method using myocardial infarction (MI) samples from the Japan BioBank, which preliminary analyses suggested to have a large case-control difference on chromosome 12. Using 1,637 case samples and 7,345 controls matched using principal component analysis based genetic matching, we detected a single region with six peaks exceeding the GWSL. That region lies on chromosome 12 between 110.1-111.7 Mb, a region that contains the BRAP gene, which was previously reported by members of our group to contain SNPs associated with risk for myocardial infarction in Japanese. Comparison of $ECDF_{cases}$ and $ECDF_{controls}$ for the top peak (p<9x10^{-8}) shows cases to have »5% more homozygous segments with length >1cM. Although this positive signal maps to a known causal variant that was detected by standard GWAS approaches, our result supports the ability of AHA to detect genomic regions in which cases and controls have different frequencies for an extended haplotype.

2813/W

Possible genetic loci associated with Chagas disease in Northeastern Brazil. *C.E.M. Kawamata[1], R.G.M. Ferreira[1], L.M. Garrido[1], L.C. Perreira[1], M.F. Feitosa[2], H. Krieger[1].* 1) Paratology, ICB, São Paulo, São Paulo, Brazil and INAGEMP; 2) Div Statistical Genomics, Washington University School of Medicine, St. Louis, MO.

Chagas disease, also known as American Trypanossomiases caused by the protozoan Trypanosssoma cruzi that is usually transmitted by Triatominae bugs, affects about 10 million people in the American continent. Previous segregation analysis showed evidence of a major gene with a small multifactorial component influencing the predisposition to the T. cruzi infection in 4697 individuals of 886 families from Northeastern Brazilian origin, collected in 1969-1970 in São Paulo, Brazil. In a follow-up study, it was carried out a genome-wide scan analysis in 69 individuals (18 cases and 51 controls). A total of 263K SNPs were genotyped, and after excluding SNPs with sex incompatibility, non-equilibrium Hardy-Weinberg and technical error 261K SNPs were analyzed by using a logistic regression analysis with an additive model. We found strong evidence of association between infection to T. cruzi with rs1746997 (minor allele frequency = 0.1667, adjusted-Bonferroni P = 0.015) on chromosome 10. This finding suggests that the susceptibility to T. cruzi infection is influenced by at least one genetic variant. Linkage studies on 15 large families are in progress in order to validate these findings. (CNPq, FAPESP).

2814/W

Association analysis of dynamic-traits via temporally-smoothed lasso. *S. Kim[1], J. Howrylak[2], E.P. Xing[1].* 1) School of Computer Science, Carnegie Mellon Univ, Pittsburgh, PA; 2) Harvard Medical School, Boston, MA.

Classical quantitative trait locus (QTL) mapping usually concerns identifying causal genetic variations that underly clinical or molecular traits such as body metrics or gene expression values. In such an analysis, it is standard to assume that the trait values follow a time-invariant distribution. In this paper, we address a new QTL mapping problem, where the trait is a time series, namely, a dynamic trait (or d-trait). Examples of a d-trait include time courses of response to a drug, growth of a tumor, and expression of a gene, which all have a developmental characteristic with a trend over time. We define a QDTL mapping problem as discovering the genetic variations that induce such temporal changes, rather than a one-time genetic effect at a single time point. Causal genetic loci underlying a d-trait are likely to influence the trait dynamics with potentially varying effect sizes over time. To detect such loci, it is important to take into account the temporal correlation in the d-trait measurements during analysis, rather than merely performing a genome-wide association (GWA) at each time point separately as in conventional methods. This problem poses a significant new challenge to current GWA methodology. We propose a new statistical method based on a temporally-smoothed regularized regression that takes advantage of the temporal correlation in a d-trait to discover causal QDTLs. Our approach first learns the temporal correlation information in the d-trait using an autoregressive model, then incorporates this information in a novel regularization function within a regression method to estimate correlated regression coefficients. Our method does not assume any parametric form of the trajectory of d-trait measurements, and has the flexibility of handling any types of stationary and nonstationary trends. We demonstrate our method on both simulated data and clinical data obtained from asthmatic children in the Childhood Asthma Management Program study.

2815/W

A powerful approach to efficiently combine p-values for gene-based association analysis. *M. Li[1,2], P. Sham[1,2,3], C. Cherny[1,2,3].* 1) Department of Psychiatry, The University of Hong Kong, Hong Kong; 2) The Centre for Reproduction, Development and Growth, The University of Hong Kong, Hong Kong; 3) The State Key Laboratory of Brain and Cognitive Sciences, The University of Hong Kong, Hong Kong.

Shifting from Single-nucleotide polymorphism (SNP)-based association analysis to gene-based analysis may be a promising solution for increasing power of genome-wide association studies (GWAS). Methodology for efficient evaluation of gene-wise association significance is still far from developed and does not suffice for the demand to handle large genome-wide dataset. Available methods resort to time-consuming permutation to account for the gene-size and linkage disequilibrium problems. We proposed a powerful association approach to efficiently combine p-values of SNPs within a gene to produce a gene-based p-value. This method employed the gene as an analysis unit. The p-value is used to evaluate gene-level association significance. We theoretically proved that the gene-based p-value is uniformly distributed U(0,1) under null hypothesis for independent SNPs. More importantly, this method could easily integrate prior weights of SNPs. Through a series of computer simulation, we demonstrated that the gene-based method was more powerful than the original SNP-based test and immune to gene size and linkage disequilibrium between markers. Moreover, our test outperformed three other alternatives including Bonferroni correction, Sidak combination test and Fisher combination test. It was even more powerful than the Logistic regression in some situations. In a test, this method (after implemented) only spent less than 1 minute in performing a genome-wide gene-based scan for 2,543,885 SNPs on an ordinary desktop computer, Intel Core™ 2 CPU 2.66GHz, RAM 1.97GB, and 32-bit Windows XP™ Professional Version 2002. To conceptually assess its performance in real data, we applied it to re-analyze three datasets of published GWAS. Our gene-based association method reported 6 more significant genes than the SNP-level association method did (FDR=0.005) in a GWAS dataset for Crohn's disease. Among the 6 genes, the two were convincingly replicated in independent samples conducted the original studies. It reported 4 more significant genes (FDR=0.01) than the SNP-level association test in GWAS for Psoriasis and Schizophrenia respectively. In conclusion, we provided a powerful method to efficient evaluate the gene-wise association significance. This method is expected to identify more disease susceptibility genes of complex diseases and will also facilitate advanced bioinformatics analysis like pathway and protein-protein interaction network enrichment analyses.

2816/W

Deep follow up genome-wide association study to identify psoriasis susceptibility genes. *Y. Li, J. Ding, G. Abecasis.* Center of Statistical Genetics, Department of Biostatistics, University of Michigan, Ann Arbor, MI.

ABSTRACT: Psoriasis is a chronic immune-mediated genetic disorder that appears on the skin, affecting about 1% of the human population. Genome-wide association studies have been conducted in an effort of identifying susceptibility loci for psoriasis. In a previous two-stage case-control study, we (Nair, Duffin, Helms and Ding et al. 2009) genotyped 438, 670 SNPs in 1,409 psoriasis cases and 1,436 controls in the first stage and then followed up 21 promising SNPs in 5,048 psoriasis cases and 5,041 controls in the second stage. In total eight replicated gene regions were confirmed to be associated with psoriasis disease. In order to further scan the above genome regions for additional psoriasis susceptibility loci, we now deeply follow up extra 7,978 SNPs in 2,855 independent psoriasis cases and 2,165 controls. Our newly follow up SNPs consist of 8 panels, including critical SNPs from 1000G in those eight replicated regions, top SNPs from a meta-analysis on 1000G imputation, and top SNPs from a meta-analysis on HapMap imputations. Our deep follow up results provide strong support for the eight associated gene regions established in the previous study. In additon, we discover some De novo psoriasis susceptibility loci. With probably the largest number of follow up SNPs in the GWAS literature, our study will help to identify high-risk individuals and shed lights on developing new therapies for psoriasis related auto-immune diseases.

2817/W

Correcting for Population Stratification in Genome-Wide Association Studies. *D.Y. Lin, D. Zeng.* Dept Biostatistics, CB #7420, Univ North Carolina, Chapel Hill, NC.

Genome-wide association studies are susceptible to the confounding effect of population stratification in that the combination of allele-frequency heterogeneity with disease-risk heterogeneity among different ancestral sub-populations can induce spurious associations between genetic variants and disease. We provide a statistically sound and computationally feasible solution to this important and challenging problem. We estimate the odds ratio of disease with a genetic variant by fitting a semi-parametric logistic regression model which includes an arbitrary function of a propensity score relating the genotype probability to ancestry-informative genomic markers. This approach provides correct control of type I error and unbiased estimates of odds ratios with proper confidence intervals even under severe population stratification. The advantages of the new method over the existing methods are demonstrated through realistic simulation studies. In particular, the new method is shown to perform nearly as well as the logistic regression analysis with known ancestry. An application to the genome-wide association data of the Wellcome Trust Case-Control Consortium (2007, Nature) further illustrates the superior performance of the new method. The relevant software is available at our website.

2818/W

Testing for Weak Signal. *P. Lipman[1], M. Cho[2], P. Bakke[3,4], A. Gulsvik[3,4], X. Kong[5], S. Pillai[5], E. Silverman[2], C. Lange[1].* 1) Dept of Biostatistics, Harvard University, Boston, MA; 2) Channing Laboratory, Brigham and Women's Hospital, Boston, MA; 3) Dept of Thoracic Medicine, Haukeland University Hospital, Bergen, Norway; 4) Institute of Medicine, University of Bergen, Norway; 5) GlaxoSmithKline, Research Triangle Park, NC.

When a statistical analysis involves multiple tests, such as a Genome-Wide Association study (GWAS) that involves upwards of one million SNP-association tests, one must correct the nominal p-values to account for the multiple opportunities to achieve statistical significance. With conservative corrections (such as the widely-used Bonferroni correction), one often fails to reject the null hypothesis when the null hypothesis may in fact be false. Despite the great success of GWAS, the majority of loci remains undiscovered, but can be identified by large-scale follow-up studies. In this communication, we develop an overall statistical test that is applied to the set of most promising association tests from the original study. The overall test will conclude whether there is evidence that the null hypothesis is in fact false in the tested set of association tests, even though no individual test achieves a global significance level. That is, we create a test that examines if one or several (weak) signals exist in the data. The conclusions of the test are used to determine whether a follow-up study on the top hits is recommended. This single test is statistically straight-forward and easily implemented. Using simulations, power is assessed under realistic scenarios of Genome-Wide Association studies. The test can also be modified to help determine the number of top hits which should be further examined in the follow-up study. We illustrate the method with an application to a chronic obstructive pulmonary disease GWAS dataset.

2819/W

Analysis of Family- and Population-Based Samples in Cohort Genome-Wide Association Studies. *A. Manichaikul[1,2], W.M Chen[1,2], K. Williams[3], Q. Wong[3], J.I. Rotter[4], S.S. Rich[1], J.C. Mychaleckyj[1].* 1) Center for Public Health Genomics, University of Virginia, Charlottesville, VA; 2) Department of Public Health Sciences, Division of Biostatistics, University of Virginia, Charlottesville, VA; 3) Collaborative Health Studies Coordinating Center, University of Washington, Seattle, Washington; 4) Medical Genetics Institute, Cedars-Sinai Med Center, Los Angeles, CA.

Cohort studies typically sample unrelated individuals from a target population, although family members of index cases may be also be recruited to investigate shared familial risk factors and to apportion genetic and environmental risk. The recruitment of family members may be incomplete or ancillary to the main cohort study, resulting in a mixed sample of independent family units, including unrelated singletons and multiplex families of 2+ members. There are multiple methods available to perform genome wide association (GWA) analysis of binary or continuous traits in families, but it is unclear whether the methods known to perform well on ascertained pedigrees, sib-ships, or trios are appropriate in analysis of a mixed unrelated cohort and family sample.

The Multi-Ethnic Study of Atherosclerosis (MESA) is an example of a cohort study of unrelated individuals enhanced by an ancillary study to recruit family members specifically for genetic analysis (MESA Family). Approximately 8400 consenting participants recruited by the original MESA cohort and MESA Family and MESA Air ancillary studies have been recently genotyped under the NHLBI SHARe program resulting in 6657 independent families with GWA data, 10.5% of which are multiplex (17.6% in African American families, 20.6% in Hispanic).

We present simulation studies based on MESA pedigree structures to compare performance of several popular methods of GWAS analysis for both quantitative and dichotomous traits in cohort studies. We evaluated approaches suitable for analysis of families, and combined the best performing methods with population-based samples either by meta-analysis, or by pooled analysis of family- and population-based samples (mega-analysis), comparing type 1 error and power. We further assessed practical considerations such as availability of software and ability to incorporate covariates in statistical modeling. Our results suggest that linear modeling approaches which accommodate family-induced phenotypic correlation, e.g. the variance component model of Chen and Abecasis (2007) for quantitative traits or generalized estimating equations as implemented by Chen and Yang (2010) for dichotomous traits, perform best in the context of combined family- and population-based cohort GWAS.

2820/W

Discovery of novel associations by re-analysis of GWAS using a family of scalable multiple locus algorithms. *J. Mezey[1,2], G. Hoffman[1], B. Logsdon[1], C. Gao[1], L. Omberg[1].* 1) Biological Statistics (BSCB), Cornell Univ, Ithaca, NY; 2) Genetic Medicine, Weill Cornell Medical College, New York, NY.

All replicated associations discovered in genome-wide association studies (GWAS) have been identified by independently analyzing each genetic marker in a study. While such individual marker techniques are straightforward to apply and have been a fruitful approach for identifying associations, it is also well appreciated that other approaches can perform better in the identification of weaker associations. We have developed a highly scalable family of multiple locus algorithms for identifying weaker associations by simultaneous analysis of all markers in a GWAS. The foundation of our family of methods is a penalized generalized linear model (GLM) that we use to analyze both case-control and continuous phenotypes, and a set of established penalties, including standard types (e.g. lasso) and non-standard but theoretically well-justified types (i.e. MC+ and mixtures). With these models, we can implement both likelihood-based model selection, using cyclic coordinate-wise descent techniques, as well as Bayesian model selection where we use approximate variational Bayes techniques to implement fast Bayesian model averaging. We have used our family of algorithms to re-analyze a number of publically available GWAS data sets, including data from studies archived in dbGaP and data collected by the Wellcome Trust Case Control Consortium. With our re-analyses, we have identified a number of novel associations not previously identified in the original studies, including associations that have also been identified in other populations, i.e. we have been able to replicate previously unreplicated associations by re-analyzing existing data. These results demonstrate that there is potentially more association information that can be extracted from existing GWAS data when applying non-standard analysis techniques.

2821/W

Meta-analysis of sex-specific genome-wide association studies. *A.P. Morris, R. Magi.* Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom.

Genome-wide association studies (GWAS) have proved to be extremely successful in mapping novel loci contributing effects to complex human traits. However, despite these successes, much of the genetic component of the variance in these traits remains unexplained. One potential source of genetic variation which may contribute to this "missing heritability" is that which has sex-specific or sex-differentiated effects. Such associations could arise, for example, if sex hormones play a role in the regulation of gene expression.

Despite recent confirmed evidence for sex-specific associations with complex traits including schizophrenia and central obesity, males and females are typically analysed together in GWAS. In these "combined-sex" analyses, allelic effects are often adjusted for gender if the trait distribution varies between males and females. However, researchers have been unwilling to undertake male- and female-specific analysis because of a fear of a loss in power because of reduced sample size as a result of stratification by sex.

Here, we review the methodology for sex-specific fixed effects meta-analysis of GWAS. Within this framework, we propose a sex-differentiated test of association, and demonstrate how we can test for heterogeneity of allelic effects between males and females. This methodology can be implemented using the "-sex" option in the freely available, open source GWAMA software (www.well.ox.ac.uk/GWAMA).

We have performed detailed simulations to compare the power of meta-analysis of gender-specific GWAS with that of the "combined-sex" approach of analysing males and females together. Our results highlight only a small loss in power of the sex-differentiated meta-analysis when allelic effects are the same in males and females. However, in the presence of heterogeneity in allelic effects between males and females, sex-differentiated meta-analysis offers substantial gains in power.

2822/W

The arcOGEN Consortium: stage 1 of a genome-wide association scan for osteoarthritis. *K. Panoutsopoulou on behalf of the arcOGEN Consortium.* Wellcome Trust Sanger Institute, Hinxton, UK.

The genetic architecture of osteoarthritis (OA) has not been characterised yet; as with many common complex traits it appears to be of a highly polygenic nature with multiple risk loci conferring small effects, detection of which necessitates large sample sizes. To enable a well-powered genome-wide association study (GWAS) for OA, we have formed the arcOGEN Consortium, a UK-wide collaboration aiming to carry out a 2-stage GWAS of 8,000 cases and 10,000 controls.

We have completed stage 1 GWAS in 3,177 knee and/or hip OA cases and have compared their genotypes against 4,894 publicly available population-based UK controls from the Wellcome Trust Case Control Consortium 2 study. Of the 514,898 single nucleotide polymorphisms (SNPs) that passed our quality control, 89 SNPs reached p values<10^{-4} (as opposed to 51 expected under the null hypothesis of no association). We took forward 102 independent SNPs with p<10^{-4} to in silico replication in three further OA GWAS (from the deCODE, Framingham and Rotterdam studies) and a subset of 52 SNPs in a UK-based GWAS (TwinsUK) comprising a total of 41,075 individuals. Based on meta-analysis results across the arcOGEN and in silico replication datasets we prioritised 36 SNPs for de novo replication in a further set of 6,255 OA cases and 8,289 controls of European ancestry (from Spain, Greece, USA and the Netherlands). None of the followed-up signals reached genome-wide significance (p<$5x10^{-8}$), but this could be due to low power caused by limited sample size, small effect sizes and phenotype heterogeneity. The strongest signal was observed for rs2277831, which lies within the *MICAL3* gene (odds ratio (OR) for the G allele 1.07 [1.04-1.11], p=$2.85x10^{-5}$).

Our study highlights possible novel OA susceptibility loci, which will require corroboration in sample sets of larger size. Stage 2 of the arcOGEN GWAS in 4,500 OA cases is currently underway. A combined analysis of the 2-stage GWAS will provide us with sufficient power to detect modest effects at common loci at the genome-wide significance level (90% power to detect an allele with frequency 0.20 and allelic OR of 1.2).

2823/W

Stratification-Score-Based Matching Outperforms Other Matching Approaches when Controlling for Confounding. *G.A. Satten[1], M.P. Epstein[2], R. Duncan[2], K.A. Broadaway[2], A.S. Allen[3].* 1) CDC, Atlanta, GA; 2) Dept of Human Genetics, Emory Univ, Atlanta GA; 3) Dept of Biostatistics and Bioinformatics & Duke Clinical Research Institute, Duke University, Durham NC.

Proper control of confounding due to population stratification is crucial for valid analysis of genetic association studies. Fine matching of cases and controls based on genetic ancestry is a strategy for control of confounding that is increasing in popularity. Information on genetic ancestry can typically be summarized by a relatively small number of genomic variables such as principal components, chosen to be significant predictors of genomic variability. However, it is difficult to match cases to controls using more than one continuous variable. Existing matching approaches therefore define some scalar measure that combines information from all of the significant components, which is then used to form matched sets. However, inclusion of ancestry components that do not predict disease status into a matching criterion can lead to inaccurate matches, and hence to an improper control of confounding. Here we propose that cases and controls be matched on the stratification score, which is the probability of disease given genomic variables [AJHG 80:921-930]. Matching on the stratification score leads to more accurate matches because case participants are matched to control participants who have a similar risk of disease, given genetic ancestry information. Using a genome-wide case-control study of schizophrenia among African-Americans, we show that confounding is resolved by our stratification-score matching approach but not by other existing matching procedures (GEM, spectral-GEM and GSM). We also use simulated data to show the superior performance of our approach. [GEM=GEnetic Matching, Amer J Hum Genet 82:453-463; spectral-GEM, Genet Epidemiol 34:51-59; GSM= Genetic Similarity Score Matching, Genet Epidemiol 33:508-517].

2824/W

A multivariate genome-wide association analysis of 10 LDL subfractions, and other lipid measures, in 1800 Caucasians. *H. Shim[1], K. Wojnoonski[2], L. Mangravite[2], M. Barber[1], M. Stephens[1,3], R. Krauss[2].* 1) Department of Human Genetics, University of Chicago, Chicago, IL; 2) Children's Hospital Oakland Research Institute, Oakland, CA; 3) Department of Statistics, University of Chicago, Chicago, IL.

Levels of plasma lipids and lipoproteins are related to risk of cardiovascular disease, and because of this considerable attention has been devoted to genome-wide association (GWA) analyses of various lipid-related measures, including plasma concentrations of LDL-cholesterol (C), HDL-C, and triglycerides (TG). These measures however fail to identify potentially important subfractions of lipoprotein particles (i.e. those defined by differences in size and density). To better understand, in detail, how genetic variants influence components of lipoprotein metabolic pathways, we performed a GWA analysis of 10 LDL subfractions measured by gradient gel electrophoresis, together with LDL-C, HDL-C, TG, apolipoprotein B, and LDL peak diameter in > 1,800 Caucasian subjects from the Pharmacogenomics and Risk of Cardiovascular Disease study. Unlike most previous analyses of these kinds of data, we analyzed all measurements jointly, rather than one at a time, providing a more detailed picture of the effect each genetic variant has on the entire lipid profile. Specifically, we use a novel Bayesian framework for multivariate association analysis, which simultaneously addresses the twin questions of *which* genetic variants affect components of the lipid system, and *how* they affect the system. We find that measuring LDL subfractions reveals associations not evident from analysis of total LDL-C. For example, rs3764261 in CETP is not significantly associated with LDL-C (Bayes Factor, BF < 1) but extremely strongly associated with the multivariate subfraction data (BF > 10^35). This is because this variant increases some subfractions and decreases others, with the effects approximately canceling one another out. In contrast, genetic variants in other genes show very different patterns: e.g., variants in APOE increase all LDL subfractions, whereas variants in SORT1 affect LDL-III and IV subfractions only. These differences among genes are evident only because of the more refined phenotyping, and point to the potential value of these kinds of analyses to identify novel genetic influences on cardiovascular risk. More generally, they illustrate the potential for detailed phenotypic measurements to enhance the ability to discover biologically meaningful associations from genome-wide analyses.

2825/W

Genetic Association Analysis of CAOD Using Mixed Models. *N.-H. Son[1], K. Song[2], S.M. Myoung[3], D.-J. Shin[4], Y. Jang[5].* 1) Cardiovascular Genome Center, Yonsei University College of Medicine, Seoul, Korea, MS; 2) Department of Biostatistics, Yonsei University College of Medicine, Seoul, Korea, PhD; 3) Department of Medical Informatics, Jungwon University, Chungbuk, Korea, PhD; 4) Cardiovascular Genome Center, Yonsei University College of Medicine, Seoul, Korea, PhD; 5) Cardiovascular Genome Center, Department of Internalmedicine, Yonsei University College of Medicine, Seoul, Korea, MD, PhD.

In genetic association studies, the complicated interaction among genes and the interaction between environmental factors and genes should be taken into consideration. However, as the number of SNPs increase, the frequently used analysis methods such as logistic regression have analytic limitation to test and interpret this complex relationships. We proposed using the mixed model approach to identify significant genotype groups and the gene-gene interactions. For the purpose of these analyses, we used data from 1,006 individuals(503 individuals who were patients with CAOD and other who were healthy) and 5 SNPs among 32 candidate SNPs examined from Cardiovascular Genome Center, Yonsei University. We defined genotype groups which are groups of individuals with same genotypes. And these observed groups were treated as random effects in a mixed model. We compared the odds of random effects model with that of mixed effect model. We could analyze genotype group-specific effects through the random effects in mixed model, and it was possible to assess the effects of SNP combination. In conclusion, the mixed model approach provided a flexible framework for identifying a significant genetic contributions that may come about through the effects of multi-locus genotypes or through an interaction between the genotype and environmental factors with the variations in diseases.

2826/W

Using covariates to improve power in genomewide association studies with related individuals. *S.A. Stanhope, M. Abney.* Department of Human Genetics, The University of Chicago, Chicago, IL.

In this paper we introduce GLOGS (Genomewide LOGistic mixed model / Score test), a procedure that simultaneously incorporates both arbitrary kinships between individuals and non-genetic susceptibility risk factors in genomewide association studies (GWASes). Because GLOGS is capable of controlling for a wider range of predictors of disease risks than alternative methods, it yields greater power in detecting associations between genetic markers and phenotypes.

We demonstrate such improvements in two simulation studies based on a dense kinship coefficient matrix, covariates and disease phenotypes observed in previously collected data. First, we examine the performance of GLOGS for cases independent of covariates. We show that GLOGS can yield up to a 4× improvement in power in comparison to FBAT. Next, we show that including non-genetic susceptibility risk factors in GLOGS can provide up to a 2× improvement in power compared to analyses omitting such effects.

To demonstrate the applicability of GLOGS to actual data collections, we perform GWASes of an asthma phenotype in the Hutterite population and an alcoholism phenotype in the GAW14 dataset. From the latter we report several markers associated with alcoholism.

Methodologically, GLOGS models disease risk in a mixed effects logistic regression framework. Kinships are expressed as an unobserved polygene represented as random effects correlated across individuals, and other risk factors are fixed effect covariates. Marker-specific score tests based on a null (no marker effect) model fit are used to evaluate associations. Since GLOGS represents kinships between individuals through a correlation matrix, it is appropriate for use on populations with either known pedigrees or inferred kinship coefficient matrices.

This study contributes to GWAS methodology through the simultaneous, tractable and general handling of both kinships between individuals and other predictors of disease risk. To promote the applied use of GLOGS, its current implementation is available for download.

2827/W

Increasing power of groupwise association test using aggregated statistic of rare variants. *J. Sul[1], B. Han[1], D. He[1], E. Eskin[1,2].* 1) Computer Science Department, University of California, Los Angeles, CA; 2) Department of Human Genetics, University of California, Los Angeles, CA.

The advent of next generation sequencing technologies allow one to discover nearly all rare variants in a genomic region of interest. This technological development increases the need for an effective statistical method for testing the aggregated effect of rare variants in a gene on disease susceptibility. The idea behind this approach is that if a certain gene is involved in a disease, any rare variants that disrupt the function of the gene are associated with the disease. In this paper, we present Rare variant Weighted Aggregate Statistic (RWAS), a method that groups rare variants and computes a weighted sum of their z-scores. We show that RWAS outperforms previous methods for the groupwise association test such as a weighed sum statistic by Madsen and Browning in the disease-risk model where each variant makes a small contribution to disease-risk. In addition, we can incorporate into RWAS prior information of which variants are likely causal, and we show that prior information can have considerable effects on the power of studies.

2828/W

A Significance Level by Empirical Distribution of the Statistic in Genome-wide Association Study. *A. Takahashi[1], N. Hosono[2], M. Kubo[2], Y. Nakamura[3], N. Kamatani[1].* 1) Laboratory for Statistical Analysis, Center for Genomic Medicine, RIKEN, Yokohama, Japan; 2) Laboratory for Genotyping Development, Center for Genomic Medicine, RIKEN, Yokohama, Japan; 3) Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

Genome-wide association study(GWAS) were widely performed, and succeeded in the identification of many genes associated with diseases. In GWAS, more than several hundred thousand Single Nucleotide Polymorphisms(SNPs) are usually genotyped, and perform the association test. In this analysis, the small p-values happen by chance because a lot of the statistical tests are performed. It is important to control the family wise error rate to capture the genes truly associated with diseases. Various significance levels in GWAS have been suggested until now. A most simple method to correct the family wise error rate is a Bonferroni's correction. However it is well known that this correction is too conservative. In addition, all SNPs were not independent because of the linkage disequilibrium(LD). The distribution of test statistics such as a trend test in GWAS is not obvious. A significance level depends on SNPs used set and population. One method to determine a significance level is to create the empirical distribution of the statistic. To assess the global type I error rate, we created the empirical distribution of the statistic by permutation test. In the Biobank Japan project, more than 30,000 Japanese people were genotyped by the Illumina Human610-Quad(610K) and the Illumina Human 550v3(550K) Genotyping BeadChips to discover susceptibility genes. At first, the two groups were created by selecting samples from 30,000 people at random. We conducted association test between two groups. The empirical distribution of the statistic under the null hypothesis can be obtained by permuting the samples at random in the two groups. We have shown the global type I error rate under the Illumina 610K/550K BeadChips in Japanese population. If sample size is small, SNPs whose minor allele frequencies are low may be observed as mono-polymorphisms. This fact means the number of effective statistical tests changes. We also have studied a significance level related to sample size.

2829/W

Testing heterogeneity of quantitative trait variance among genotype classes guides fine mapping of association signals. *F. Takeuchi, N. Kato.* National Ctr for Global Health & Med, Tokyo, Japan.

Purpose. Single nucleotide polymorphisms (SNPs) identified in genome-wide association studies (GWAS) are supposedly markers in linkage disequilibrium (LD) with some causal variant(s), and fine mapping is a current hot topic. For efficient fine mapping, we want to know whether the variant(s) accounting for a GWAS signal is included in the common SNP(s) (minor allele frequency, MAF, ≥5%) showing the strongest association, or will emerge once association testing is extended into low frequency variants (MAF, 0.5-5%). To answer the question, we propose a statistical test that examines if the association of a SNP with a quantitative trait (QT) is due to LD with (possibly unknown) low frequency variants. **Proposed method.** We test the heterogeneity of QT variance (ie, heteroscedasticity) among genotype classes of a SNP, using the Bartlett's test (significance level 0.05), after applying rank-based inverse normal transformation to the QT distribution in the whole study population. **Results.** To quantify the QT variance, we modeled a marker SNP with alleles *A* and *a* and a single causal variant with alleles *B* and *b*, assuming that the QT is normally distributed with the unit variance and the mean equal to 2*d*, *d* or zero within individuals of genotype *B/B*, *B/b* or *b/b*, respectively. If the low frequency allele *B* is always linked to allele *A*, individuals with *A/A* genotype have either of three genotypes, *B/B*, *B/b* or *b/b*, thus show a wide QT distribution; the QT variance inflates proportionally to d^2 and the variance of the causal genotype within the individuals. For SNPs identified in simulated GWAS, heteroscedasticity was detectable (power >0.8) when the SNP is in LD with one primary causal variant that has a frequency lower than the SNP by >8-fold. Finally, we could validate the usefulness of the test by real data for the *APOE* locus associated with LDL-cholesterol level. **Conclusion.** Testing heteroscedasticity can guide fine mapping by suggesting the next step after studying association of common SNPs exhaustively in a locus. If heteroscedasticity is significant for the common SNP(s) showing the strongest association, we recommend extending the study into low frequency variants, which should include the variant causing heteroscedasticity. Alternatively, if heteroscedasticity is not significant (when tested in >5,000 samples), the leading SNP(s) are causal, or there are multiple low frequency causal variants together canceling heteroscedasticity.

2830/W

A Sequential Test Algorithm for DNA Pooling/Bootstrap-Based Studies. *J.I. Velez Valbuena, M. Arcos-Burgos.* Med Genetics Branch, NHGRI/NIH, Bethesda, MD.

DNA pooling is a practical way to reduce the cost of large-scale association studies to identify susceptibility loci. In contrast to individual genotyping, in DNA pooling we can combine samples from *N* cases and *M* controls into two single pooled sample tests and estimate the allele frequency of hundred of thousand SNPs using high-throughput genotyping technologies. Selection of disease-associated SNPs is performed based on their *P*-value after a statistical test has been run. For the subset of those SNPs determined to be significant, all individuals are then genotyped to corroborate results from the pooled samples.

The strategy described above has been successfully used for years. However, there are situations in which either it is not possible to recruit the number of patients (cases) needed based on power estimations or replication of DNA pools is important. In both situations, the limitation is the availability of DNA samples.

When using sequential testing, formally presented in 1945 as sequential probability ratio test (SPRT), units of interest are sequentially included as they are generated, and a statistical test is run at every stage. In the context of DNA pooling, units would be represented by the pooled samples coming from each group of cases and controls while using a bootstrapping re-sampling strategy.

We have developed a SPRT algorithm for identifying disease-associated SNPs when comparing cases and controls via DNA pooling that is at least as powerful as the strategy previously described, but that needs less DNA samples than the strategy described above. Along with our algorithm, we also provide a way to estimate the number of stages needed, i.e., the number of SNP-chip pairs to stop the algorithm achieving a desired significance and power levels. We illustrate how our approach works using GWAS on Oppositional Conduct Disorder (OCD) and Attention Deficit Hyperactivity Disorder (ADHD).

2831/W

Covariate Adjustment in Case-Control Studies. *C. Xing.* McDermott Ctr, Univ Texas SW Med Ctr, Dallas, TX.

A conventional wisdom in classic linear regression is that adjusting for covariates associated with the response variable can improve the precision of estimates by reducing the residual variance; however, covariate adjustment in logistic regression models always leads to a loss of precision. Nonetheless, this loss of precision does not always result in a loss of power. When the genetic and risk/preventive environmental factors are independent and do not have interaction effects on the disease, it is always more efficient to adjust for the predictive covariates. Recently, Kuo and Feingold (Genet Epidemiol 2010, 34:246-253) compared the power of three logistic regression models to detect genetic effects, concluded that "the most commonly used approach to handle covariates—modeling covariate main effects but not interaction—is almost never a good idea", and recommended modeling only the genetic factors without covariate adjustment in genome-scanning. A person's genetic background is determined from birth, thus in most cases it is not unreasonable to assume it is independent of his/her subsequent environmental exposure, which has been a key assumption in some study designs to investigate gene-environmental interaction. If we assume that only a small proportion of genetic variants interact with the known environmental factors on disease susceptibility, then, contrary to the conclusion by K&F, we recommend adjusting for predictive covariates at the genome-scanning stage.

2832/W

Where do true positives rank in GWAS? *D.V. Zaykin.* Dept Biostatistics, NIEHS/NIH, Res Triangle Park, NC.

A standard calculation in the design of GWAS is a sample size determination needed to achieve adequate power at the genome-wide level of significance. An alternative approach is to calculate the probability that a true positive will rank among a specific number of best results, when they are sorted by an association statistic. The rank-based approach allows one to find the number of most significant results to follow up on, as determined by the desired probability of capturing a true association. The rank-based approach is appealing, since it provides guidance for the number of SNPs needed in a replication study. Unlike the power-based approach, it does not require specification of a particular significance level. The usual evaluation of ranking probabilities is via simulations and non-standard numerical methods. I give a very simple but highly accurate approximation for computing ranking probabilities. The approximation allows for linkage disequilibrium and is applicable to multiple associated SNPs. Further, I describe a straightforward relation of ranking probabilities to power at the GWAS level.

2833/W

eQTL mapping in peripheral blood CD4+ lymphocytes identifies replicable, novel asthma susceptibility loci, augmenting standard genome-wide association testing. *S. Sharma, A. Murphy, V. Carey, B. Himes, J. Howrylak, B. Raby.* Dept Respiratory Epidemiology, Channing Laboratory, Boston, MA.

Rationale: Genome-wide association studies (GWAS) have yet to identify the majority of genetic variants involved in the pathogenesis of complex diseases like asthma, perhaps in part due to lack of meaningful data to guide functional prioritization of the large number of markers tested. Expression quantitative trait loci (eQTL) mapping should enable such prioritization by identifying those variants that impact phenotypic diversity in transcript abundance. Using asthma as a model, we sought to evaluate this possibility by testing the cis-acting regulatory variants identified from an eQTL mapping study of peripheral blood CD4+ lymphocytes (a cell-type implicated in the pathobiology of asthma) for association with asthma susceptibility in two well-characterized populations. **Methods**: The eQTL study was performed in a subset of 200 non-Hispanic white subjects with mild-moderate persistent asthma participating in the Childhood Asthma Management Program (CAMP), for whom GWAS genotype data (Illumina HumanHap 550k chip) were available. CD4+ lymphocyte gene expression profiles were generated using the Illumina HumanRef8 v2 array. eQTL testing was restricted to those variants mapping within 50kb of transcript, resulting in identification of 6,977 cis-acting regulatory variants in 1,8311 genes (FDR<0.05). We then tested these variants for association with asthma susceptibility in 403 parent-child trios participating in CAMP using family-based association tests in PBAT. Significant associations were then tested for replication in 600 parent-child trios participating in the Genetics of Asthma in Costa Rica Cohort. **Results**: Cis-acting regulatory polymorphisms demonstrated reproducible association with asthma in both cohorts. We first confirmed the previously-reported association of chromosome 17q regulatory variants (i.e. the ZPBP2/GSDMB/ORMDL3 locus) with asthma susceptibility (CAMP p=0.0007, Costa Rica p=0.000003, fisher's combined p=4.4 x10-8). However, additional variants in other biologically plausible candidates were also observed including FADS2 (Fisher's combined p=0.0002) and TNFSF12 (Fisher's combined p=0.005). These represent novel asthma susceptibility targets warranting validation in independent cohorts. **Conclusions**: Our results suggest that eQTL mapping of regulatory variation is a powerful method for identifying disease-susceptibility loci. Using this method, we have identified two novel asthma susceptibility loci.

2834/W

Model-Based Multifactor Dimensionality Reduction for detecting gene-gene interactions in case-control data in the absence and presence of noise. *K. Van Steen[1,2], T. Cattaert[1,2], M.L. Calle[3], S.M. Dudek[4], J.M. Mahachie John[1,2], F. Van Lishout[1,2], V. Urrea[3], M.D. Ritchie[4].* 1) Systems and Modeling Unit, Montefiore Institute, University of Liege, Grande Traverse 10, 4000 Liège, Belgium; 2) Bioinformatics and Modeling, GIGA-R, University of Liege, Avenue de l'Hôpital 1, 4000 Liège, Belgium; 3) Department of Systems Biology, University of Vic, Carrer de la Sagrada Família 7, 08500 Vic, Spain; 4) Ritchielab, Center for Human Genetics Research, Vanderbilt University, 519 Light Hall, 2215 Garland Avenue, Nashville, TN 37232-0700, USA.

Analyzing the combined effects of genes and/or environmental factors on the development of complex diseases is a great challenge from both the statistical and computational perspective, even using a relatively small number of genetic and non-genetic exposures. Several data mining methods have been proposed for interaction analysis, among them, the Multifactor Dimensionality Reduction Method (MDR), which has proven its utility in a variety of theoretical and practical settings. Model-Based Multifactor Dimensionality Reduction (MB-MDR), a relatively new MDR-based technique that is able to unify the best of both non-parametric and parametric worlds, was developed to address some of the remaining concerns that go along with an MDR-analysis. Whereas the true value of MB-MDR can only be revealed via extensive applications of the method in a variety of real-life scenarios, here we investigate the empirical power of MB-MDR to detect gene-gene interactions in the absence of any noise and in the presence of genotyping error, missing data, phenocopies and genetic heterogeneity. For the considered simulation settings, we show that the power is generally higher for MB-MDR than for MDR, in particular in the presence of genetic heterogeneity, phenocopies and epistasis models involving markers with low minor allele frequencies.

2835/W

Contingency table test for multiple-categories with arbitrary weights on the cells. *R. Yamada, T. Kawaguchi.* Statistical Genetics, HGC, Kyoto University, Kyoto, Japan.

Test of independence between two factors is one of the most basic tests for many fields. In genetics, it has been in regular use for genotype-phenotype correlation. The simplest case of genotype-phenotype correlation handles two categorical phenotypes (cases vs. controls) and three diplotypes of diallelic genetic variants, ie., 2x3 table test. Even for this simple table, multiple tests, Pearson's test with two degrees of freedom (df), genetic mode-specific tests with 1 df (dominant test, recessive test , additive test). Sometimes MAX3 test or MAX test, which is consisted of the three mode-specific tests, is also used. All of these tests analyze the same table and they output different result because they weighi cells of the table differently.

In the recent genetic epidemiology studies, the phenotypes can be multi-categorical with disease stages or grades and combinations of multiple conditions of diseasee criteria, and the number of categories of genotypes can be more than three. Therefore it is beneficial to handle NxM tables in the same way with the genetic mode-specific tests for 2x3 tables.

When we generalized the idea for 2x3 tables to NxM tables, we evaluated the structure of tests geometicly.In the case of 2x3 tables, Pearson's test of 2 df and other tests of 1 df were in the relation where Pearson's test's statistics drew elliptic contour lines in 2-dimensional space and the others did straight lines.We generalized two-dimension to df-dimension for NxM tables, where df = (N-1)x(M-1) and gave the method to calculate statistics for NxM tables with arbitrary weights on the celles.

2836/W

Bayesian Analysis of Rare Variants in Genetic Association Studies. *N. Yi, D. Zhi.* Dept Biostatistics, Univ Alabama, Birmingham, AL.

Recent advances in next-generation sequencing technologies facilitate the detection of rare variants, making it possible to uncover the roles of rare variants in complex diseases. As any single rare variants contain little variation, association analysis of rare variants requires statistical methods that can effectively combine the information across variants and test their overall effect. We here propose a novel Bayesian generalized linear model for analyzing multiple rare variants within a gene or genomic region in genetic association studies. Our model can deal with complicated situations that have not been fully addressed by existing methods, including issues of disparate effects and non-functional variants. Our method jointly models the overall effect and the weights of multiple rare variants and estimates them from the data. This approach could produce different weights to different variants based on their contributions to the phenotype, yielding an effective summary of the information across variants. We evaluate the proposed method and compare its performance to existing methods on extensive simulated data. The results show that the proposed method performs well under all situations and is uniformly more powerful than existing approaches.

2837/W

Stability selection analysis of GWAS uncovers importance of second messenger signalling pathways in genetic predisposition to rheumatoid arthritis. *H. Eleftherohorinou[1,2], C. Hoggart[1], V. Wright[2], M. Levin[2], L. Coin[1].* 1) Department of Epidemiology and Biostatistics, Imperial College London, St Mary's Campus London, United Kingdom; 2) Department of Paediatrics, Division of Medicine, Imperial College London, St Mary's Campus London, United Kingdom.

Background: Genome-wide association studies (GWAS) have largely identified genes associated with disease on the basis of single-SNP *P* values. Meta-analysis of multiple GWAS has been applied the last couple of years to increase the sample size and therefore the power of discovery, but in essence it follows the same concept as the single-SNP approach and as such it suffers from the same limitations. Aim: We recast the question of how to associate genes with disease as a variable selection problem. We introduce a stability selection procedure in a pathway-based setting to identify functionally relevant genes which are robustly selected in predictive models of case-control outcome in two rheumatoid arthritis (RA) studies. Methods: We examined 700 pathways assembled from public databases and we used the cumulative trend statistic (CTT)[1] to assess their evidence of association with the disease. We collected the genes within associated pathways and we applied Hlasso[2], a logistic regression and variable selection algorithm, under subsampling and constant tuning of the regularization parameters, shape λ and penalty f'. Selection probabilities, over 1000 subsamples and 100 parameter sets, were calculated for genes blocks as a means to address for large gene size and LD structure. The procedure is called stability selection[3]. It is based on the principle that while tuning the amount of regularization, the truly relevant variables exhibit higher and stable selection probabilities. Subsampling is used to control the finite sample familywise type I error rate. Results: We identified 45 genes from the Wellcome Trust Case Control Consortium RA study and 54 genes from the North American Rheumatoid Arthritis Consortium (NARAC) study. 30 of these genes were selected in both studies ($P_{overlap\_by\_chance}<10^{-6}$), of which 21 are novel associations with RA. The novel associations implicate genetic variation in second messenger signalling pathways through cyclic AMP, inositol phosphate and calcium in RA susceptibility. Conclusion:The marriage of the biologically driven pathway information with a robust variable selection methodology yields markedly improved and consistent results across two independent datasets of RA. Concurrency of findings is achieved both at the pathway and at the gene level. References [1]Eleftherohorinou et al,PLoS One, 2009 [2]Hoggart et al,PLos Genetics, 2008 [3]Meinshausen N and Buhlmann P, Journal of the Royal Statistical Society (to appear).

2838/W

HLA Associations with Birth Date and Age and Applications to Disease Association Studies. *L. Gragert[1], M. Maiers[1], W. Klitz[2].* 1) Bioinformatics, National Marrow Donor Program, Minneapolis, MN; 2) Public Health, University of California, Berkeley, CA.

In order to test for HLA disparities based on birth date and age, we split a control sample of European-American donors recruited by the NMDP between 1997 and 2002 into two parts and ran HLA association studies. Comparing the HLA of donors under 50 with those over 50, we found several significant HLA associations. The HLA haplotype most associated with older age was A*02-B*18-DR*04 with an odds ratio of 0.582 and a P-value of 0.0006 after Bonferroni correction. We also found a B-DR haplotype associated with younger age, B*35-DR*02 with an odds ratio of 1.25 and a P-value of 0.013. When comparing donors born before 1950 with those born after 1950, we also detected several significant associations, some of which were distinct from the age association analysis. HLA differences based on age may come about from protective and/or predisposing effects of HLA for disease, results in older subjects having a different HLA makeup than younger subjects. HLA differences based on birth date may reflect demographic changes in populations over time, a result of new immigration or higher levels of admixture in younger subjects. Further investigation is needed to test hypotheses for the causes of these HLA disparities. We have previously described significant HLA associations within self-identified race/ethnic (SIRE) categories for geography, gender, and population substructure. Matching controls for all of these factors when conducting HLA disease association studies and must be considered part of the research protocol to avoid erroneous conclusions.

2839/W

Shared Loci for Leprosy and Crohn's Disease: Clues to Inflammation and Immunity? *F.O. Vannberg[1], H.S. Wong[1], S. Gochait[2], D. Malhotra[2], S.O. Sow[3], R.M. Pitchappan[4], R.N.K. Bamezai[2], A.V.S. Hill[1].* 1) Wellcome Trust Human Genetics, Univ Oxford, Oxford, United Kingdom; 2) National Centre of Applied Human Genetics, School of Life Sciences, Jawaharlal Nehru University, New Delhi 110067, India; 3) Institut Marchoux, Bamako, Mali, West Africa; 4) Centre for Excellence in Genomic Sciences, Madurai Kamaraj University, Madurai 625021, India.

Our group have identified novel mycobacterial susceptibility loci using genome-wide approaches. A subset of the leprosy susceptibility loci overlap with those of Crohn's disease, including the 13q14.11 locus. This locus contains the gene *C13orf31* (rs3764147, P=6.1x10-8, OR= 1.59, 95%CI 1.34-1.89), and this SNP was found to consistently replicate in leprosy case control populations from New Delhi (India), Kolkotta (India) and from the west African country of Mali. The MHC locus near HLA-DRB1 associates with leprosy (rs1071630, P=4.9x10-14, OR=0.43, 95%CI 0.35-0.54), and lies within 10 kb of the MHC association signal for ulcerative colitis, another form of inflammatory bowel disease. We did not replicate the previous leprosy association with *NOD2*, although we did find that another innate immune receptor did associate with leprosy (*TLR1*, rs5743618, I602S, P=5.7x10-8, OR=0.31, 95%CI 0.20-0.48). We further go on to perform genetic association analysis between our genome-wide association study of leprosy and that of the Wellcome Trust Case Control Consortium Crohn's disease study. This formalized analysis identifies overlapping genetic susceptibility loci between these diseases to better define molecular pathways involved in immunity to intracellular pathogens and to the excessive inflammation which defines Crohn's disease.

2840/W

Gene-based analysis identifies novel loci shared by Crohn's disease and ulcerative colitis. *K.I. Morley[1,2], L. Jostins[1], D.G. Clayton[3], J.C. Barrett[1], UK IBD Genetics Consortium.* 1) Human Genetics, Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Cambridge, United Kingdom; 2) Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, School of Population Health, The University of Melbourne, Melbourne, Australia; 3) Cambridge Institute for Medical Research, Medical Genetics, Wellcome Trust/MRC Building, Addenbrooke's Hospital, Cambridge, CB2 0XY, United Kingdom.

Genome-wide association (GWA) studies of Crohn's disease (CD) and ulcerative colitis (UC) have identified over 50 associated genomic regions. This research has provided insight into how irregularities in the innate and adaptive immune systems predispose to these conditions, and has also demonstrated that there are both common and independent biological mechanisms contributing to each disease. Joint association analysis of CD and UC may improve power to detect loci shared between them, but must allow for the fact that different variants within a gene region may show a different degree of association to each trait. We used a recently developed variable selection method, an extension of the elastic net, to conduct genome-wide gene-based analysis of a data set combining 1,748 CD cases (WTCCC), 2,361 UC cases (WTCCC2), and 5,417 controls. We replicated many of the known loci for CD and UC (such as *IL23R*, *NOD2*, and *NKX2-3*), and also identified a number of novel loci exceeding a genome-wide gene-based significance threshold of $3x10^{-6}$. These novel loci include a region on chromosome 11q13.1, where *CTSW* (cathepsin W, $p = 8x10^{-12}$) is located, expression of which is regulated by *IL-2* and occurs primarily in cytotoxic lymphocytes. We also identified a locus at chromosome 16p13 that encompasses *DEXI* ($p = 2x10^{-7}$), a gene transcribed in response to the powerful anti-inflammatory drug dexamethasone and *CIITA* ($p = 2x10^{-8}$), which regulates transcription of class II major histocompatibility complex genes. Replication of these findings is ongoing in an independent collection of 5,000 inflammatory bowel disease cases. These results demonstrate the utility of our gene-based approach to joint association of related diseases, a method that can be extended to include additional related diseases, or applied to non-autoimmune disease phenotypes.

2841/W

Identifying phenotypes of interest from noisy measurements: Mood responses to a single dose of amphetamine. *B. Engelhardt[3], A. Hart[2], M. Stephens[1,2], H. de Wit[4], A. Palmer[2,4].* 1) Department of Statistics, Univ Chicago, Chicago, IL; 2) Department of Human Genetics, University of Chicago, Chicago, IL; 3) Department of Computer Science, University of Chicago, Chicago, IL; 4) Department of Psychiatry and Behavioral Neuroscience, University of Chicago, Chicago, IL.

Phenotypes of interest can be difficult to measure and quantify. Complex phenotype information is often summarized in such a way as to maximize the possibility of detecting meaningful genetic associations, but the phenotypic interpretation of the summarization is not always clear. We present our approach to complex phenotype summarization within the scope of a genome-wide association study involving response to amphetamine in humans. Propensity to drug addiction may be linked to the mood-altering response to a drug, but drug response is a difficult phenotype to quantify. In this study, we sought to find genetic markers that influence response to d-amphetamine, which is known to be a heritable trait. Healthy young adults participated in three sessions in which they received, in randomized order, placebo, 10mg, and 20mg of d-amphetamine. The participants then completed self-reported mood questionnaires, and their physiological reactions were assessed, every 30 minutes for a three-hour period. Because of the variability inherent in these complex data, no single question or measurement simply quantified the reaction to the drug across all individuals. To identify a stronger signal of drug response, we smoothed the data by summarizing the time points in various ways, then we applied a method for sparse factor analysis (SFA) to these data. SFA is a statistical model that identifies a small set of factors that, through a weighted linear combination, approximate the survey responses of each individual. Each survey question may or may not contribute weight to a particular factor. By looking at questions that contribute substantial weight to a factor, we can identify the phenotype captured by that factor. Previously, principal components analysis with rotations was applied in lieu of SFA, but we have found that SFA produces factors with a higher level of sparsity, leading to greater interpretability in the factors. On these data, SFA found a set of factors that are individually identifiable as drug response or other phenotypes of interest. Furthermore, when we analyzed association between these factors and the available genotypes in a Bayesian framework, we identified a number of possible associations near genes of interest. This study shows the promise of using SFA on complex data to extract meaningful signals of phenotype, which were substantiated by the quality of the associations we identified.

2842/W

Scan Statistics for Pathway-based Genome-wide Association Study. *T. Nishiyama[1, 2], K. Takahashi[3], T. Tango[3], S. Takami[4], H. Kishino[4].* 1) Doctor of Public Health Program in Biostatistics, National Institute of Public Health, Wako, Japan; 2) Clinical Trial Management Center, Nagoya City University Hospital, Nagoya, Japan; 3) Department of Technology Assessment and Biostatistics, National Institute of Public Health, Wako, Japan; 4) Laboratory of Biometry and Bioinformatics, Graduate School of Agriculture and Life Sciences, University of Tokyo, Tokyo, Japan.

Despite great success of genome-wide association studies (GWAS) in identification of genes underlying complex diseases, the current GWAS have focused only on examining significance on a SNP-by-SNP basis. One of the biggest challenges facing GWAS is a lack of sufficient power to detect small effects as significant. To overcome this limitation, gene set analysis (GSA) methods of analyzing SNP data in GWAS have recently been proposed. However, GSA methods of GWAS do not use gene-pathway structure information, represented by gene products as graph nodes and graph edges between these nodes. Instead of focusing only on the SNPs that are grouped as gene-sets, we propose genome-wide association analysis that incorporates pathway structure information. As a proof of concept, we performed GWAS on a set of 868 cases and 1194 controls genotyped for almost 545080 SNPs, based on pathway structure information on Pathway Commons. To detect clustering of significantly associated SNPs on Pathway Commons pathway, scan statistics were applied with a variable window size. We treated the smallest P values as our statistic of interest and determined its overall significance level. We applied this method based on existing statistics used for GSA methods, such as Gene Set Enrichment Analysis (GSEA). The results demonstrated that this new method for GWAS was able to identify biologically meaningful sub-pathways associated with the trait examined. The performance of our scan-statistics approach is extensively compared with GSA methods.

2843/W

Novel methods for detecting associations between continuous trait and rare variants: Application to the 1000 genome project. *M. Ladouceur[1,2], C. Greenwood[1,2], Y. Aulchenko[3], B. Richards[1,2].* 1) Department of Human Genetics, McGill University, Montreal, Quebec, Canada; 2) Divison of Clinical Epidemiology, Montreal Jewish General Hospital, Montreal, Quebec, Canada; 3) Erasmus Medical Center, Rotterdam, Holland.

For many complex traits, common variants describe a small proportion of the genetically determined variance. Recent evidence suggests that multiple rare variants may account for a larger proportion of this susceptibility. However, these variants are hard to identify using conventional single nucleotide-based association methodology unless extremely large samples are available. Therefore new methodologies are required to dissect the contribution of rare base pair variants to common disease susceptibility. We are developing novel methods to identify the contribution of rare variants to continuous phenotypes, using exome sequencing data from the 1000 genomes project. We simulate various continuous trait based on the genotypic information of each individual, to reflect different plausible scenarios. The phenotypes are simulated using several sets of exclusive assumptions: 1) the effects of the rare variants on the traits are deleterious 2) the effects of the rare variants are distributed following a gamma distribution, and finally 3) that the effects of the rare variants can be mostly deleterious but some will have a protective effect. Several methods are tested to find the association between gene and trait. We test the genetic association using a random effect model, where the genotypic information is used as a method to cluster groups of individuals. Another approach allows inclusion of gene-based genomic kinship information in a random effect model. Finally, different models where each variant receives a specific weight will be computed. A genetic score per individual will be calculated as the sum of rare alleles across the genetic loci of interest of the number of minor allele found at a particular SNP divided by its weights. The genetic score per individual is regressed against the value of their phenotype. To assess the p-value of that score, we use a permutation test. Comparison with standard approach will be perform.

2844/W

Assessing statistical significance in PLINK segmental sharing test. *J. Kwan, S. Cherny, P. Sham.* HKU, Hong Kong, Hong Kong, Hong Kong.

Genome-wide association studies (GWAS) have been used for identifying the genetic causes of many complex traits like Alzheimer's disease, schizophrenia, as well as various cancers. Given that nearly a million genotypes are generated for each individual in a typical case-control GWAS, it is natural to ask how the GWAS data could be fully utilized. Traditionally, GWAS are known to be suffered from the weak detection of rare risk variants. Resequencing of candidate loci or entire genome can certainly uncover such alleles, but this would require additional genotyping resources. Imputation of genotypes at loci untyped in the study but typed in the HapMap project does provide some extra information, but not necessary for the discovery of rare variants. Purcell et al. (2007) introduced a segmental sharing analysis between pairs of unrelated individuals using GWAS data. Their idea is based on family-based linkage analysis in which one would like to know if any chromosomal region is shared more among affected members than among affected and unaffected members within pedigrees, and the segmental test can be regarded as its extension when these familial relationships become distant and varied. If one can trace back to the infinite past, every case or control in a GWAS should actually belong to one of the many descendants of a remote common ancestry. In other words, all individuals within a GWAS should be considered related members within one large pedigree rather than totally unrelated. The PLINK approach of segmental sharing analysis is to compute a (linkage) test statistic S for each independent locus (i.e., in approximate linkage equilibrium with other tested loci) across the genome, asking whether there is a higher rate of case/case sharing than expected at each position, and to look for extended regions with statistic S significantly deviated from the expectation. However, whether the statistic S observed is significant remains a crucial question. One standard approach is to generate an empirical distribution of the statistic S under the null hypothesis via permutation. An alternative approach is to derive a SNP-wise p-value threshold characteristic to the sample of interest to balance the possibility of getting excessive false positive or false negative results. Here, we derived and proposed a threshold for the PLINK segmental sharing analysis by extending the theoretical framework of Lander and Kruglyak (1995) for family-based linkage analysis.

2845/W

Empirical-Bayesian Testing for Association in Family-based Association Study. *T. Wang, C.Y. Lin, K. Pradhan, K. Ye.* Department of Epidemiology and Population Health, Albert Einstein Col Medicine, Bronx, NY.

With availability of new genotyping technology, in particular SNP arrays as well as the coming next-generation sequencing, efforts of mapping genes of human diseases/traits have been focusing on genetic association study. Family-based and population-based studies are two commonly-used designs of genetic association study. In contrast to population-based study, family-based study is robust to bias due to population stratification. However, family-based study is often less powerful than population-based study because part of association information (information between families) is not used due to its susceptibility to the bias of population stratification. To improve power, a two-stage algorithm has been proposed to fully make use of association information in genome-wide association studies. This algorithm can keep good control of the global type-I error rate, but p-values of individual loci are still subject to population stratification in that loci with substantial population stratification tend to have smaller p-values than loci without population stratification. This makes difficult to explain the association results for individual loci, because individual p-values are no longer comparable. To overcome limitations of current approaches, we propose a new Empirical-Bayesian test for combining association information within and between families. In this test, the population stratification parameter is not canceled out by conditioning on parental genotypes, but it is estimated from data, and then a shrinkage parameter is defined by this population stratification parameter so that the amount of shrinkage to the statistic based on information within families is estimated from data. By simulations, we showed this test can substantially improve the power, while it keeps good control of type I error rate in presence of subtle local population stratification.

2846/W

Inference Techniques for Large Samples: An Unbiased Estimator Without Using Likelihood. *Y.E. Maruvka[1], N.M. Shnerb[1], D.A. Kessler[1], J. Wakeley[2], Y. Bar-Yam[3], S. Solomon[4], G. Yaari[5].* 1) Physics, Bar-Ilan University, Ramat Gan, Israel; 2) 2Department of Organismic & Evolutionary Biology, Harvard University, Cambridge, MA 02138, USA; 3) New England Complex Systems Institute, Cambridge, MA 02138, USA; 4) 4Racah Institute of Physics, Hebrew University of Jerusalem, Jerusalem 91904, Israel; 5) 5Department of Ecology and Evolutionary Biology, Yale University, New Haven, Connecticut 06520, USA.

Progress in genetic sequencing techniques challenge scientists analysing genetic polymorphism. The current methods based on maximal likelihood are geared towards small samples, and thus have conceptual and technical problems in dealing with large samples. Here we present a paradigmatically new approach to analysing large samples inspired by mean-field theory of statistical mechanics. The key behind our approach is to find some quantity (summary statistic - SS) that behaves almost deterministically, despite the randomness of the entire genetic tree. By comparing the measured quantity to the expected value for this quantity, one may infer the demographic parameters. We used various SS to infer a range of parameters. We used the number of haplotypes (NOH) (i.e. distinct genetic sequences) in the genetic sample as our SS. We derived an expression for the NOH in the genetic sample, which is an almost deterministic quantity. However, from this single number it's not possible to infer the two parameters of a growing population: the current population size and growth rate. We solved this by defining the NOH for subsets of the whole genetic sequence. Due to the independence of the mutations along the genome, using a shorter genetic sequence is parallel to reducing the mutation rate. Thus, we obtained a function F(l) of the NOH vs. the mutation rate (sequence length). By fitting this measured function to our formula for the NOH, we were able to infer the current population size and growth rate, with small error bars, and as opposed the current methods, without bias in the growth rate. For real cases, where repeated mutations occur, we developed a simulation-obtained function to replace the analytical one. We applied our method to Chinese HVR1 sequences of the mtDNA and retrieved the demographic and mutational parameters. Another almost deterministic quantity that we used is the number of lineages as a function of time (NLFT). Looking backward in time, genetic lineages coalesce stochastically, but still the average NLFT is an almost deterministic quantity (in large samples only). Therefore, by comparing the average NLFT of some real sequences to the NLFT expected by the formula that we derived, one can infer the demographic parameters. We applied our method both to simulated and real datasets, and demonstrated that in comparison to the common methods, our method is faster, has smaller error estimations, and most significantly, has an unbiased estimator.

2847/W

Capitalizing on Admixture in Genome-wide Association Studies: A Two-stage Testing Procedure and Application to Height in African-Americans. *G. Kang[1], G. Gao[1], S. Shete[2], D. Redden[1], B. Chang[3], T. Rebbeck[3], J. Barnholtz-Sloan[4], N. Pajewski[1], D. Allison[1].* 1) The University of Alabama at Birmingham, Birmingham, AL; 2) M. D. Anderson Cancer Center, University of Texas, Houston, TX; 3) School of Medicine, University of Pennsylvania, Philadelphia, PA; 4) Case Comprehensive Cancer Center, Case Western Reserve University, Cleveland, Ohio.

As genome-wide association studies expand beyond populations of European ancestry, the role of admixture will become increasingly important in the continued discovery and fine-mapping of variation influencing complex traits. Though admixture is commonly viewed as a confounding influence in association studies, approaches such as admixture mapping have demonstrated its ability to highlight disease susceptibility regions of the genome. In this study, we illustrate a powerful two-stage testing strategy designed to uncover trait-associated single nucleotide polymorphism in the presence of ancestral allele frequency differentiation. In the first stage, we conduct an association scan using predicted genotypic values based on regional admixture estimates. We then select a subset of promising markers for inclusion in a second-stage analysis, where association is tested between the observed genotype and the phenotype conditional on the predicted genotype. We prove that, under the null hypothesis, the test statistics used in each stage are orthogonal and asymptotically independent. Using simulated data designed to mimic African-American populations in the case of a quantitative trait, we show that our two-stage procedure maintains appropriate control of the family-wise type I error rate (FWER), and has higher power under realistic effect sizes than the one-stage testing procedure in which all markers are tested for association simultaneously with control of admixture. We apply the proposed procedure to a study of height in 201 African-Americans genotyped at 108 ancestry informative markers. The two-stage procedure identified two statistically significant markers rs1985080 (PTHB1/BBS9) and rs952718 (ABCA12). PTHB1/BBS9 is downregulated by parathyroid hormone in osteoblastic cells, and is thought to be involved in parathyroid hormone action in bones and may play a role in height. ABCA12 is a member of the superfamily of ATP-binding cassette (ABC) transporters and its potential involvement in height is not clear.

2848/W

Association Analyses of Mitochondrial Genes Polymorphisms with Femoral Neck Compression Strength Index. *N. Yu[1], T. Xu[1,4], Y. Cheng[1,4], Y. Pei[3], L. Zhang[3], J. Chen[1,2], H. Liu[1], J. Li[1], R. Kirk[1], Q. Tian[1], P. Yu[1], J. Hamilton[1], Y. Liu[1], H. Deng[1].* 1) University of Missouri - Kansas City, kansas city, MO; 2) Laboratory of Molecular and Statistical Genetics, College of Life Sciences, Hunan Normal University, Changsha, Hunan, P.R. China; 3) Institute of Molecular Genetics, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an, P.R. China; 4) Institute of Clinical Pharmacology, Central South University, Changsha, Hunan, P.R.China.

Introduction: Femoral neck compression strength index (CSI) is a novel parameter that integrates bone density, bone size, and body size [CSI = (BMD×FNW)/Weight]. It may have the potential to improve hip fracture risk assessment. However, the genetic factors underlying variations in CSI remain unknown. Recent studies found that mitochondrial dysfunction was associated with ageing-related phenotypes such as osteoporosis, suggesting potential role of mitochondrial genes on bone metabolism.

Materials and Methods: We performed association analyses for CSI in a sample of 2,258 unrelated US Caucasians (1728 females and 558 males) genotyped for Affymetrix genome-wide human SNP array 6.0. We focused our analyses on 20,131 mitochondrial SNPs due to the important role of mitochondria on bone metabolism. Association statistical analyses were carried out using PLINK software package (version 1.07).

Results: We identified four SNPs, (rs6782625, rs1842033, rs17020648 and rs17020592) that were significantly associated with CSI in males only (p = 2.35×10^{-6}, 2.40×10^{-6}, $2.40\times10^{-6}$and 2.45×10^{-6}, respectively). These four SNPs are located at the CMC1 (COX assembly mitochondrial protein homolog) gene. Importantly, the SNP rs17020592 was replicated in a Chinese sample including males sample (combined p = 2.21×10^{-6}). Interestingly, none of the SNPs in the CMC1 gene was associated with hip BMD in Caucasians or Chinese.

Conclusions: In this focused study of mitochondrial SNPs, we found that the CMC1 gene was consistently associated with CSI in US Caucasians and Chinese, independent of BMD. The CMC1 gene may affect bone metabolism by regulating the mitochondrial Cu-Zn superoxide dismutase activity. Our findings may provide new insights into pathogenesis of osteoporosis.

Key words: osteoporosis, CMC1, compression strength index, hip fracture, mitochondria.

2849/W

Delineation of plausible sets of causative SNPs in GWAS associated regions. *M. Reppell, C. Ma, M. Boehnke, L.J. Scott.* Department of Biostatistics, University of Michigan, Ann Arbor, MI.

Current GWAS show clear evidence of association in multiple regions throughout the genome. Each associated region will likely have a single SNP with the strongest evidence for association, but multiple additional strongly associated SNPs are often present. For a given region our goal is to identify the most plausible set of causal SNPs based on the evidence for association, with the assumption that the causal variant is among the analyzed variants. Biological follow-up experiments can be time consuming and costly and thus, in the absence of variants annotated as functional, for each signal we would like to quantitate the trade-off between number of SNPs selected for follow-up and probability of including the causal SNP within the selected set. We have taken a simulation based approach, initially using data from the *MMAB* region on chromosome 12, recently identified in large scale meta-analyses of high density lipoprotein cholesterol (HDL-C) concentrations. Using an additive model and data for ~2500 Finnish subjects from the FUSION study, we chose an effect size and randomly generated quantitative traits for each of the 459 SNPs in the *MMAB* region, assuming each SNP in turn was causal. We then tested all SNPs for association with each simulated quantitative trait. We compared the difference in association strength between the simulated causal SNP and the most strongly associated SNP; simulation runs where no SNP achieved genome wide significance (p-value < $5x10^{-8}$) were excluded from further analysis. Our results showed that when 0.25% of the trait variance was explained by the causative SNP, this SNP's p-value was among the best 10 p-values in 55% (43/78) of our simulations and within one $\log_{10}$ unit of the most strongly associated SNP in 69.2% (54/78) of simulations. For 2.5% of trait variance explained, these numbers increased to 74.4% (31513/42356) and 97.0% (41085/42356), respectively. This work shows that as effect size increases so does our ability to select smaller sets of SNPs that are likely to contain the causal variant for biological follow-up. We currently are analyzing other GWAS associated regions to determine more general rules for selection of a plausible set of causal SNPs based primarily on regional patterns of linkage disequilibrium and accounting for imputation quality.

2850/W

Describing and automating best-practices for pathway analysis in genome-wide association studies. *N. Tintle[1], A. Bekmetjev[1], R. Pruim[2].* 1) Math Dept, Hope College, Holland, MI; 2) Math and Stat Dept, Calvin College, Grand Rapids, MI.

Pathway (gene set) analysis is an increasingly popular method for the analysis of data from case-control genome-wide association studies (GWAS). In contrast to traditional approaches which seek to evaluate phenotype association for each SNP individually, pathway analysis is used to establish the significance of biologically related sets of SNPs (e.g. all the SNPs related to genes in a particular biological pathway). While many different implementations of pathway analysis have been proposed, little practical advice on best practices for pathway analysis is available. Additionally, there is a noticeable lack of software tools available which automate the multi-step pathway analysis process. In this presentation we present results of theoretical, simulated and real data analyses comparing a variety of approaches to pathway analysis yielding a set of best-practices. Additionally, we describe a series of freely available R scripts we have written that provide a semi-automated pathway analysis environment for case-control GWAS data.

2851/W

Detecting Rare Variants: Bayesian Group Risk Index. *M. Wilson, D. Thomas, D. Conti.* Department of Preventative Medicine, University of Southern California, Los Angeles, CA.

We are interested in investigating the involvement of multiple rare variants within a given group (i.e. gene or region) by conducting analyses of individual groups with two goals: (1) to first determine if the group of rare variants is associated with risk; and (2) conditional upon the group being associated we wish to identify specific genetic markers within the group that are driving the association. In particular, we seek a formal integrated analysis that achieves both of our goals. For rare variants with minor allele frequency (MAF) less than .05 there is very little power to statistically test the divergence of observed counts between cases and controls for each variant. Thus, genetic association studies are often limited to detecting association within a subset of only the common genetic markers. However, it is very likely that some associations exist in the rare variants themselves that many not necessarily be represented in the set of common markers. Our framework aims at developing a risk index based on multiple rare variants in a group or region and in turn detecting associated regions based on the indices. Our analytical strategy is novel in that we use a Bayesian approach to incorporate model uncertainty in the selection of which variants to include in the index as well as if the variant is involved as a protective or risk contributing factor. Addiitonally, the approach allows for inference at both the group and variant specific levels. Using a set of study-based simulations, we show that our methodology has added power over the more commonly used weighted sum indices to detect risk groups and to identify the specific markers driving the association.

2852/W

Do VEGF and eNOS gene haplotypes influence the development of retinopathy of prematurity in premature infants? *K. Yanamandra[1], S.A. Ursin[1], H. Chen[1], A. Pramanik[1], J.A. Bocchini Jr.[1], R. Dhanireddy[2].* 1) Dept Pediatrics, LSU Med Ctr, Shreveport, LA; 2) Dept. Pediatrics, UT Health Science Ctr, Memphis, TN.

Retinopathy of prematurity (ROP) is a multifactorial disease with multiple genes and environment playing a major role in the etiology of the disease. Early gestations and low birth weights are risk factors in the development of the disease. Ventilated very low birth weight infants have immature retina and are susceptible to ROP. The immature vasculature leads to retinal detachment in extreme cases. Vascular endothelial growth factor (VEGF), being a major mediator of angiogenesis, and Nitric oxide (NO), being an upstream and downstream regulator of VEGF mediated angiogenesis, have been suggested to play a role in the development of diabetic retinopathy. Our laboratory has been studying the etiology of ROP in ventilated very low birth weight infants since 2003. Presently we have been investigating the role of VEGF and endothelial nitric oxide synthase (eNOS) gene polymorphisms in the development of ROP. We genotyped 360 premature infants (56 infants with ROP and 304 infants without) for the presence of single nucleotide polymorphisms (SNPs) using PCR-RFLP methods: T-460C in the promoter region of VEGF gene, T-786C in the promoter region of eNOS gene and G894T in exon 7 of the coding region. Our results revealed a significant increase of variant haplotype (VEGF-460T/eNOS-786C/eNOS 894T) frequency over the other haplotype (-460C/-786T/894G) in premature infants with ROP compared to those infants without ROP (odds ratio 2.5, 95% CI 1.7-3.7, p=<0.000001). Our data reveal a strong association of variant haplotype of VEGF/eNOS genes suggesting that these genes are playing a significant role in the etiology of ROP in these premature infants. Data and statistics on the individual genotypes and their associations with the disease will be presented.

2853/W

Augmented variable selection of GWAS findings for genetic associations of hypertension by a composite test incorporating marker information content. *J. Zhou[1], J. Chu[1], S. Wang[1], W. Yang[1], CC. Gu[1,2].* 1) Division of Biostatistics, Washington University, St Louis, MO 63108; 2) Department of Genetics, Washington University, St Louis, MO 63108.

Backgrounds: GWAS studies employing case-control designs typically focus on individual SNPs that are most different in allelic or genotypic frequencies between the two groups. We hypothesize that cases chromosomes are originated from different subpopulations than those in controls and the differences in fine LD structures between the 2 groups contain disease-related location information that could be used to improve detection of disease variants. Methods: The method of marker ambiguity score (MAS) was developed to measure the information content that a genetic marker possesses in discriminating fine local LD structures (Gu et al., 2006). It quantifies disagreement between SNP memberships in LD blocks derived in different subpopulations. We present here a composite test (CT) combining conventional single-SNP test (SST) result and MAS score for each individual SNP for more informative selection of important SNPs. Permutation (1000 iterations) then followed to obtain empirical p-values. We applied the method to a GWAS study of hypertension in 9315 Caucasian samples and compare the results with those by SST. Results: We report results on Chr5 (168082 SNPs) and Chr2 (220741 SNPs), with most and least number of significant SNPs by SST at 10-5, respectively. We considered a lower significance thresholds to examine the method's ability to prevent premature exclusion of potentially important SNPs. Briefly, (1) at a significance level of 0.01, on Chr2, we had 2568 significant SNPs by CT and 2693 by SST with 1879 only detected by CT; on Chr5, there were 1862 and 1740 significant SNPs by CT and SST respectively, with 1762 only by CT; (2) on both chromosomes, significant SNPs detected by CT were more likely associated with know genes, with most pronounced change On Chr5, where number of SNPs associated with a known gene increased from 79 SNPs to 765 by CT; (3) among the "novel" findings by CT, 12 synonymous and 9 missense SNPs were found on Chr5 while 4 synonymous and 3 missense SNPs were on Chr2; (4) among the "novel" ones detected by CT only, many were associated with genes with promising roles in HT including ALK (transmembrane receptor protein kinase activity pathway), which was detected in an independent CA sample (n=592) of GWAS analysis of HT. Conclusion: Our analyses provide interesting evidence that incorporating marker information content in GWAS analysis will improve selection of potentially important variants in GWAS studies of hypertension.

2854/W

GREAT.stanford.edu: From low input and high throughput to concrete functional hypotheses output. *G. Bejerano[1], C. McLean[2], D. Bristor[1], M. Hiller[3], S. Clarke[4], B. Schaar[3], C. Lowe[5], A. Wenger[2].* 1) Dev Biol/Computer Sci, Stanford Univ, Stanford, CA; 2) Computer Science, Stanford University, Stanford, CA; 3) Developmental Biology, Stanford University, Stanford, CA; 4) Genetics, Stanford University, Stanford, CA; 5) Biomolecular Science and Engineering, UC Santa Cruz, Santa Cruz, CA.

Recent technological advances in DNA sequencing provide an unprecedented view of the regulatory genome in action. We can now sequence all binding events of transcription factors and complexes, examine the dynamics of chromatin marks, assay for open chromatin modifications between healthy and disease samples, human vs. chimp samples, and more. High throughput technologies also enable genome wide association studies for different human traits and diseases, and the inference of recent evolutionary events including population specific selective sweeps and human-specific changes in functional non-coding DNA content. Once a set of genomic regions of interest is defined, attempts to interpret this data must currently resort to the use of computational tools developed for microarray analysis. However the one-probe-per-gene methodology is a poor proxy for the rich regulatory landscape of the human genome. More often than not these analyses fall short, forcing researchers to manually scrutinize but a handful of their copious data. Our lab has recently published (McLean et al, Nat. Biotechnol., May 2010) the Genomic Regions Enrichment of Annotations Tool (GREAT), providing the first computational tool that properly models whole genome cis-regulatory data. Our published work focused on successfully applying GREAT to reinterpret ChIP-Seq datasets of multiple transcription-associated factors in different developmental contexts. Here we significantly extend the same methodology, demonstrating how to analyze high throughput datasets of particular interest to Human Geneticists: Genome Wide Association studies (GWAS), epigenetic markers and key transcription factors from human cancer cell lines, as well as evolutionary datasets such as Human Accelerated Regions (HARs), and genomic sweeps in different human populations inferred from HapMap data. For each of these, GREAT recovers many known functions that are missed by microarray-based tools, as well as generates novel testable hypotheses. Many of these datasets are rich in genomic regions putatively regulating components of particular pathways and cellular processes, some known, others novel. Some cancer related datasets show interesting similarities to target sets of different drugs. GREAT incorporates rich biological annotations from 20 ontologies and is available to the Human Genetics community as an intuitive web tool at http://great.stanford.edu/. Direct submission is also available from the UCSC Genome Browser.

2855/W

A novel method for estimating the overlap of eQTLs between two tissues, with application to skin and lymphoblastoid cells. *J. Ding, G.R. Abecasis.* Biostatistics, Univ Michigan, Ann Arbor, MI.

Gene transcript levels can serve as an intermediate phenotype that bridges genotypes and more complex organismal phenotypes, including common diseases. Genome-wide association studies of gene expression in several human tissues have identified thousands of genetic loci impacting the expression of specific transcripts. Each of these loci is called an expression quantitative trait locus (eQTL). Although it is expected that many eQTLs will be tissue specific, the exact proportion of eQTLs that are tissue specific or shared between tissues remains unknown.

A simple measure of the tissue specificity of eQTLs can be obtained by examining the overlap of eQTL lists from two different tissues. Unfortunately, this naïve approach will likely underestimate the true proportion of overlapping signals. We have developed a more accurate method. Our multi-step procedure first generates a list of potential eQTLs and then uses unbiased estimates for eQTL effect sizes to estimate the expected number of replicating eQTLs for a specific sample size. The proportion of overlapping eQTLs can then be interpreted in this context. When applied to compare cis-eQTLs detected in an analysis of 57 skin biopsies and in a panel of ~400 lymphoblastoid cell lines, our method shows that 70-80% of eQTL are shared between tissues, a much larger proportion than the naïve estimate of 30-40%.

Our results provide guidance to researchers contrasting eQTL results across tissues and a specific means to accurately estimate the proportion of overlapping eQTLs between tissues.

2856/W

A Hierarchical Model Approach to Map Population/Tissue Specific eQTLs. *X. Wen[1], M. Stephens[1,2].* 1) Dept Statistics, Univ Chicago, Chicago, IL; 2) Dept Human Genetics, Univ Chicago, Chicago, IL.

Recent studies have shown that the correlation between genetic variant and gene expression may behave in a population-specific or tissue-specific manner. To investigate the population/tissue specificity of eQTLs, we propose a hierarchical mixture model approach. Our model not only can efficiently map tissue/population specific eQTLs, but also has the ability to assess the probability of the tissue/population specificity of all eQTLs. More importantly, the proposed model allows researchers to investigate biological features that are potentially causal to eQTL specificity. We demonstrate our method by both simulation and real data application (e.g. Stranger's data for population specific eQTL mapping).

2857/W

Association of Gene-Gene Interactions with Venous Thromboembolism (VTE): A Pathway-Directed Candidate-Gene Case-Control Study. *J.A. Heit[1], S.M. Armasu[2], T.M. Petterson[2], D.N. Rider[2], J.M. Cunningham[3], M. de Andrade[2].* 1) Internal Med/Hematology Res, Mayo Clinic, Rochester, MN; 2) Biomedical Statistics and Informatics, Mayo Clinic, Rochester, MN; 3) Experimental Pathology, Mayo Clinic, Rochester,MN.

While bivariate interactions between Factor V Leiden, prothrombin G20201A and hereditary deficiency of antithrombin, protein C and protein S compound VTE risk, whether other gene-gene interactions are associated with VTE is largely unknown. Thus, our goal is to test gene-gene interactions for an association with VTE. Cases (n=1486) were Mayo Clinic European-American patients of non-Hispanic ancestry with objectively-diagnosed VTE in the absence of active cancer, venous catheter or antiphospholipid antibodies. Controls (n=1439) were Mayo Clinic outpatients without VTE who were frequency-matched on case age, gender, race, MI/stroke status and state of residence. We selected candidate genes relevant to the anticoagulant, procoagulant, fibrinolytic and innate immunity pathways. For these genes (n=754), we selected all non-synonymous coding SNPs with minor allele frequency ≥ 0.005; the remaining SNPs were selected using an LD tagging algorithm (Carlson et al, AJHG 2004); 500 successful ancestry-informative markers were also included. Leukocyte genomic DNA was genotyped using a custom Illumina Infinium iSelect chemistry and platform, including appropriate controls. For these analyses, all pairwise SNP-SNP interactions for 12,497 SNPs were tested as well as SNPs uncovered using a 2-stage procedure. In addition, we tested all Factor V Leiden -, prothrombin G20210A - and ABO non-O blood group - SNP interactions. The mean ± SD case and control ages were 54.7± 6.3 and 55.5±15.7 years, respectively, and 1504 were female. Analysis of ancestry-informative markers gave no evidence of population stratification. Among almost 72 million pairwise interactions tested, 7018 could be evaluated for the 2nd stage ($p < E-4$). Of these, 516, 50 and 5 SNP-SNP interactions reached p-values £ 1E-5, 1E-6, and 1E-7 respectively; 373 reached the Bonferroni statistical significance threshold (£ 7.1E-6). The gene-gene pairwise interaction with p-value £ 1E-8 was: Arachidonate 5-lipoxygenase-activating protein (ALOX5AP) - Angiotensin I converting enzyme 1 (ACE 1). Gene-gene pairwise interactions with the lowest p-value for Factor V Leiden and ABO non-group O were Lymphocyte membrane glycoprotein (CD44) (7.4E-5), and Phospholipid scramblase 1 (PLSCR1) (5.3E-5), respectively. Our pairwise gene-gene interaction analyses suggest potential associations between VTE and novel genes and gene pathways for future testing in replication studies.

2858/W

A direct approach to surrogate variable analysis for high dimensional data. *S. Lee[1], W. Sun[1,2], F.A. Wright[1,2], F. Zou[1,2].* 1) Biostatistics, University of North Carolina, Chapel Hill, NC; 2) Carolina Center for Genome Sciences, University of North Carolina, Chapel Hill, NC.

In modern high dimensional datasets, such as genome-wide gene-expression measured by microarray or RNA-seq, unobserved environmental, demographic, and technical factors can have a negative effect on estimating and testing the effects of experimental design variables (i.e. observed variables). These hidden factors are a source of systematic variation and can induce dependence among observations which can prevent proper estimation and valid type I error control. Recently, "surrogate variable analysis" (SVA) has been proposed to tackle this problem. To estimate hidden factors which are possibly correlated with observed variables, a current version of SVA either conducts PCA on a subset of measurements, or performs a weighted PCA based on presumed associations of measurements with hidden factors vs. associations with the variables of interest. This method, however, has limited usage if signals of observed variables are spread across all measurements. Furthermore, the steps of finding subsets or computing weights add variability to estimation, and prevent efficient adjustment and elucidation of the theoretical properties of this approach. We propose a simple and direct SVA approach which skips the additional step of SVA and uses all measurements simultaneously. We first show that the hidden factors can be successfully reconstructed from naïve estimates of observed variables and residuals from the linear model where only observed variables are included. Extensive simulation and real data analysis will then be used to demonstrate the advantages of the proposed method over the existing SVA approach.

2859/W

Large-scale time series LC-MS/MS analysis of monocyte proteome from patients with severe traumatic injury. *H. Gao[1,4], A. Kaushal[1,4], W. Qian[2,4], D. Camp[2,4], W. Xu[1,4], J. Seok[1,4], R. Davis[1,4], R. Tompkins[3,4], W. Xiao[1,3,4].* 1) Stanford Genome Technology Center and Biochemistry Department, Stanford Univ, Stanford, CA; 2) Pacific Northwest National Laboratory, Richland, WA; 3) Massachusetts General Hospital, Harvard Medical School, Shriners Hospital for Children, Boston, MA; 4) Inflammation and Host Response to Injury Program.

Severe traumatic injury has long been a significant health care burden due to complications. To date, little is known about the underlying molecular mechanism responsible for the development of complications. Here we explore the monocyte proteome (including 13,095 peptide features) from 100 patients with severe injury collected at 12 hours, 1, 4, 7, 14, 21, and 28 days after injury using dual channel liquid chromatography coupled with tandem mass spectrometry technique, in order to identify proteomic profiles of those developing complications and to predict clinical outcomes early. We integrate the time series linear mixed model with a binary probit regression approach to identify proteomic features with significant differential expression across time or between clinical outcomes. After demonstrating its superior performance using both simulation and spike-in experiments, we apply our approach to this large-scale data set and detect 569 proteins with differential expression over time at 1% false discovery level. We compare them with the results from genome-wide expression analysis of those samples and discover that while a fraction of genes have distinctive trends between gene expression and protein expression, more than 60% show the similar variation pattern across time. Pathway analysis reveals that those genes are mainly related to integrin signaling, ILK signaling, Fcy receptor-mediated phagocytosis, and virus entry via endocytic pathways.

2860/W

Allelic Expression Imbalance to Detect the Cis-acting Regulatory SNPs. *R. Xiao[1], M. Boehnke[2], L. Scott[2].* 1) Dept of Biostatistics and Epidemiology, Univ of Pennsylvania, Philadelphia, PA; 2) Dept of Biostatistics, Center of Statistical Genetics, University of Michigan, Ann Arbor, MI.

Testing for association between gene expression and SNPs identified by genomewide association studies (GWAS) can help understand the relationship between these SNPs and the trait of interest and identify the gene(s) most likely to influence the trait. Allelic expression imbalance (AEI) between the two alleles of a gene can be used to detect cis-acting regulatory SNPs (rSNP) in individuals heterozygous for a transcribed SNP (tSNP). The use of AEI is complementary to testing for SNP-gene expression association and has the advantage of testing both alleles within the same environment in each individual. Analysis of the AEI data depends on linkage disequilibrium (LD) between the rSNP and tSNP and whether we know linkage phase. In this study, we propose five tests to detect the association between the potential rSNP and the AEI when LD between the rSNP and tSNP is incomplete (D' < 1) and there is no phase information between them. We show that the relative power of the tests depends strongly on the magnitude of the LD between the rSNP and tSNP, and whether the two SNPs have similar allele frequencies, and less strongly on the AEI effect size of the rSNP and the number of tSNP heterozygotes. We further demonstrate that the impact of a second ungenotyped rSNP on the relative power of these tests depends on the LD structure of the three SNPs, but almost never invalidates the proposed tests nor substantially changes the rankings of the tests.

2861/W

Identification of SNP-gene expression associations with adaptive methods. *A. Ziegler[1], T. Zeller[2], A. Schillert[1], S. Blankenberg[2], S. Szymczak[1].* 1) Inst Med Biometry & Statistics, Univ Lübeck, Lübeck, Germany; 2) Department of Medicine II Cardiology, Johannes Gutenberg University, Mainz, Germany.

Single nucleotide polymorphism-gene expression association studies (SNP-eQTL analysis) are performed to clarify the molecular function of genetic variants identified in genome-wide association (GWA) studies. The aim of these studies is to detect differences in gene expressions given genotype. Standard statistical approaches such as the analysis of variance (ANOVA) or the Kruskal-Wallis test can lead to false positive associations since model assumptions are often violated in SNP-eQTL studies. In fact, the empirical type I error level can be up to 99% when the nominal error level is 5%. A promising alternative for controlling the type I error are adaptive statistical methods. Here, characteristics of the distributions are estimated in the first step. These information about skewness and tail weights are then used to select an appropriate test statistic in the second. We propose a new adaptive test for the analysis of SNP-eQTL associations and demonstrate its validity in Monte-Carlo simulation studies. Using CADomics, a GWA study of coronary artery disease with 2000 cases and 3000 controls in combination with global monocyte transcriptome data (n=1500), we identified several biologically promising SNP-eQTL associations.

2862/T

A simple scalable association hypothesis test combining gene-wide evidence from multiple polymorphisms. *D. Vaidya, R. Mathias, L. Becker, D. Becker.* Medicine, Johns Hopkins University, Baltimore, MD.

Introduction: In high-density single-nucleotide polymorphism (SNP) scans, tests of SNP-phenotype association hypotheses are reported, however there is biological interpretation only for genes that span multiple SNPs. The current practice of using Bonferroni-corrected significance thresholds is overly conservative and yet only polymorphism-specific, not gene-wide. We demonstrate and validate a method of combining gene-wide evidence using data for high-density lipoprotein cholesterol (HDLC).

Methods and Results: In a family based study (N=1782 from 482 families), we used 1000 phenotype-permuted datasets to determine the correlation of z-test statistics for 592 SNP-HDLC association tests comprising 14 genes previously reported to be associated with HDLC. We generated gene-wide p-values using the distribution of the sum of correlated z-statistics. Of the 14 genes, CETP was significant ($p=4.0*10^{-5}$ <0.05/14), while PLTP was significant at the borderline ($p=6.7*10^{-3}$ <0.1/14). Gene-wide p-values that were more significant than Bonferroni-corrected p-value for the most significant SNP in the 11 of 14 genes (p=0.023). Gene-wide p-values calculated from SNP correlations derived for 20 simulated normally distributed phenotypes reproduced those derived from the 1000 phenotype-permuted datasets (Spearman correlation = 1.00).

Conclusion: We have validated a simple scalable method to combine polymorphism-level evidence into gene-wide statistical evidence. High-throughput gene-wide hypothesis tests may be used in biologically interpretable genomewide association scans. Gene-wide association tests may be used to meaningfully replicate findings in populations with different linkage disequilibrium structure, when SNP-level replication is not expected.

2863/T

Integration of sequence and array data in a population and haplotype-based model of SNPs and CNVs. *L.JM. Coin.* Epidemiology and Biostatistics, Imperial College, London, Greater London, United Kingdom.

Read depth analysis has been proposed as a method for detecting copy number variants from second generation sequence data. However, the resolution of this approach strongly depends on coverage. In particular, the resolution of current single-sample methods may be limited on low-coverage population sequencing projects such as the 1000 genomes project. To overcome this, we extended our previously published algorithm, cnvHap, which learns the local CNV haplotype structure in the entire population, to low-coverage sequence data. We used dense array CGH data collected on Hapmap samples to demonstrate the improvement in resolution available from this approach. We have previously quantified the improvement in CNV genotyping accuracy from integrating multiple genotyping and CGH platforms in a single probabilistic model (cnvHap). In order to investigate whether existing array data can improve CNV genotyping accuracy from low coverage sequence data, we also integrated array and sequence data in our model, and ascertained improvements in CNV genotyping accuracy.

2864/T

A New Multi-Phenotype Approach on Genome-Wide Association Studies (GWAS) to Identify Novel Pleiotropic Genes that Affected Multiple Quantitative Traits: Bone Metabolism and Glucose Homeostasis, in Caucasian Populations. *Y. Hsu[1,2], X. Chen[3], D. Karasik[1], LA. Cupples[4], J. Meigs[5], DP. Kiel[1].* 1) Hebrew SeniorLife Inst Aging Res and Harvard Medical School, Boston, MA; 2) Integrative Molecular and Physiological Sciences Program, Harvard School of Public Health, Boston, MA; 3) Department of Environmental Health, Harvard School of Public Health, Boston, MA; 4) Department of Biostatistics, Boston University School of Public health, Boston, MA; 5) Massachusetts General Hospital, Boston, MA.

Pleiotropy occurs when gene codes for a product has a signaling function on various targets. Previous studies have proposed to simply look-up on the overlaps of association signals among univariate GWAS across multiple traits to identify pleiotropic genetic effects. However, the correlation among traits may also lead to overlap in false positive/negative signals. In addition, due to moderate genetic effects, it is inefficient to detect pleiotropy by univariate analytical framework. Adopting multivariate methods may overcome these disadvantages of univariate GWAS and improve statistical power. We propose here a new approach to test for pleiotropy on GWAS using a two-stage strategy: in the first stage, we performed a multi-phenotype GWAS by modeling traits simultaneously using our newly developed method, empirical-weighted linear-combined test statistics (eLC); and then, we tested the pleiotropy using a simplified structure equation modeling on selected SNPs from the first stage. eLC is to directly combine correlated test-statistics with a weighted sum of univariate statistics to maximize the heritability of the overall association tests. Using GWA16 simulated dataset, our eLC approach has outperformed the simple look-up on the overlaps among univariate GWAS and traditional multivariate methods (such as MANOVA, GEE and PCA). A recent rodent study showed that bone biomarker, osteocalcin, was involved in endocrine regulation of glucose homeostasis, which provided directly evidence linking the skeleton to the pathogenesis of diabetic complications. Significantly genetic correlations for bone mineral density (BMD) with fasting glucose and insulin in the Framingham Study indicates that shared genetic determinants may regulate both bone and energy metabolism. We applied our approach to data from the Genetic Effects for Osteoporosis (n=32,000) and Meta-analysis of Glucose and Insulin traits (n=36,610) consortia to identify pleiotropy on both BMD and glycemic phenotypes. Several pleiotropic effects were found, i.e. SNPs in or near NPSR1, TNFRSF11B and TGFBI genes. In conclusion, we proposed a powerful approach to identify pleiotropy from GWAS meta-analysis and our results reveal novel pleiotropic genes to further elucidate the link between the skeleton and energy metabolism.

2865/T

The winner's curse in meta-analysis of genome-wide association studies. *H. Nakaoka, H. Miura, I. Inoue.* Division of Molecular Life Science, School of Medicine, Tokai University, Isehara, Kanagawa, Japan.

Meta-analysis is a useful tool to enhance the statistical power to detect gene-disease associations by combining results from the original and subsequent replication studies. Recently, consortium-based meta-analyses of several genome-wide association (GWA) datasets have discovered new susceptibility genes of common diseases. Assessing consistency or heterogeneity of the associations across studies is an important aim of meta-analysis. There are two commonly used procedures for combining results from independent studies: fixed effects model (FEM) meta-analysis assumes that effect sizes are homogeneous across studies and all differences are due to chance and random effects model (REM) meta-analysis can incorporate between-study heterogeneity. We illustrated properties of test for and measures of between-study heterogeneity and effect of between-study heterogeneity on conclusions of meta-analyses via simulation study.

Our simulation shows that the use of FEM could increase the type I error rate even to the extent that the between-study heterogeneity could not be fully identified by Cochran's *Q* test and two measures (I^2 and H_M^2). We should note that the power of REM meta-analysis of GWA datasets (assuming that five to 20 GWA studies are combined and the total case-control sample size ranges from 5,000 to 20,000) to detect a small genetic effect (odds ratio [OR] = 1.4 under dominant model) decreases as between-study heterogeneity increases and then the mean of OR of the simulated meta-analyses passing the genome-wide significance threshold would be upwardly biased (referred to as "winner's curse" phenomenon). The estimates of mean OR were upwardly biased especially in simulation scenarios whose powers of detecting gene-disease associations were low. Additionally, we examined approaches to overcome and adjust the biases due to the winner's curse phenomenon.

The implication from the results of this simulation study is: i) the use of REM rather than FEM would be preferable to control false positive rate; ii) a study design of consortium-based meta-analysis of GWA datasets considering the presence of between-study heterogeneity would be constructed; and iii) further data accumulation after initial findings from GWA meta-analyses is needed to estimate unbiased effect sizes of the discovered gene-disease associations.

2866/T

The PhenX Toolkit: Facilitating the use of common measures in genomics research. *H. Pan[1], D. Jackman[1], V. Bakalov[1], K. Chang[1], A. Flynn[1], W. Huggins[1], J. Levy[1], D. Nettles[1], Y. Qin[1], H. Ray[1], P. Schad[1], N. Whitehead[1], M. Zmuda[1], H. Junkins[2], E. Ramos[2], L. Strader[1], C. Hamilton[1].* 1) Research Computing Division, RTI International, Research Triangle Park, NC; 2) National Human Genome Research Institute, Bethesda, MD.

To facilitate cross-study comparisons, PhenX (consensus measures for Phenotypes and eXposures) created a Toolkit of common measures for researchers to use when designing genomics-based studies. The PhenX Toolkit provides the user with a web-based interface for searching, browsing and selecting PhenX Measures and protocols. For each PhenX Measure, the Toolkit provides the user with a brief description of the measure, the rationale for selecting the measure, protocol(s) for collecting the measure, and supporting documentation. The Toolkit contains over 200 measures (15 research domains). Measures for the six remaining domains will be included in the Toolkit by the end of 2010. To expand its utility, the PhenX Toolkit has extended its browse and search capabilities and its collaborative efforts. The “Smart Query Tool” provides the option to use either a high-specificity search through measure and protocol names, synonyms and keywords or a full-text search. The “Data Collection Worksheet” will enable Toolkit users to integrate PhenX measures into their studies more easily while the Data Dictionary provides users with variable names, identifiers and many attributes in several formats. Mapping PhenX measures and variables to related research efforts will facilitate data interoperability, thus helping Toolkit users combine and/or harmonize data. To demonstrate its utility, PhenX has mapped PhenX measures, protocols and/or variables to studies in dbGaP, the Public Population Project in Genomics’s (P3G) Data Schema and Harmonization Platform for Epidemiological Research (DataSHaPER), and electronic Medical Records and Genomics (eMERGE), and the results of mapping will be presented. PhenX measures are accessible using the caBIG CDE browser. Logical Observation Identifiers Names and Codes (LOINC) is developing LOINC codes for PhenX measures. These codes will make enable identification of PhenX variables in electronic medical records (EMRs), clinical data repositories and other resources, thus facilitating cross-study analysis. The PhenX Toolkit provides the research community with freely available, well-established, low-burden, high quality measures, and the bioinformatics support to use them effectively. Broad acceptance and use of PhenX Measures can facilitate identification of genes associated with common diseases, as well as gene-gene and gene-environment interactions. Supported by: NHGRI, Award No. U01 HG004597.

2867/T

Elucidating the molecular etiology of asthma using genome-wide association study pathway analysis. *J.R. Huyghe[1], G. Van Camp[1], K. Van Steen[2].* 1) Department of Biomedical Sciences, University of Antwerp, Belgium; 2) Department of Electrical Engineering and Computer Science (Montefiore Institute), University of Liège, Belgium.

Genome-wide association studies (GWAS) have resulted in new insights in the biology of a substantial number of complex phenotypes. However, for most of the investigated phenotypes, the identified common SNPs cumulatively explain just a fraction of the total heritability. Yet, evidence exists that hundreds of common variants with very small effects account for a large portion of the missing heritability [1]. Their identification would require impossibly large sample sizes and by performing single SNP analysis these variants will be missed. In addition, gene-gene and gene-environment interactions, together with genetic and allelic heterogeneity may dilute power. Therefore, several investigators advocate GWAS Pathway Analysis (GWASPA) as a potentially more powerful complementary analysis to single SNP analysis [2,3]. In a GWASPA, pathway database information and, possibly, prior information regarding disease biology, is harnessed to statistically combine signals across sets of related genes. This study aims to carry out a GWASPA on the data from SHARP, the SHARe (SNP Health Association Resource) Asthma Resource Project, a large GWAS for asthma based on case-parent trios as well as independent cases [4]. Several different strategies for GWASPA have been proposed [2,3]. For any GWASPA, a number of important choices need to be made that affect the outcome of the analysis: i) How to define pathways? Which pathway database resource to use? Should prior information regarding pathways involved be used, or should an “agnostic” approach be used in which all pathways are considered? ii) How to map SNPs to genes? iii) How to score the pathways and to formally assess statistical significance? Moreover, several hurdles need to be taken, including adequately handling varying numbers of genes per pathway, varying numbers of SNPs per gene, varying SNP densities across the genome, linkage disequilibrium, multiple independent signals in one gene, and multiple testing. Special attention will be given to the multiple testing problem. In particular, strategies will be explored to reduce the computational burden of permutation tests. Methods to assess significance for case-parent trio data will be explored as well. We will present and motivate our analytic strategies. [1] Purcell, S.M. et al. (2009), Nature 460: 748-752; [2] Wang, K. et al. (2009), Am. J. Hum. Gen. 84: 399-405; [3] Holmans, P. et al. (2009), Am. J. Hum. Gen. 85: 13-24; [4] dbGaP accession number phs000166.v2.p1.

2868/T

Multiethnic genetic association studies improve power for locus discovery. *S.L. Pulit[1,2], B.F. Voight[2], P.I.W. de Bakker[1,2,3,4].* 1) Division of Genetics, Department of Medicine, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA; 2) Program in Medical and Population Genetics, Broad Institute of Harvard and MIT, Cambridge, MA; 3) Department of Medical Genetics, University Medical Center Utrecht, The Netherlands; 4) Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, The Netherlands.

To date, genome-wide association studies (GWAS) have focused predominantly on populations of European ancestry. The limitation of focusing on strictly European samples is exemplified by an association to type II diabetes recently discovered in East Asian samples. The association (in the *KCNQ1* locus) had little power to be detected in European samples (where the polymorphism had a minor allele frequency of ~5%), but had improved power to be discovered ($p < 5x10^{-8}$) in East Asian samples (at MAF ~40%) and replicated at a more liberal threshold in a European cohort. An approach for future GWAS would be to focus further efforts on cohorts of multiple ethnicities. This approach leverages the idea that population genetic drift may have elevated some variants to higher allele frequency in different populations, boosting statistical power to detect an association. Based on empirical allele frequency distributions from eleven worldwide populations represented in the new HapMap Phase 3 resource as well as pilot data from the 1000 Genomes Project, we simulate a range of genetic models to quantify the power of association studies in multiple ethnicities relative to studies that exclusively focus on samples of European descent. In each of these simulations, we perform a first phase of GWAS in strictly European samples (to mimic the first wave of GWAS already completed in samples of European ancestry) followed by a second GWAS phase in any one of the populations represented in HapMap 3 or the 1000 Genomes Project, as well as multiethnic population panels. We find that nontrivial power gains can be achieved by conducting future whole-genome studies in worldwide populations, where, in particular, African populations and multiethnic cohorts contribute the largest relative power gains for low-frequency alleles (<5%) of moderate effect that suffer from low power in samples of European descent. Our results emphasize the importance of broadening an imminent second wave of genetic studies to worldwide populations to ensure efficient discovery of genetic loci contributing to phenotypic trait variability, especially for those traits for which large numbers of samples of European ancestry have already been collected and tested.

2869/T

The use of abdominal adipose tissue gene expression and genotype data to identify novel genetic pathways involved in metabolic syndrome. *J.L. Min[1], R.X. Menezes[2], M. van Iterson[1], L. Parts[3], P.A.C. 't Hoen[1], G.J.B. van Ommen[1], J.T den Dunnen[1], J.M. Boer[1].* 1) Center for Human and Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands; 2) Epidemiology and Biostatistics, VU Medical Centre, Amsterdam, The Netherlands; 3) Wellcome Trust Sanger Institute, Hinxton, United Kingdom.

Genome-wide association studies of complex traits have identified reproducible associations with modest effect sizes, with often unknown functional basis. The integrated analysis of genotypic and expression data for association with complex traits could identify novel genetic pathways involved in complex traits. However, linkage disequilibrium between SNPs, the abundance of eQTLs and confounding variation in gene expression data may decrease power to detect eQTL associations. Power to find eQTL associations can be greatly improved by accounting for global variation using factor analysis, and by analysing groups of genes rather than individual genes. Menezes (2009) *et al*. analysed DNA copy number and gene expression data from the HapMap individuals and detected additional associations using genesets. On abdominal fat gene expression data from 52 individuals kindly provided by the MolPAGE consortium, we re-analysed the genotype (Illumina 317k arrays) and expression data (Affymetrix hgu133plus2 arrays) by modelling genotype as a function of factor-analysis corrected gene expression levels using genesets. These genesets comprise expression levels of genes that are i) within a 1Mb interval of the SNP or ii) that belong to the same pathway or gene ontology class. Preliminary results showed 308 (uncorrected) and 434 (factor-corrected) significant SNP-geneset associations (FDR P < 0.01) whereas the gene-by gene model detected 650 (uncorrected) and 831 factor-corrected significant SNPs (FDR P < 0.01). The geneset model found 20 (31 SNPs) and 47 (68 SNPs) independent SNP-geneset associations (r^2 < 0.5) in addition to the gene-by-gene model. Of these, 25 and 37 SNPs were associated with multiple expression probesets. In addition, we tested 205 genesets that were grouped by KEGG pathway. Of these, 13 genesets were significantly associated with 13 SNPs (4 loci) that were in *cis* with at least one of the probesets in the geneset. These loci were overlapping with the 1 Mb window geneset or gene-by-gene eQTL associations suggesting the influence of a single expression probe in *cis* and a lack of pathway effects in *trans*. Our results strongly support *cis*-regulatory variation in abdominal fat extending over multiple genes that can be detected using genesets. Additional eQTL associations were found in the factor-corrected expression data. Using a similar approach, we will explore whether a group of SNPs - belonging to the same pathway - influences gene expression variation.

2870/T

Novel statistical methods for combining genome-wide linkage and association analyses provide evidence of different genetic architecture for autism in the presence or absence of intellectual disability. *V. Vieland[1], J. Hallmayer[2], Y. Huang[1], A. Pagnamenta[3], D. Pinto[4], H. Khan[4], A.P. Monaco[3], A.D. Paterson[4], S.W. Scherer[4], J.S. Sutcliffe[5], P. Szatmari[6], Autism Genome Project.* 1) Battelle Ctr Math Med, Res Inst Nationwide Child Hosp, Columbus, OH; 2) Dept of Psychiatry, Division of Child and Adolescent Psychiatry and Child Development, Stanford Univ School of Medicine, Stanford, CA; 3) Wellcome Trust Centre for Human Genetics, Univ of Oxford, Oxford UK; 4) The Centre for Applied Genomics and Program in Genetics and Genomic Biology, The Hospital for Sick Children and Dept of Molecular Genetics, Univ of Toronto, ON; 5) Department of Molecular Physiology and Biophysics, Vanderbilt Kennedy Center, and Centers for Human Genetics Research and Molecular Neuroscience, Vanderbilt Univ, Nashville, TN; 6) Department of Psychiatry and Behavioural Neurosciences, McMaster Univ, Hamilton, ON.

Using novel adaptations of the PPL (posterior probability of linkage) framework to simulaneously analyze genome-wide linkage data on 1,069 multiplex autism families (with 10K genotypes) together with 1,129 autism trios (with 1M genotypes), we set out to identify genes involved in autism spectrum disorders (ASD), with a particular focus on whether different genes might be involved in causing ASD in the presence or absence of lower IQ (LIQ). We found multiple linked loci, and because the PPL can accumulate evidence against linkage as well as in favor of linkage, we were able to divide loci into those linked in LIQ families but unlinked in normal IQ (NIQ) families or vice versa. The LIQ group showed stronger and more numerous linkage peaks, suggesting more major gene effects. We then conducted genome-wide association analysis based on the posterior probability of trait-marker disequilibrium (PPLD), using PPLs from the multiplex families as prior linkage information going into PPLD analyses in the trios. PPLD-based evidence implicating specific genes was low even under the linkage peaks, however, overall the NIQ group yielded more loci with PPLD's > 10%, suggesting the possibility of greater contributions from multiple genes of smaller individual effect. In aggregate our results suggest that both the specific genes involved and also the genetic architecture of ASD may differ between the LIQ and NIQ families. Sequencing of particular candidate genes (CDH8, PTCHD1) implicated in these analyses and supported by independent evidence is ongoing.

2871/T

Integration of genome-wide SNP and expression profiling data to identify candidate genes for brain arteriovenous malformation hemorrhage. *S.M. Weinsheimer[1], L. Pawlikowska[1], H. Xu[2], A.S. Achrol[1,3], B. Stamova[2], P.Y. Kwok[4,5], S. Sidney[6], J. Zaroff[6], C.E. McCulloch[7], F.R. Sharp[2], W.L. Young[1,8], H. Kim[1,7].* 1) Center for Cerebrovascular Research, Department of Anesthesia and Perioperative Care, University of California, San Francisco, San Francisco, CA; 2) Department of Neurology and MIND Institute, University of California, Davis, Sacramento, CA; 3) Department of Neurosurgery, Stanford University, Palo Alto, CA; 4) Cardiovascular Research Institute, University of California, San Francisco, San Francisco, CA; 5) Institute for Human Genetics, University of California, San Francisco, San Francisco, CA; 6) Division of Research, Kaiser Permanente Northern California, Oakland, CA; 7) Department of Epidemiology and Biostatistics, University of California, San Francisco, San Francisco, CA; 8) Departments of Neurology and Neurological Surgery, University of California, San Francisco, San Francisco, CA.

Background: Brain arteriovenous malformations (BAVMs) occur throughout life, but are an especially important cause of intracranial hemorrhage (ICH) in young adults. To identify candidate genes associated with ICH presentation in BAVM patients, we have performed a genome-wide association study (GWAS) and a global profiling of blood gene expression in BAVM patients. Integrating data from these two genomic approaches may more accurately identify functional genes and expression quantitative trait loci (eQTLs) relevant for BAVM hemorrhage. **Methods:** We compared results from our GWAS study (with 127 ICH and 211 non-ICH patients) and blood gene expression profiling study (with 20 ICH and 20 non-ICH patients) to determine whether the genes and gene pathways identified in each study were similar. Pathway analysis was performed using WebGestalt software to provide a functional interpretation of the genes. We tested for significant overlap in genes identified between the two studies, and searched for eQTLs using linear regression in the subset of 12 patients (3 ICH, 9 non-ICH) with both genotype and expression data available. **Results:** Our GWAS identified 582 SNPs associated with ICH presentation (*P*<0.001). Of these, 372 (64%) SNPs mapped to +/- 100kb of 349 genes. Blood gene expression profiling identified 493 genes associated with ICH presentation (false discovery rate £0.1, fold-change≥1.2). Twelve genes (3.4% of 349) overlapped in the two approaches, although this number is not greater than expected by chance (*P*=0.23). However, all 12 genes are expressed in normal brain tissue, and 10 of these genes were also significantly decreased in the blood of hemorrhagic BAVM patients compared to unruptured patients. Although the majority of genes did not overlap between the two studies, they belong to similar functional pathways including Wnt signaling, MAPK signaling, T cell receptor signaling, and Natural killer cell mediated cytotoxicity pathways. eQTL analysis for the 12 overlapping genes resulted in 1 significant trans eQTL (Beta=0.74; *P*=2.25E-08) for the *LARGE* gene, and 2 marginally significant trans eQTLs (Beta=0.44; *P*=9.6E-08) for the *CLTA* gene. **Conclusions:** Comparison of results from GWAS and blood gene expression profiling studies revealed several candidate genes and pathways for BAVM hemorrhage. Integration of genotype and gene expression datasets may be a powerful tool to identify novel eQTLs that influence the expression of genes involved in BAVM hemorrhage.

2872/T

A rigorous statistical framework for integrating multiple heterogeneous existing data sets. *J. Bukszár[1], A.N. Khachane[1], K. Åberg[1], Y. Liu[2], J.L. McClay[1], P.F. Sullivan[2], E.J. van den Oord[1]*. 1) Virginia Commonwealth Univ, Richmond, VA; 2) University of North Carolina, Chapel Hill, NC.

During the past decade, databases related to the genetic basis of complex diseases have grown exponentially. Examples are meta-analyses of genome linkage scans, published candidate gene studies, disease-specific biochemical pathways, and genome-wide association studies. Integrating these existing data into novel data collection studies has huge potential to increase the likelihood of identifying the genes affecting susceptibility to common diseases. This is because these data provide a freely available independent source of information and the convergence of evidence from data generated by different technologies reduces the risk of false discoveries. Because of the volume and heterogeneity of existing databases, there have been several efforts towards developing systematic data integration methods. A limitation of many of the proposed methods is that they lack a solid statistical basis. This hampers the optimal use of existing data in empirical research. For example, most approaches produce a prioritization score that indicates the disease relevance of a gene. However, it is typically very hard to assess the quality of that score, there are no tools to check whether the assumptions made to calculate the scores are accurate, and methods specifying how to optimally integrate these scores into novel data collections are lacking. Here, we propose a rigorous statistical framework for integrating multiple heterogeneous existing data sets into novel studies. Our framework can handle existing data and novel data collections generated by any kind of technology/activity and providing information about genetic units at any level (e.g., SNPs, genes, regions). Furthermore, it can be used in the case of a small number of relatively large genetic effects as well as scenarios involving a very large number of small effects. The end product of the data integration process is the compound local true discovery rate (ceTDR) that can be interpreted as the probability that a genetic marker is associated with the disease after taking all existing and novel data into account. Because it is formalized in a mathematical way, we can prove that our framework has numerous desirable properties. We demonstrate our framework using a large meta-analysis of schizophrenia GWAS. Results show that several of the external data sets were informative and that after integrating these data, SNPs that were likely to have effects were much more reliably separated from those without effect.

2873/T

The extent and nature of epistasis among expression quantitative trait loci (eQTLs). *W. Bush, S. Turner.* Ctr Human Gen, Vanderbilt Univ, Nashville, TN.

Epistasis is often proposed as an important part of complex disease architecture, but the detection and confirmation of epistasis has proven difficult. An often cited rationale for examining epistasis is that combinations of genetic variants may influence gene expression in complex dynamic systems. Recent studies have explored and identified single nucleotide polymorphisms that alter the expression of genes in lymphoblastoid cell lines isolated from multiple human subpopulations -- so called eQTLs. Often, multiple SNPs near a gene of interest are identified, some of which likely represent independent genetic effects. In this work, we analyze the degree and extent to which there is non-linear interaction among eQTL SNPs for the genes they regulate. We identified genes with more than one eQTL SNP with ANOVA p-values < 5e-3 from the work of Veyrieras et al. eQTL SNPs were pruned so that no two SNPs have an r-squared > 0.1 to minimize haplotype effects. We then fit multi-locus association models for each gene, and statistically test the influence of SNP-SNP interactions using R^2 difference tests. From this analysis, we assess the role that regional (or cis) epistasis plays in altering expression of human genes, which could ultimately lead to testable multi-locus models for studies of human phenotypes.

2874/T

Non-independence and transmission bias of pathway-based allele combinations. *J. Haines, W. Bush.* Ctr Human Genetics, Vanderbilt Univ Medical Ctr, Nashville, TN.

Epistasis is thought to be a pervasive part of complex phenotypes due to the dynamics and complexity of biological systems, and a further understanding of epistasis in the context of biological pathways may provide insight into the etiology of complex disease. The International HapMap Project characterizes the structural dependencies between alleles of the human genome in multiple human populations. In this study, we use genotype data from the HapMap project to characterize potential dependencies between alleles of genes that have related biological functions. Using a collection of KEGG pathways, we performed chi-square tests to identify non-independence between the genotypes of SNP pairs from functionally related genes within parental Caucasian and Yoruba samples. We further refine this list of SNP pairs by testing for the over- or under-transmission of pseudo-haplotypes to offspring in the sample using a haplotype-based TDT test. From these analyses, we identify pathways enriched for non-independent genotypes in both Caucasian and Yoruba populations. We also identify a set of 863 SNP pairs (representing 453 gene pairs) with consistent non-independence of genotypes and consistent transmission distortion (by TDT) of the same pseudo-haplotype in both Caucasian and Yoruba populations. These results represent allele pairs with strong evidence of epistasis within the context of a biological function, potentially due to co-selection of alleles in response to the environment.

2875/T

Initialization Parameter Sweep in ATHENA: Optimizing Neural Networks for Detecting Gene-Gene Interactions in Simple and Complex Disease Models. *E. Holzinger, C. Buchanan, S. Turner, E. Torstenson, S. Dudek, M. Ritchie.* Ctr Human Gen Res, Vanderbilt Univ, Nashville, TN.

Recent advances in genotyping technology have led to the generation of an enormous quantity of genetic data. Traditional methods of statistical analysis have proved insufficient in extracting all of the information about the genetic components of common, complex human diseases. A contributing factor to the problem of analysis is that amongst the small main effects of each single gene on disease susceptibility, there are non-linear, gene-gene interactions that can be difficult for traditional, parametric analyses to detect. In addition, exhaustively searching all multi-locus combinations has proved computationally impractical. Novel strategies for analysis have been developed to address these issues. The Analysis Tool for Heritable and Environmental Network Associations (ATHENA) is an analytical tool that incorporates grammatical evolution neural networks (GENN) to detect interactions among genetic factors. Initial parameters define how the evolutionary process will be implemented. This research addresses how different parameter settings affect detection of disease models involving interactions. In the current study, we iterate over multiple parameter values to determine which combinations appear optimal for detecting interactions in simulated data for both a simple two-locus model, as well as a more complex three-locus model.

2876/T

Detection of SNP-SNP interactions in case-parent trios, with applications to a study of oral clefts. *H. Schwender[1,2], S.-C. Jin[3], T. Wu[3,4], R.A. Redett[5], G. Raymond[5], Y.H. Wu-Chou[6], H. Wang[5], X. Ye[7,8], S. Huang[9], V. Yeow[10], S.S. Chong[11], S.H. Jee[12], J.B. Hetmanski[3], K.Y. Liang[2], T.H. Beaty[3], K. Ickstadt[1], I. Ruczinski[2].* 1) Department of Statistics, TU Dortmund University, Dortmund, Germany; 2) Department of Biostatistics, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA; 3) Department of Epidemiology, Bloomberg School of Public Health, John Hopkins University, Baltimore, MD, USA; 4) Peking University Health Science Center, Beijing, China; 5) School of Medicine, Johns Hopkins University, Baltimore, Maryland, USA; 6) Chang Gung Memorial Hospital, Taoyuan, Taiwan; 7) Mt. Sinai Medical School, New York, New York, USA; 8) Wuhan University, Wuhan, China; 9) Peking Union Medical College, Beijing, China; 10) KK Women's and Children's Hospital, Singapore; 11) National University of Singapore, Singapore; 12) Epidemiology and Health Promotion, Yonsei University, Seoul, Korea.

Numerous methods have been proposed for the detection of interactions between genetic markers, particularly SNPs, in population-based association studies. However, only a few such procedures have been devised for case-parent trio data. One of these exceptions is an extension of the genotypic transmission disequilibrium test (gTDT) to two-way epistatic interactions. Another example is trio logic regression, an adaptation of logic regression originally developed for population-based designs, which makes it possible to detect higher-order SNP-SNP interactions.

In this presentation, we first show how a permutation-based gTDT procedure can be employed to identify the most significant two-way interactions in genotype data from a case-parent study concerned with cleft lip with or without cleft palate. We then investigate potential higher-order interactions using trio logic regression, and propose a resampling method that applies trio logic regression to several subsets of the data, and summarizes the results over the different applications. This approach stabilizes the results of trio logic regression, and quantifies the contribution of the detected interactions to overall disease risk. We demonstrate via a simulation study that this approach has the capability to identify disease-associated SNP-SNP interactions, even if their actual effect size is modest. This procedure is also applied to a set of SNPs from 10 candidate genes in the case-parent trio study concerned with oral clefts. In this application, an interaction of 3 SNPs from the FGF10 gene is identified that yields an odds ratio of about 6.1 and a p-value of $9.68 * 10^{12}$.

2877/T

A comparison of multifactor dimensionality reduction and L1-penalized regression to identify gene-gene interactions in genetic association studies. *S.J. Winham[1], A.A. Motsinger-Reif[1,2].* 1) Department of Statistics, North Carolina State University, Raleigh, NC; 2) Bioinformatics Research Center, North Carolina State University, Raleigh, NC.

In recent years, research in the area of genetic epidemiology has exploded, creating a wealth of high-dimensional data and new analytical challenges. In addition, much evidence suggests that common, complex diseases may be due to complex etiologies such as gene-gene and gene-environment interactions, and these types of interactions are difficult to identify in high-dimensional data using traditional statistical approaches. Therefore data-mining approaches designed to sift through large amounts of data are gaining popularity for association studies, performing variable selection and statistical modeling simultaneously. One of the most commonly used data-mining approaches to evaluate potential gene-gene interactions is Multifactor Dimensionality Reduction (MDR), designed specifically to address this problem. However, variable selection is a hot topic in the field of statistics, and a number of penalized regression techniques have emerged, like the Least Absolute Shrinkage and Selection Operator (Lasso). Lasso has exploded in popularity within the statistical community, and is now being applied for variable selection in human genetics, including extensions for interactions in GWA studies. In this study we compare the performance of both MDR and Lasso to detect gene-gene interactions. We consider two different Lasso approaches, the traditional (ungrouped) L1 penalty (TL1) and the group L1 penalty for categorical data (GL1). Through simulation, we compare the performance of MDR, GL1, and TL1 in regards to power to identify gene-gene interactions, the number of identified loci, and true and false positive rates under a wide range of genetic models and effect sizes. Unsurprisingly, we find that each method has both advantages and disadvantages, and our results are context dependent. GL1 is frequently superior to TL1, which tends to over-fit, identifying false positive as well as true positive loci. MDR has higher power to detect interactions for models which also exhibit independent main effects; for both types of Lasso the main effects tend to dominate and the interaction effects are missed. When the model is purely epistatic, GL1 outperforms MDR and TL1 for lower minor allele frequencies, whereas MDR outperforms GL1 and TL1 for higher frequencies. The results of these simulations provide general recommendations of when each of the three approaches might be best suited for detecting and characterizing interactions with different mechanisms.

2878/T

Dose Genetic Regulation of IgE Begin In-Utero - Evidence from T_H1/T_H2 Gene Polymorphisms and Cord Blood Total IgE. *H.-J. Tsai[1,3], X. Hong[1,2], X. Liu[1,2], L. Arguelles[2], R. Kumar[4], G. Wang[2], N. Kuptsova-Clarkson[2], C. Pearson[5], K. Ortiz[5], A. Bonzagni[5], S. Apollon[5], L. Fu[2], J. Pongracic[4], R. Schleimer[6], P. Holt[7], H. Bauchner[5], X. Wang[1,2].* 1) Dept Pediatrics, Northwestern Univ Sch Med, Chicago, IL; 2) The Mary Ann and J. Milburn Smith Child Health Research Program, Children's Memorial Hospital and Children's Memorial Research Center, Chicago, IL; 3) Division of Biostatistics and Bioinformatics, Institute of Population Health Sciences, National Health Research Institutes, Zhunan, Taiwan; 4) Division of Allergy and Immunology, Children's Memorial Hospital,Chicago, IL; 5) Department of Pediatrics, Boston University School of Medicine and Boston Medical Center, Boston, MA; 6) Division of Allergy-Immunology, Northwestern Feinberg School of Medicine, Chicago, IL; 7) Division of Cell Biology, Telethon Institute for Child Health Research, West Perth 6872, Western Australia.

Background: Elucidation of early life factors is critical to understand the development of allergic diseases which mostly manifest in the first few years of life. In contrast to numerous genetic studies of blood total IgE, few studies were conducted to identify genetic determinants of cord blood IgE (CBIgE). Objective: To test associations between genes involved in the T_H1/T_H2 pathway and CBIgE obtained at birth in a large U.S. inner-city birth cohort. Methods: CBIgE, was measured by Phadia ImmnunoCAP. It was analyzed as a continuous outcome (log_{10}-transformed CBIgE) and a binary outcome (detectable CBIgE $\geq$ 0.1kU/L). The association of each SNP with the two outcomes was tested using multiple tobit regression and logistic regression models, respectively, with adjustment of pertinent covariates, ancestry proportion, and multiple testing. Ethnic heterogeneity and gene-gene (GxG) interactions were also explored. Results: Three SNPs (rs1800925, rs2069743 and rs1295686) in IL13 gene were significantly associated with CBIgE levels (p £$6x10^{-4}$, pFDR<0.05). These SNPs jointly influenced CBIgE level in a dose-response manner (ptrend=$9.4x10^{-8}$). Significant associations were observed for the SNPs in IL13RA1 (rs5956080) and STAT6 (rs11172106) genes. Ethnicity-specific effects were observed for SNPs in IL5 (rs4143832) and GATA3 (rs570613) genes. Several statistically significant GxG interactions were detected (i.e. IL13 and IL4R, IL13 and STAT6, IL13RA1 and JAK2, STAT3 and JAK1) in relation to the two CBIgE outcomes. Conclusion: Our data demonstrated that multiple SNPs were individually and jointly associated with CBIgE, with evidence of gene-gene interactions and ethnic heterogeneity. Our finding suggests genetic regulation of IgE may begin in-utero.

2879/T

Accelerating Detection of Genetic Interactions in Genome-wide Association Studies using Graphics Processing Units. *S. Chikkagoudar[1], K. Wang[2], M. Li[1].* 1) Department of Biostatistics and Epidemiology, University of Pennsylvania School of Medicine, Philadelphia, PA; 2) Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, PA.

Most of the published Genome-wide association studies (GWAS) report results from single marker based analysis in which each SNP is analyzed individually. There is growing evidence that gene-gene interactions are important contributors to genetic variation in complex human diseases. However, detecting gene-gene interactions in whole-genome level remains a challenge due to the lack of powerful and computationally efficient methods. The most commonly used method for studying such interactions is to use a regression framework in which a pair of markers and their interaction terms are included as predictors. However, such analysis is computationally intensive when a large number of markers are available for testing, such as when analyzing typical GWAS datasets. It is infeasible to test for all pairwise interactions using traditional CPU computing even with small-scale computing clusters. However, gene-gene interaction analysis is parallelizable in nature. Parallel computing until recently meant using a computing cluster having multiple nodes or CPUs. The costs of building and using a computing cluster may run in hundreds of thousands of dollars. An emerging economical scientific computing paradigm is to use Graphics Processing Units (GPUs) that are present in graphic cards of most desktop computers for general purpose computing. A GPU is a processor that was traditionally used for accelerating graphical operations. A typical graphics card has several processors as well as its own dedicated memory. The computing power offered by the latest graphic cards is comparable to that of a computing cluster with hundreds of core processors, but the GPU programming approach for parallel computing is much cheaper. Our software package analyzes gene-gene interaction using the power of GPU computing to analyze several SNP pairs in parallel. The algorithm is scalable and can handle large datasets. First, the dataset is split into several fragments of a fixed size, where each fragment contains a different set of SNPs. Second, all the SNP pairs within each fragment are analyzed in parallel, and then the SNP pairs between fragments are analyzed in parallel. Finally, we report the results for those SNP pairs that meet a certain p-value threshold. We used a NVIDIA Geforce 9800GT card that costs $120 to test the algorithm. Our algorithm achieves a speed up of 5.8 times over its CPU implementation for a test dataset containing one fragment of 224 SNPs and 1077 individuals.

2880/T

Genome wide study of cleft palate case-parent trios considering maternal environmental exposures. *T.H. Beaty[1], I. Ruczinski[1], J.B. Hetmanski[1], K.Y. Liang[1], T. Wu[1,10], P. Patel[1], R.A. Redett[2], H. Schwender[1], S.C. Jin[1], J.C. Murray[3], M.L. Marazita[4], R.G. Munger[5], A.J. Wilcox[6], R.T. Lie[7], E.W. Jabs[2,8], Y.H. Wu-Chou[9], P.K. Chen[9], H. Wang[10], X. Ye[8,11], S. Huang[12], V. Yeow[13], S.S. Chong[14], S.H. Jee[15], B. Shi[16], K. Christensen[17], K.F. Doheny[18], E.W. Pugh[18], H. Ling[18], A.F. Scott[2].* 1) Dept Epidemiology, Johns Hopkins Univ,Sch Pub Hlth, Baltimore, MD; 2) Johns Hopkins Univ, Sch of Medicine, Baltimore MD; 3) University of Iowa, Dept of Pediatrics, Iowa City IA; 4) University of Pittsburgh, Sch Dental Medicine, Pittsburgh PA; 5) Utah State University, Logan UT; 6) NIEHS/NIH, Durham NC; 7) University of Bergen, Bergen Norway; 8) Mount Sinai Medical Sch, New York NY; 9) Chang Gung Memorial Hospital, Taoyuan Taiwan; 10) Peking University Health Sciences Center, Sch Pub Hlth, Beijing PRC; 11) Wuhan University, Wuhan PRC; 12) Peking Union Medical College, Beijing PRC; 13) KK Womens and Childrens Hospital, Singapore, Singapore; 14) National University of Singapore, Singapore, Singapore; 15) Yonsei University, Seoul, Korea; 16) Sichuan University, West China Sch of Stomatology, Chengdu, PRC; 17) University of Southern Denmark, Odense, Denmark; 18) Center of Inherited Disease Research, Johns Hopkins Univ. Baltimore MD.

Cleft palate (CP) is a complex and heterogeneous birth defect which shows strong evidence of genetic control, although multiple causal genes are likely and several environmental risk factors (generally maternal exposures) may interact with such genes. We conducted a genome wide association study (GWAS) using 550 case-parent trios drawn from an international consortium to screen for genetic risk factors while testing for gene-environment (GxE) interaction with 3 common maternal exposures: smoking, alcohol consumption during the peri-conceptual period and multivitamin supplementation during pregnancy. Conventional transmission disequilibrium tests (TDT) for all autosomal and X-linked SNPs showed none achieving genome wide significance in this sample of 550 trios when maternal exposures were ignored. We used PBAT to screen all autosomal markers for GxE interaction with (1) a 2 df test for gene effects (G) and GxE interaction considered together, and (2) a 1 df test for GxE interaction alone. Markers in 16 different genes/regions showed substantial increases in statistical significance, giving p-values$<10^{-6}$ in either the 2 df or the 1 df test. Conditional logistic regression models were then used to estimate the odds ratio for SNP effects with and without exposure [i.e. OR(CP|G no E) and OR(CP|G and E)], where the target allele was fixed as the apparent high-risk allele. Five genes showed compelling evidence of GxE interaction with maternal exposures: MLLT3 and SMC2 with maternal alcohol consumption; and OBSCN, TBK1 and ZNF236 with maternal smoking. ACOXL and BAALC also showed suggestive evidence of GxE interaction with maternal vitamin supplementation. While most of these genes appeared to represent “pure interaction” where both the high-risk allele and the maternal exposure were necessary to see any increase in OR(CP|G and E), OBSCN seems to represent “quantitative interaction” where OR(CP|G no E) was statistically significant and exposure enhanced the effect of these markers.

2881/T

Study of a Polynesian genetic isolate underscores gene environment interaction in biliary atresia. *A. Henrion Caude[1], M. Girard[1], A.L. Leutenegger[2], M. Besnard[3], A.S. Jannot[1], D. Vernerey[1], M. Sahbatou[2], P. Chune[4], C. Tetaria[4], A. Munnich[1], O. Bernard[5], S. Lyonnet[1], E. Jacquemin[5].* 1) Inserm U781, Department of Genetics, Hôpital Necker-Enfants Malades, Université René Descartes, Paris, FRANCE; 2) Fondation Jean DAUSSET - C.E.P.H. - Paris, FRANCE; 3) Centre Hospitalier territorial, Papeete, Tahiti, POLYNESIE FRANCAISE; 4) Banque d’ADN de Polynésie, Papeete, Tahiti, POLYNESIE FRANCAISE; 5) Department of Pediatric Hepatology, Hôpital Bicêtre, Kremlin-Bicêtre, FRANCE.

Despites a century-old characterization of biliary atresia (BA), nothing is commonly assumed about heritability of this disease, its onset, its susceptibility factors nor its physiopathology. In this study, we provide the clues to thoroughly revisit BA as a dynamic complex disease, related to genetic ancestry. Among the 152,866 children born in French Polynesia between 1979 and 2009, 40 had biliary atresia accounting for an incidence of 26.1/100,000 live births, which is the highest incidence worldwide. Epidemiologic data and follow-up study over 30 years period reveal statistically significant two-dimensional influence of environment. We further identified that temporality of BA significantly fitted a sinusoidal model. In all 40 patients, we also studied genealogic and clinical data. Genome-wide analyses of 250,000 SNPs in 24 patients and their parents were performed. Genetic isolate was evidenced by genealogy, inbreeding coefficient calculation (F=0.045) and principal component analysis. Contribution of environmental factors was integrated into an original statistical modeling. While homozygosity mapping revealed no significantly shared region, suggestive linkage to chromosome 2q13.1 was identified in patients with environmental constraint. Transmission disequilibrium test analysis further identified suggestive association with SNPs from 14 loci. Using bioinformatics, we could identify that the majority of genes that were encompassed in those loci were involved in the same highly significant morphogenetic pathway, specific of liver. Our findings provide a novel view of a complex genetic make-up in an isolate population underlying BA. As such, our design unveils an approach to study the contribution of both ethnic and environmental factors in a rare disease with a complex genetic background.

2882/T

Empirical tests for compositional epistasis. *T.J. VanderWeele.* Department of Epidemiology, Harvard School of Public Health, Boston, MA.

The term “epistasis” is sometimes used to describe some form of statistical interaction between genetic factors and is alternatively sometimes used to describe instances in which the effect of a particular genetic variant is masked by a variant at another locus. The latter use of the term is sometimes referred to as “compositional epistasis.” In general statistical tests for interaction are of limited use in detecting compositional epistasis. It is, however, shown that there are relations between empirical data patterns and compositional epistasis that have not been previously noted. These relations can sometimes be exploited to empirically test for compositional epistasis in the sense of the masking of the effect of a particular genetic variant by a variant at another locus. The methods are illustrated by an example concerning possible epistatic interaction between HLA-DRB1 and R620W PTPN22 polymorphisms on anti-CCP-Positive rheumatoid arthritis.

2883/T

Epistatic relations between MAP2K4 and PTPN22 in development of rheumatoid arthritis. *K. Shchetynsky[1], M. Ronninger[1], B. Ding[2], L. Klareskog[1], L. Alfredsson[2], L. Padyukov[1].* 1) Rheumatology Unit, Department of Medicine, Karolinska Institutet/Karolinska University Hospital Stockholm, Sweden; 2) Institute for Environmental Medicine, Karolinska Institutet, Stockholm, Sweden.

HLA-DRB1 shared epitope (SE) alleles and PTPN22 alleles have been established as the two major genetic risk factors for autoantibody-positive rheumatoid arthritis (RA). Recently, MAP2K4 locus has been identified as being in statistical interaction with HLA-DRB1 in RA patients with anti-citrullinated protein antibody (ACPA). MAP2K4 encodes a dual specificity protein kinase (MKK4) that has been earlier demonstrated to promote JNK activation in rheumatic diseases. The aim of this study was to address possible epistasis between MAP2K4 and PTPN22 genes. This study was based on genome-wide association study data for 1921 RA cases and 1079 healthy controls included in the Swedish Epidemiological Investigation of Rheumatoid Arthritis (EIRA) study population. The analysis was performed on 22 SNPs from MAP2K4 locus and rs2476601 from PTPN22. To assess genetic interaction, the attributable proportion due to interaction (AP) was calculated. AP was interpreted as the proportion of RA cases that can be attributed to the interaction *per se* in specified combinations of alleles. We performed separate analyses according to ACPA status among the cases as well as according to presence of SE alleles in the cohort. In our analysis, SNPs rs8078439, rs9895159, rs2013868, rs2169161, rs7212043 from MAP2K4 gene, when combined with rs2476601 from PTPN22 provided a more than additive increase in risk of ACPA-positive RA (AP 0.33 [95% CI 0.08-0.57], AP 0.49 [95% CI 0.19-0.79], AP 0.47 [95% CI 0.25-0.69], AP 0.46 [95% CI 0.24-0.68], AP 0.5 [95% CI 0.3-0.71], respectively). Additionally, rs9895159, rs2013868, rs2169161, rs7212043 demonstrated significant interaction with rs2476601 regarding ACPA-positive RA in absence of SE alleles (AP 0.61 [95% CI 0.16-1.05], AP 0.5 [95% CI 0.12-0.88], AP 0.46 [95% CI 0.05-0.87], AP 0.54 [95% CI 0.19-0.89], respectively). However, none of these interactions could be observed for ACPA-negative RA. This study implies that combinations of polymorphisms in MAP2K4 and PTPN22 may characterize a novel genetic risk factor for autoantibody-positive RA. These epistatic relations may also point at a signalling pathway important in RA development.

2884/T

Gene-by-Environment Interaction in Studies Based on Nuclear Families. *M. Shi, DM. Umbach, CR. Weinberg.* Biostatistics Br, NIEHS, Res Triangle Park, NC.

Several proposed methods for examining haplotype-exposure interactions use data from affected individuals and their parents. Unfortunately, that design cannot assess the effects of exposure, limiting the interpretability of identified interactions. Motivated by the Two Sister Study, an ongoing study of families affected by young-onset breast cancer, we consider a design that supplements the case-parents design with an unaffected sibling who is not genotyped but provides exposure data. If, in the population at large, inheritance is Mendelian and haplotypes do not influence propensity for exposure, then this tetrad structure permits the study of genetic effects, exposure effects, and gene-by-exposure interactions. In the presence of exposure-related population stratification, the traditional gene-by-environment analysis of data from the case-parent design may be biased, a phenomenon not well recognized by investigators. We document this bias and then show that it can be ameliorated by careful adjustment using the tetrad design. When some genotypes or some family members are missing, one can use available software to infer the list of possible diplotypes for family members and then use multiple imputation to estimate parameters and perform statistical tests. We present findings from simulation studies to compare the performance of the tetrad design with that of the case-parents and the discordant sib-pairs designs in testing gene-by-exposure interaction. We compare both type I error rates and power under a range of scenarios. We also identify circumstances under which one can improve efficiency by additionally studying one or more unaffected offspring.

2885/T

A likelihood approach to testing for and estimating additive gene-environment interaction in case-control studies. *S. Wang[1], L. Sheppard[1,2], B. Weir[1,3].* 1) Department of Biostatistics, University of Washington, Seattle, WA; 2) Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA; 3) Department of Genome Sciences, University of Washington, Seattle WA.

The recent development of genome-wide genotyping technology has provided new opportunities in finding major genetic factors that contribute to the risk of complex diseases and also spawned numerous studies of gene-environment interaction (G X E). However, to date there are relatively few examples of replicated and biologically plausible G X E findings in the literature. One possible reason for this phenomenon is the lack of adequate statistical power in many studies. Another possible reason is that many G X E studies have been limited to testing for multiplicative interaction due in part to the fact that multiplicative models are more accessible than additive models. Despite the lack of studies, additive interaction is considered to be more biologically relevant than multiplicative interaction. We propose a likelihood ratio test (LRT) for statistical additive interaction between genetic and environmental factors in case-control studies. This proposed LRT is useful not only for detecting additive gene-environment interaction, it also produces a maximum likelihood estimate (MLE) of interaction contrast ratio (ICR, a measure of additive interaction) and provides confidence intervals (CIs) for the ICR estimate based on profile likelihood. To illustrate this method we apply it to the data set obtained from a case-control study of venous thromboembolism in relation to factor V Leiden allele and oral contraceptive (OC) use (Botto and Khoury 2001 and reference therein). Our proposed LRT finds no evidence of statistical additive interaction between factor V Leiden allele and OC use at a significance level of 0.05. The MLE of ICR obtained from our proposed likelihood approach equals to that obtained from odds ratios. The profile likelihood-based 95 percent CI for this ICR estimate is skewed to the left and contains the value zero. We also conduct simulation studies to compare the performance of the profile likelihood-based CI resulting from our proposed LRT with that obtained from the best method currently available for estimating CIs of additive interaction estimates. The results of these simulation studies show that our proposed method and this available method perform equally well for estimating CIs of ICR estimates. However, as our proposed method is oriented for hypothesis testing, it can be used more conveniently for screening a large number of combinations of SNPs and environmental exposures to detect interactions in genome-wide association studies.

2886/T

Gene-gene associations in the apoptosis pathway and breast cancer risk. *R. Abo[1], A. Cox[2], MWR. Reed[2], M. Parry[2], S. Rigas[2], N. Camp[1].* 1) Univ Utah, Salt Lake City, UT; 2) University of Sheffield, Sheffield, UK.

Missing heritability in common diseases may be due to rare variants and gene-gene effects. Haplotype analyses provide more power for rare variants and joint analyses across genes can address multi-gene effects. The recent CASP8 variant (Cox et al. 2007) and haplotype (Shephard et al. 2009) associations with breast cancer provide CASP8 as a candidate for potential gene-gene effects with other apoptosis genes. Here we present a gene-gene data-mining analysis based on three genes that encode proteins that interact in the extrinsic apoptosis pathway: CASP8, TNFRSF10B and TNFRSF10A. Breast cancer cases and controls were from Sheffield, UK (n=1843). The method for analysis is an extension of our haplotype-mining software, and allows construction of multi-locus SNP sets at two genes and tests joint gene-gene effects and interactions between single variants or haplotype combinations. A Monte Carlo framework is used to provide statistical significance assessment of the joint and interaction statistics. All three gene pairs were analyzed using the gene-gene mining approach based on 100,000 simulations for assessment of empirical p-values. For all three gene pairs, several results reached the most significance possible empirical p-value with 100,000 simulations (P<1.0 x 10-5). All of the top results consisted of a two-locus haplotype at one gene interacting with a single variant in the other gene. For CASP8-TNFRSF10A, four interactions were suggested: three identified with interaction odds ratios statistics (IOR=5.86, 4.72, 3.45 with corresponding P<1.0 x 10-5 for the first two and P=8.0 x 10-5); the fourth was identified with the correlation statistic (P=4.0 x 10-5). The CASP8-TNFRSF10B analysis also produced four interactions with the IOR statistic (IOR=3.27-7.40, all P < 1.0 x 10-5). Five interactions were suggested in the TNFRSF10A-TNFRSF10B analysis, four with IOR and one with the correlation statistic. Two of these findings suggested a positive epistatic effect in disease risk (IOR=3.97, 4.53, both P < 1.0 x 10-5), while the other two findings suggested a negative effect (IOR=0.255, 0.217, both P < 1.0 x 10-5). The correlation statistic achieved P=7.0 x 10-5. These results illustrate the potential of our gene-gene approach to identify statistical interactions between biologically relevant genes that may be responsible for contributing to breast cancer risk. Assessment of construction-wide significance is now required to interpret these findings.

2887/T

Gene-environment interactions in genome-wide association studies: A comparative study of tests applied to empirical studies of type 2 diabetes. *M.C Cornelis[1], E.J Tchetgen[2,3], L. Liang[2,3], L. Qi[1], N. Chatterjee[4], F.B Hu[1,2], P. Kraft[2,3].* 1) Nutrition, Harvard School of Public Health, Boston, MA; 2) Epidemiology, Harvard School of Public Health, Boston, MA; 3) Biostatistics, Harvard School of Public Health, Boston, MA; 4) Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health and Human Services, Rockville, MD.

The most effective statistical approach to investigating gene-environment (GXE) interactions in the context of genome-wide association studies (GWAS) remains unresolved. Using two case-control GWAS of type 2 diabetes (T2D), we present a comparative study of five tests for interactions: (i) standard logistic-regression-based case-control; (ii) case-only; (iii) semi-parametric maximum-likelihood estimation (semiMLE) (iv) an empirical-Bayes (EB) shrinkage estimator; and (v) a two-stage test. We also compared two joint tests of genetic main effects and GXE interaction: (i)joint and ii) semiMLE joint tests. Elevated body mass index (BMI) was the exposure of interest and modeled as a binary trait based on our findings of inflated Type I error in tests of interaction when the environmental main effect is misspecified. Single nucleotide polymorphisms (SNPs) with the most significant GXE interactions using the standard test were also strongly correlated with the exposure among controls. A similar, but less dramatic, pattern was observed for the EB and two-stage tests, while the case-only and semiMLE interaction tests were not correlated with tests of G-E independence among controls. Both joint tests detected markers with known marginal effects, and yielded similar or markedly smaller P values for SNPs in TCF7L2 (marginal P=3.4E-7 and 1.5E-4 versus joint P<8.3E-7 and 2.3E-7 in men and women, respectively). However, a marginal effect model adjusted for BMI produced similar results to those of the joint tests. In summary, our findings suggest that statistical methods which exploit G-E independence are efficient and robust options to investigating GXE interactions in GWAS. In contrast, tests that incorporate the standard GXE interaction parameter are liable to detect markers that are associated with the exposure by chance and should be interpreted with caution. Finally, joint tests of genetic main effects and GXE interaction can be powerful approaches to enhancing the detection of disease loci.

2888/T

Gene-Gene Interaction between ROBO1 and RORA in Neovascular Age-related Macular Degeneration. *G. Jun[1,2,3], M. Nicolaou[1], M.A. Morrison[4], I.K. Kim[4], D.A. Schaumberg[5], M.G. Kotoula[6], E.E. Tsironi[6], F. Zacharaki[6], G.S. Hageman[7], L.A. Farrer[1,2,3,8,9], M.M. DeAngelis[4].* 1) Medicine, Boston University School of Medicine, Boston, MA; 2) Ophthalmology, Boston University School of Medicine, Boston, MA; 3) Biostatistics, Boston University School of Public Health, Boston, MA; 4) Ophthalmology, Massachusetts Eye & Ear Infirmary, Boston, MA; 5) Division of Preventive Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; 6) Medicine, University of Thessaly, Larissa, Greece; 7) Ophthalmology & Visual Sciences, John A. Moran Eye Center, Salt Lake, UT; 8) Neurology, Boston University School of Medicine, Boston, MA; 9) Epidemiology, Boston University School of Public Health, Boston, MA.

ROBO1 is a strong candidate gene for neovascular age-related macular degeneration (AMD) based upon its location under a linkage peak on chromosome 3p12, its expression pattern, and its purported function in a pathway that includes RORA, a gene previously associated with neovascular AMD risk. Previously, we observed that expression of ROBO1 and RORA was down-regulated among neovascular AMD cases, as compared to unaffected siblings. Thus, we hypothesized that AMD risk is increased by an interaction of these two genes. Using Taqman and Sequenom assays, we genotyped 31 single nucleotide polymorphisms (SNPs) in ROBO1 and tested association for neovascular AMD (unaffected = normal, AREDS category 2, or AREDS category 3; affected = neovascular AMD) in two cohorts including 226 discordant and 87 concordantly affected sibpairs from New England and 261 unrelated subjects from central Greece (139 affected and 121 unaffected). We selected 5 SNPs (rs730754, rs8034864, rs12900948, rs17237514, rs4335725) in RORA that previously showed association with neovascular AMD in three diverse cohorts and 16 SNPs in ROBO1 that were moderately significant in the family cohort ($P < 0.05$) to test gene-gene interaction. Tests of all models including one of the 16 ROBO1 SNPs, one of the 5 RORA SNPs and an interaction term in the two cohorts analyzed separately using the program UNPHASED revealed significant interaction between 9 SNPs in ROBO1 and rs8034864 in RORA after adjustment for multiple testing (meta $P < 6 \times 10^{-4}$). No other SNPs in RORA showed significant interaction with ROBO1 SNPs at the permuted significance threshold of $P < 0.001$. These findings suggest that the effects of the ROBO1 and RORA genes on neovascular AMD risk are not independent and that further functional studies of these genes are warranted.

2889/T

A machine learning approach for genome-wide interaction analysis. *U. Masao, T. Gen.* Yamagata University, Yamagata, Japan.

Nowadays genome-wide association studies (GWAS) using single nucleotide polymorphisms (SNPs) to identify human diseases-susceptible genes have been popularly employed. However, geneticists come to recognize that gene-gene (epistatic) interactions should be considered rather than single-locus weak effects (Cordell 2009, Nat Rev Genet). It may be inevitable that, without allowing for the gene-gene interaction, we will miss a large proportion of genetic component contributing to heritability for human complex common diseases. Direct application of commonly used methods for the recent GWAS, such as the Bonferroni's correction, to gene-gene interactions analysis incurs several problems. Among them, computational burden and difficulty in setting overall p-value for testing are extremely serious. The former restricts the algorithm to be much simple, whereas the latter is a theoretical problem that no efficient and universal approach is proposed so far. The main reason is that the number of SNPs is too large (typically 300k-1,000k SNPs should be handled) and, hence, that of the interactions between SNPs turns out to be ultra huge, indicating that Bonferroni's correction can be undesirably conservative. Instead of conventional hypothesis testing, we developed a new software implementing a logistic regression-based machine learning approach, which allows to complete an exhaustive search for SNP by SNP interaction in non-filtered large GWAS data within several days under common computing environments and to avoid multicollinearity due to these highly-correlated SNPs. Our fast program was applied to find some novel genetic components in the WTCCC (Wellcome Trust Case-Control Consortium) data.

2890/T

GxG interactions potentially account for the blood pressure linkage peak on chromosome 2 in the Old Order Amish. *M. Montasser, Y. Chang, Y. Wang, J. O'Connell, A. Shuldiner, B. Mitchell, N. Steinle.* Endocrinology Diabetes & Nutri, Univ Maryland Sch Med, Baltimore, MD.

Blood pressure is a complex trait regulated by environmental and genetic components, as well as the interaction between them.While the environmental components are very well established, the genetic components remain largely unknown. We previously identified significant evidence for linkage of a QTL on chromosome 2q31-34 to diastolic blood pressure (DBP) in 660 Old Order Amish (OOA) with a LOD score of 3.36; this locus was repeatedly identified by several other groups. To fine map this QTL, we genotyped 3,052 SNPs within the 40Mb linkage region in 762 OOA individuals and performed variance component association analysis. All analyses were adjusted for the effect of age, age2, sex, and excluded those who are taking hypertension medication. Single SNP association analysis did not identify any highly significant results after correcting for multiple testing. We then examined interactions between each pair of these SNPs using the generalized multifactor dimensionality reduction (GMDR) method. Several rounds of searching identified 10 pairs involving 19 different SNPs showing evidence for interaction. The best models identified by GMDR were then tested using regression models implemented under the variance component method to allow incorporation of the linkage and family components. In conditional linkage analysis, each of these pairs individually accounted for up to 6% of the variability of DBP, and reduced the LOD score by up to 34%. A combination of 6 of the 10 pairs accounted for 18% of the total variation in DBP and reduced the LOD score by 85%. These SNPs have relatively high minor allele frequencies (>0.15) and are located in intergenic regions, introns of known genes (in PARD3B, ABI2 , CREB1, FAM126B, LOC729245, MARCH4, MPP4, and SPAG16), and in coding exons (SLC4A3 and BARD1). Cross examination of eQTLs or loci that are associated with expression levels showed that 15 of the 19 SNPs are either eQTLS themselves or are in high LD with other SNPs that are known eQTLs that influenced expression of at least one gene. These results, if replicated in other populations, suggest that accounting for gene by gene interactions is an under-explored approach in searching for genes involved in complex traits and may explain at least some of the missing "heritable" component of complex traits, such as blood pressure and hypertension.

2891/T

Evidence for interaction is scale dependent: Searching for interaction in genome-wide association data on multiplicative and additive scales. *L. Petukhova[1, 2].* 1) Department of Dermatology, Columbia University, New York, NY; 2) Department of Epidemiology, Columbia University, New York, NY.

The term 'interaction' has a number of connotations within the fields of genetics and epidemiology. In genetics, interaction is most commonly thought of as epistasis, which is traditionally defined as the masking of effects of one locus, by a second locus. In epidemiology, this concept is expressed as 'effect measure modification'. Furthermore, epidemiologists draw a distinction between statistical interaction, a departure from the underlying form of a statistical model, and synergy, which is mechanistic and thus biologically meaningful. Finally, in genetics literature it is common to see tests of interaction performed on a multiplicative scale and rarely is evidence assessed on an additive scale. However, epidemiologists have demonstrated that the choice of statistical model will influence whether or not there is evidence for statistical interaction and may or may not correspond to the underlying causal model. We have recently completed a genome-wide association study in alopecia areata in which we identified at least eight regions in the genome with statistically significant association (p<5x10-7). Two of these regions contain three genes which are each ligands of NKG2D, an activating receptor of natural killer (NK) cells. In order to evaluate statistical interaction, we first identified a set of SNPs that are likely to harbor mechanistic interaction because they share a common biological pathway. Specifically, we identified at set of 51 SNPs with at least nominal association (p<1x10-3) to AA, from within 28 genes in the NK cell mediated cytotoxicity pathway. We then use conditional logistic regression to search for evidence of interaction on both multiplicative and additive scales among the SNPs in this pathway and demonstrate that evidence for statistical interaction is driven by the choice of statistical model.

2892/T

Pathway-based candidate genes analyses revealed evidence of sex-gene interactions, epistasis, and novel pathways for essential hypertension. *P.B. Shih[1], A. Kamburov[1], J.H. Moore[2], B. Rana[1], M. Mahata[1], S. Mahata[1], T. Ideker[1], D.T. O'Connor[1].* 1) School of Medicine, University of California, San Diego, San Diego, CA; 2) Department of Genetics, Dartmouth Medical School, Lebanon, NH.

Essential hypertension is a highly heritable polygenetic disease of unknown etiology. While the GWAS have uncovered credible genetic loci, together these loci only explained less than 10% of phenotypic variance in common diseases such as hypertension. To address the issue of "missing heritability", we examined the hypertension genetic complexity introduced by effect modifiers such as gene-by-gene (epistasis) and gene-by-sex interactions. We also utilized hypertension differentially expressed microarray data to examine the "interconnectedness" pathway candidates have with these disease reporter genes. Using an extreme-phenotype case-control study design, we assessed gene-by-sex interactions and epistatic relationships of 49 hypertension candidate genes chosen from 4 well-studied hypertension pathways both by non-parametric method (MDR) and parametric models. The MDR analysis revealed a two-locus model of IL6 with CHGA to successfully predict hypertension cases, and significant gene-by-sex interactions for COMT, IL6, and CHGA (permutated p-value <0.001). These associations were confirmed using parametric tests both as categorical and quantitative blood pressure trait. Furthermore, regression models demonstrated the BP residues explained in this study population were increased by 4% of the total variance when accounting for the interaction effects. In cella experiments confirmed the functional nature of epistasis (IL6 and CHGA) , while 2 Y-chromosomal markers verified sex-specific influence on genotype-phenotype correlations. Moreover, hypergeometric analysis of the PPI network and over-representation analysis using the pathway databases demonstrated candidate genes' close connection with hypertension reporter genes, uncovered additional protein partners our pathway candidate genes interact biochemically with, and revealed novel pathways these genes participate in. Together, our data confirmed the important roles epistasis and gene-by-sex interactions play in heritable risk of essential hypertension. The significant interconnectedness between pathway candidate genes and hypertension differentially expressed reporter genes suggested the disease-modulating effects the pathway candidates have beyond their known biochemical pathways. Consideration of epistasis, gene-by-sex interactions, and candidate genes' roles in the global genetic architecture of hypertension susceptibility may be the key to finding the "missing heritability".

2893/T

Robust Detection of Genetic Associations incorporating gene-environment interaction and independence. *E. Tchetgen.* Department of Epidemiology Harvard Sch Pub Healt, 677 Huntington ave, Boston, MA.

We consider the detection and evaluation of genetic effects in the presence of gene-environment interaction and independence. Whereas ordinary logistic regression cannot exploit the fact that the genetic variant and the environmental factor are unrelated in the underlying population of interest, we present a novel approach which makes explicit use of this assumption to improve detection and evaluation accuracy of genetic associations. For a binary genetic variant, our method which uses both cases and controls, entails =85tting a constrained retrospective logistic regression, in which the genetic variant plays the role of the response variable, and both the disease indicator and the environmental exposure are the independent variables. The regression model constrains the parameters corresponding to the environmental exposure odds ratio association with the genetic variant among the controls to be null, thus explicitly encoding the gene-environment independence assumption. The main appeal of the proposed retrospective regression approach is that (i) it is easy to implement with standard software, (ii) it readily accounts for multiple environmental exposures of a polytomous or of a continuous nature, and also allows for any additional observed covariates, (iii) it is more efficient than ordinary logistic regression (iv) unlike ordinary logistic regression, and the pro=85file likelihood approach of Chatterjee and Carroll(2005), our approach does not require a model for the odds ratio association of the environmental exposure with the disease outcome; as a result it is agnostic and thus completely robust to possible model misspeci=85cation which can happen with an exposure of a polytomous or of a continuous nature.

2894/T

Exhaustive search of gene-gene and gene-environment interactions for obesity related traits across three ethnic cohorts of women. *D.R. Velez Edwards[1], A. Naj[2], W. Zheng[1], W. Wen[1], X.O. Shu[1], Y. Gao[3], W. Lu[4], Y.B. Xiang[3], K. Monda[5], K. North[5], M. Neuhouser[8], M. Vitolins[7], J. Manson[6], M. O'Sullivan[5], E. Rampersaud[2], T.L. Edwards[1].* 1) Vanderbilt Epidemiology Center Institute of Medicine and Public Health, Vanderbilt University, Nashville, TN; 2) Hussman Institute for Human Genomics, University of Miami, Miller School of Medicine, Miami, FL, USA; 3) Department of Epidemiology, Shanghai Cancer Institute, Shanghai, 200032, China; 4) Shanghai Center for Disease Prevention and Control, Shanghai Institute of Preventive Medicine, Shanghai, 200336, China; 5) Department of Obstetrics and Gynecology, University of Miami, Miami, FL, USA; 6) Harvard Medical School, Boston, MA; 7) Department of Epidemiology & Prevention, Division of Public Health Sciences, Wake Forest University Health Sciences, Winston-Salem, NC; 8) Fred Hutchinson Cancer Research Center, Seattle, WA.

Genome-wide association studies of BMI and obesity have associated several SNPs, some of which interact with physical activity. However, no large evaluation of gene-gene (GxG) or gene-environment (GxE) interactions has been performed. We conducted a search of GxG and GxE interactions in post-menopausal African-American (AA) and Hispanic (HP) women from the Women's Health Initiative SNP Health Associated Resource GWAS study. GWAS data from Chinese women in the Shanghai Women's Health Study, Breast Cancer Study, Endometrial Cancer Study, and Breast Cancer Survivor Study (N=6932) were used to investigate the top 30 GxG interaction models from HP and AA analyses with interaction term p<9x10-10. Single SNP linear regression on BMI adjusted for MDS-derived axes of ancestry was run in race-stratified data with 871512 SNPs available from AA (N= 8264) and 837668 SNPs from HP (N=3510). 70880 SNPs (MAF>0.1) associated with BMI (p<0.1) in AAs (mean Beta: 1x10-5) and 64494 SNPs in HPs (mean Beta: 3x10-5) and were used for GxG analyses without LD pruning. GxG analysis was run using linear regression for 2.5 billion pairwise SNP combinations in AA and 2 billion in HP, adjusted for ancestry. The most consistent evidence from GxG interaction terms across all ethnic groups was for SNPs in *SLCO1B1* (rs7966613) and *ADCYAP1* (rs1610274)(AA p= 7x10-9; Chinese p=2x10-3). This result was not observed in HP; however, GxG interactions were observed at other SNPs within these genes (p=2x10-3). *SLCO1B1* and *ADCYAP1* are involved in energy homeostasis via thyroid hormone activity from human genetic studies, mouse models, and cell culture. Tests of GxE interaction at all SNPs for physical activity (met-hrs/wk), energy intake (kcal/day), and smoking were run with linear regression in AA and HP adjusted for ancestry, followed by meta-analysis of all GxE interaction terms. The strongest evidence for concordant GxE interactions in AA and HP was for smoking and *CSMD1* (rs10104470; Q statistic p= 0.8, beta=2x10-4, p=3x10-7). *CSMD1* has been associated with metabolic syndrome, methamphetamine dependence, and smoking cessation. The strongest evidence for GxE association within a cohort was in AA for dietary energy and *PFTK1* (rs17479541; SNP beta=-0.02, kcal beta=7x10-6, SNPxkcal beta=1x10-5, p=4x10-7), a gene involved in thyroid hormone pathways and adipogenesis. No results survive Bonferroni correction; however, validation of these results with independent AA and HP cohorts are ongoing.

2895/T

Incorporating Covariates in Association Mapping Using General Pedigree Data. *Y.-F. Chiu, C.-Y. Lee.* Biostatistics & Bioinformatics, National Hlth Res Inst, Zhunan, Miaoli, Taiwan.

As complex diseases are often involved a number of genetic and environmental factors, incorporating these factors into linkage mapping can improve the power to detect disease loci or the efficiency of estimating disease loci. Moreover, the incorporation of covariates provides information that can be used to characterize disease loci, which is helpful for understanding disease etiologies and mechanisms and for identifying population subgroups that may have particularly high disease risks. Previously, we developed two approaches to incorporate covariates into linkage disequilibrium mapping in the case-parent design. The approaches, including parametric and nonparametric modeling, are robust in that no assumption about the underlying genetic model is required, other than the assumption that there is no more than one disease gene in the chromosomal region. In addition to the estimate of a disease locus, the magnitudes of the associations between the genetic effect at the disease locus and covariates can also be assessed. In practice, data are often available for extended pedigrees with multiple nuclear families or relative pairs, it would be desirable to have the association mapping approaches that can use all potentially informative data. In the present study, we will extend these approaches to general pedigrees to make full use of the data available, so as to improve the efficiency of estimate for a disease locus. By making full use of pedigree data, one can also estimate the relative risks among different relatives, which is informative for uncovering the underlying genetic model of a disease. Our simulation studies evaluated the efficiency in estimating the disease locus when using general pedigrees compared to trios data. The proposed approach was applied to a young-onset hypertension data sample and the relative efficiency of estimating the locus of young-onset hypertension using either trios only or the whole family data was assessed.

2896/T

Marker-set Analysis for Genetic Main Effects and Gene-environment Interactions via Gene-trait Similarity Regression. *JY. Tzeng[1,2], D. Zhang[1].* 1) Department of Statistics, North Carolina State Univ, Raleigh, NC; 2) Bioinformatics Research Center, North Carolina State Univ, Raleigh, NC.

Modern association studies of complex traits, such as GWAS or sequencing studies, demand statistical tools that are cable to detect small-effect variants, model complex interaction effects, and have convincing speed performance. In this work, we introduce a similarity-based regression method to perform marker-set analysis. The method uses genetic similarity to aggregate information from multiple polymorphic sites (e.g., SNPs or a mixture of different polymorphisms), and regresses trait similarities for pairs of unrelated individuals on their genetic similarities to access the gene-trait association. The association is detected using a score test whose limiting distribution is derived. The proposed method can account for covariates, has the capacity to model both main and interaction effects, and is computationally efficient. We also show that the gene-trait similarity regression does not require phase sequence and that it explicitly models the non-additive effects among markers. These features makes it an ideal tool for evaluating association between phenotype and marker sets defined by haplotypes, genes or pathway in whole-genome analysis.

2897/T

A powerful two-stage method for genome-wide analysis of gene-gene interactions in case-control data. *Z. Bochdanovits[1], J. Peánka[2], P. Heutink[1], A. van der Vaart[2].* 1) Dept Clinical Genetics, VU Univ Medical Ctr, Amsterdam, Netherlands; 2) Department of Mathematics, Vrije Universiteit, Amsterdam, the Netherlands.

Functional gene x gene interaction (epistasis) is a biologically very plausible explanation for, part of, the "missing heritability" of complex traits. Although this possibility is widely acknowledged, a full screen for epistatic effects on a genome-wide scale is still considered unfeasible because statistical power would be seriously compromised by correcting for multiple testing. Previously we have suggested that a possible way to avoid this problem is to ascertain pairs of variants that are a priori more likely to be involved in functional gene-gene interactions and test only these for association with a complex phenotype. Here we present the formal procedure for detecting genome-wide significant gene x gene interactions based on a pre-test to select pairs of loci that exhibit non-equilibrium two locus genotype frequencies in controls, followed by an adjusted test statistic for interaction that corrects for the correlation between the pre-test and interaction test induced by re-using the controls in the second step. Multiple testing correction is only necessary for the number of test in the second step, while type I error is correctly maintained. We show that the procedure has superior power compared to a related one step interaction test followed by multiple testing correction for all pairs involved provided that the ratio of controls vs. cases is larger than approximately two. The overall power of the method depends on true interactions inducing two-locus disequilibrium between the loci in the controls. We show that realistic interaction effect sizes on disease susceptibility induce sufficient two-locus disequilibrium in controls to be detected in the pre-test, while gene pairs with strong additive effects of either loci are much less likely to pass the pre-test. Because only the chi-square test based pre-test is performed for all pairs of loci, the procedure is computationally feasible and efficient while the reduction of the number of tests in the second step conveys increased overall power making this procedure suitable to analyze genome-wide data.

2898/T

A Mann-Whitney based whole genome-wide association study finds significant gene-gene interaction for Type 2 diabetes. *Q. Lu[1], C. Wei[1], C. Ye[1], R.C. Elston[2].* 1) Dept of Epidemiology, Michigan State Univ, East Lansing, MI; 2) Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH.

With the merge of large amount data from whole genome-wide studies, interest has been moved towards whole genome-wide search for gene-gene interaction. The importance of gene-gene interaction has long been recognized. However, identifying interactions has been a great challenge, especially when involves millions of genetic markers. Most of the currently used statistical tools are not originally designed for whole genome gene-gene interaction search, and are subject to issues such as multiple testing, computational inefficiency, and incapability of capturing high order interaction. We here proposed a Mann-Whitney based approach for whole genome-wide gene-gene interaction search. It extends traditional univariate Mann-Whitney test to assess the joint association of multiple loci, considering all levels of possible interactions. With only one overall significant test is conducted, it avoids issue of the multiple testing. The approach adopts a computationally efficient algorithm, and thus is feasible to conduct whole genome-wide gene-gene interaction analysis with a reasonable time on a high performance personal computer. We evaluated the approach using both simulation and real data application. By applying the approach on 24 Type 2 diabetes (T2D) susceptibility genes, we identified a four locus model strongly association with T2D in the Wellcome Trust (WT) study (permutation P-value<0.001), and replicated the finding in the Nurses' Health Study/ Health Professionals Follow-up (NHS/HPFU) study (P-value=3.03E-11). Furthermore, we conducted a whole genome-wide gene-gene interaction search on nearly 500K loci. The approach identified four loci joint associated with T2D (P-value=1.29E-5) in WT study. The significance leave of this association reaches 4.01E-6 in the NHS/HPFU study.

2899/T

A Novel Statistic for Genome-wide Interaction Analysis. *X. Wu[1], H. Dong[3], L. Luo[2], Y. Zhu[2], G. Peng[2], J. Reveille[4], M. Xiong[2].* 1) Deptof epidemiology , Bengbu Medical College at Bengbu, Bengbu, Anhui, China; 2) Human Genetics Center, University of Texas School of Public Health, Houston, TX 77030; 3) Laboratory of Theoretical Systems Biology and Center for Evolutionary Biology, State Key Laboratory of Genetic Engineering, School of Life Science and Institute for Biomedical Sciences, Fudan University, Shanghai 200433, China; 4) Division of Rheumatology, Medical School, University of Texas Health Science Center at Houston, Houston, TX 77030.

Although great progress in genome-wide association studies (GWAS) has been made, the significant SNP associations identified by GWAS account for only a few percent of the genetic variance, leading many to question where and how we can find the missing heritability. There is increasing interest in genome-wide interaction analysis as a possible source of finding heritability unexplained by current GWAS. However, the existing statistics for testing interaction are less powerful for genome-wide interaction analysis. To meet challenges raised by genome-wide interactional analysis, we have developed a novel statistic for testing interaction between two loci (either linked or unlinked). The null distribution and the type I error rates of the new statistic for testing interaction are validated using simulations. Extensive power studies show that the developed statistic has much higher power to detect interaction than classical logistic regression. The results identified 44 and 211 pairs of SNPs showing significant evidence of interactions with FDR < 0.001 and 0.001 < FDR < 0.003, respectively, which were seen in two independent studies of psoriasis. These included five of interacted pairs of SNPs in genes LST1/NCR3, CXCR5/BCL9Land GLS2, some of which were located in the target sites of miR-324-3p, miR-433, and miR-382, as well as 15 pairs of interacted SNPs that had nonsynonymous substitutions.

2900/T

A Novel Statistic for Testing Genetic Interactions between Linked Loci. *J. Zhao[1], X. Wu[2], Y. Zhu[2], M. Xiong[2].* 1) Biostatistics & Epidemiology, Univ Oklahoma HSC, Oklahoma City, OK; 2) University of Texas HSC at Houston, Houston, TX.

Despite the success of GWAS in identifying single SNP associations for complex diseases, the positive associations identified so far explained only a small proportion of phenotypic variations in the studied phenotypes. The etiology of human complex disorder, however, involves multiple genes and their interactions. Modeling complex genetic interactions is clearly important to fully explain inter-individual variability in complex disease phenotypes. Up to date, identification of gene × gene interactions at a genome-wide level remains a challenge for human genetic studies, partly because of lack of powerful statistical methodologies. We have previously reported a LD-based test statistic in detecting gene × gene interactions between two unlinked loci. Here we report another novel test statistic for testing genetic interactions between linked loci at a genome-wide level. The statistic defines gene × gene interaction as a stochastic dependence between two interacting loci in leading to disease susceptibility. The performance of the new statistic including null distribution, statistical power and type 1 error rates at different significance levels, were evaluated using extensive simulation studies. The proposed novel statistic was also assessed in comparison with three existing methodologies in testing genetic interactions: logistic regression, the "fast-epistasis" program implemented in PLINK, and the haplotype odds ratio method. Our results showed that the proposed novel test statistic has a higher power in detecting genetic interactions. Finally, we applied the proposed statistic to two GWAS datasets to identify genome-wide genetic interactions for rheumatoid arthritis. Dozens of genetic interactions were identified and replicated in the two independent studies.

2901/T

A Faster Pedigree-Based Generalized Multifactor Dimensionality Reduction Method for Detecting Gene-Gene Interactions. *G.B. Chen[1], J. Zhu[2], X.Y. Lou[1].* 1) Section on Statistical Genetics, Department of Biostatistics, University of Alabama, Birmingham, AL; 2) Institute of Bioinformatics, Zhejiang University, Hangzhou, China.

We proposed a faster pedigree-based generalized multifactor dimensionality reduction algorithm, called PedGMDR II (PII), to detect gene-gene interactions underlying complex traits. Inheriting from our previous framework of PedGMDR (PI), PII is applicable to handle both dichotomous and continuous traits in pedigree-based designs and allows for covariate adjustment. Compared with PI, this faster version can theoretically halve the computing burden and memory requirement. To evaluate the performance of PII, we performed comprehensive simulations across a wide variety of experimental scenarios, in which we considered two study designs, discordant sib pairs and mixed families with varying size, and five common factors that may potentially affect statistical power: minor allele frequency, missing rate of parental genotypes, covariate effect, gene-gene interaction, and scheme to adjust phenotypic outcomes, leading to a total of up to 4096 scenarios. Simulations showed that PII gave a well controlled type I error rates against population admixture. In general, PII had a higher average power than PI in simulations for both dichotomous and continuous traits, and the advantage was more pronounced for continuous traits. Given a magnitude of interaction effect, PII appeared to be less sensitive to changes in other factors. Applied to the Mid-South Tobacco Family study, PII detected a significant interaction with a P-value of 5.4×10^{-5} between two taster genes, *TAS2R16* and *TAS2R38*, responsible for nicotine dependence. In conclusion, PII is a faster supplementary version of our previous PI for detecting multifactor interactions.

2902/T

Using Biological Knowledge To Discover Higher Order Interactions In Genetic Association Studies. *G. Chen[1], D. Thomas[1], A. Presson[2].* 1) Preventive Medicine, USC, Los Angeles, CA; 2) Biostatistics and Pediatrics, UCLA, Los Angeles, CA.

The recent successes of genome-wide association studies (GWAS) have revealed that many of the replicated findings have explained only a small fraction of the heritability of common diseases. One hypothesis that investigators have suggested is that higher-order interactions between SNPs or SNPs and environmental risk factors may account for some of this missing heritability. Searching for these interactions poses great statistical and computational challenges. We propose a novel method that addresses these challenges by incorporating external biological knowledge into a fully Bayesian analysis. The method is designed to be scalable for high-dimensional search spaces (where it supports interactions of any order) because priors that use such knowledge focus the search in regions that are more biologically plausible and avoid having to enumerate all possible interactions. We provide several examples based on simulated data demonstrating how external information can enhance power, specificity, and effect estimates in comparison to conventional approaches based on maximum likelihood estimates. We also apply the method to data from a GWAS for breast cancer, revealing a set of interactions enriched for the Gene Ontology terms growth, metabolic process, and biological regulation.

2903/T

A Novel and Comprehensive Method To Find Complex Interactions. *S. Prabhu, I. Pe'er.* Computer Sci, Columbia Univ, New York, NY.

Despite considerable success in exposing the genetic basis of many diseases, genome wide association studies have made limited progress in explaining their heritability. One popular hypothesis as to why single locus methods have had limited success in this area speculates that complex interactions between distant loci might be responsible for some of the missing heritability. In such a case, while the marginal effect of any single contributing locus might be statistically insignificant (amid genome-wide testing burden), when seen in combination with its interacting counterparts their effect becomes apparent.

However, designing association studies for complex interactions have hitherto been plagued by problems arising from their combinatorics. Specifically, two fundamental limitations have been:

(a) Computational - the total number of interactions increases exponentially with the number of loci. For datasets of just a million typed SNPs, even testing all low-arity (2-way, 3-way) interactions presents a considerable computational burden. (b) Statistical - testing all interactions independently incurs a multiple testing burden that can be prohibitive. However, we demonstrate that in most realistic scenarios computational infeasibility sets in much earlier than the statistical bound.

We present a novel method that uses Probably Approximately Correct (PAC) learning techniques to search for complex interactions. Our algorithm seeks telltale patterns in randomly chosen groups of cases that are indicative of interaction effects. By repeated random sampling and examination of a few summary statistics for each sample, we are able to uncover any existing interaction in the case cohort with arbitrarily high probability. In other words, without resorting to search space reductions (e.g. shortlisting a few loci based on marginal effect, functional significance, etc.) or brute-force enumeration of all possible combinations of loci, our method comprehensively searches for interactions under various disease models at a very small fraction of the erstwhile computational cost. On synthetic case-control datasets (whole genome sequences with 16M SNPs, or genotypes with 1M SNPs), our method reveals 2-way, 3-way and 4-way interactions when marginal effects of each contributing locus are completely unremarkable ($p<0.4$).

2904/T

Predicting Patient Outcomes from Clinical and Genome-Wide Data. *M.M. Barmada[1], S. Visweswaran[2], P. Hennings-Yeomans[2], K. Bui[2], G. Cooper[2].* 1) Dept Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; 2) Department of Biomedical Informatics, University of Pittsburgh, Pittsburgh, PA.

Clinical classification and prediction are key components of clinical care. Even modest improvements in classification and predictive performance have the potential to significantly improve patient outcomes and reduce healthcare costs. Traditional clinical data are becoming increasingly available in electronic form. In addition, in the foreseeable future genome-wide patient-specific data are likely to become available as part of the electronic health record to inform clinical care. Both sources of data provide significant opportunities for using statistical and machine-learning methods to improve classification and prediction over what is currently possible. However, the sheer magnitude of the number of variables in these data (in the hundreds of thousands or millions) presents formidable computational and modeling challenges. It is therefore important to explore new ways to use traditional clinical data and genome-wide data together to improve clinical classification and prediction. We propose a new approach for using both types of data to perform clinical classification and prediction. The new method builds on our previous work in Bayesian outcome prediction, which has yielded positive results when applied using just traditional clinical data. That previously developed method, however, is not able to handle computationally the huge number of variables introduced by genome-wide data. The new Bayesian method is computationally tractable, yet still able to model important interactions among the predictor variables. Using data from the Framingham SHARe resource, we examine how clinical and genome-wide data can be used to predict the onset of major cardiovascular events and death from all causes. We compare the new Bayesian method to a set of traditional prediction methods with regard to computational tractability and predictive performance in terms of discrimination and calibration.

2905/T

Gene-smoking interactions on chromosomes 5 and 18 in age-related macular degeneration revealed through genome-wide environmental interaction analysis. *W.K. Scott[1], A.C. Naj[1], W.H. Cade[1], P.J. Gallins[1], P.L. Whitehead[1], I. Konidari[1], M.D. Courtenay[1], L.M. Olson[2], K.L. Spencer[2], N.C. Schnetz-Boutoud[2], S.G. Schwartz[3], J.L. Kovach[3], A. Agarwal[4], G. Wang[1], J.L. Haines[3], M.A. Pericak-Vance[1].* 1) Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL; 2) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN; 3) Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, Naples, FL; 4) Vanderbilt Eye Institute, Vanderbilt University Medical Center, Nashville, TN.

Age-related macular degeneration (AMD) is the most common cause of irreversible vision loss in older adults in developed countries. AMD has a complex etiology comprising several genetic (*CFH, ARMS2, CFB/C2, CFI, TIMP3*) and environmental (cigarette smoking, estrogen use, diet) risk factors. The major genetic factors have been identified using linkage analysis and association mapping (*CFH* and *ARMS2*), candidate gene testing (*CFB/C2*, *CFI*), and genome-wide association screening (*TIMP3*). While these factors explain a substantial portion of the risk of AMD, additional factors (including gene-gene and gene-environment interactions) remain to be discovered. To this end, we conducted a genome-wide association and gene-environment interaction study of 1013 white AMD cases and 601 white unaffected controls using the Affymetrix Genome-Wide SNP Array 6.0. Environmental risk factor information was collected on 830 cases and 486 controls by self-administered questionnaire. After quality control checks, 671,524 SNPs were retained for analysis. Main effects analysis using logistic regression and an additive genotype model (adjusting for age and sex) detected genome-wide significant associations ($p<5x10^{-8}$) at 3 known loci: *CFH* ($p=1.3x10^{-24}$), *ARMS2* ($p=2.8x10^{-20}$), and *CFB/C2* ($p=8.8x10^{-9}$). Gene-environment interaction was tested by including pairwise interaction terms between genotype and smoking history (ever/never) in the logistic regression models. No interactions were genome-wide significant, but the strongest interactions were detected in an intergenic three-SNP block on chromosome 18 ($p=5.3x10^{-7}$ at rs1707364) and an intergenic three-SNP block on chromosome 5 ($p=5.4x10^{-6}$ at rs4865735). Unfolding these interactions by stratification on smoking history revealed significant, opposite effects at each locus: the minor alleles at the chromosome 18 locus increase risk of AMD in ever smokers (OR=1.4, 95% CI: [1.1-1.8]) but decrease risk in never smokers (OR=0.55, 95% CI: [0.42-0.73]. A similar pattern was observed at chromosome 5: the minor alleles increase risk in non-smokers (OR=2.1, 95% CI: [1.3-3.3] but decrease risk in ever smokers (OR= 0.53, 95% CI: [0.37-0.75]. These results suggest that gene-environment interactions with no detectable main genetic effects may explain some risk of AMD. Additional studies to replicate and extend these findings are needed to establish the biological mechanisms underlying the interactions.

2906/T

Copy number haplotype inference with Hidden Markov Model and localized haplotype clustering. *Y. Lin[1], S. Hsu[2], C. Tang[1], W. Hsieh[2].* 1) computer science, National Tsing Hua University , Hsinchu, Taiwan; 2) Statistics, National Tsing Hua University , Hsinchu, Taiwan.

Copy number polymorphisms and aberrations can now be studied at high resolution using genome-wide SNP arrays. Most of the tools detect the copy number alteration regions by either segmentation approach or a dynamic Hidden Markov Model. The total copy number or allele specific copy number is inferred based on the probe intensities designed to measure the quantity of either allele. Hidden Markov Model based approaches can only provide discrete states and are not accurate for somatic mutations since the sample is a mixture of heterogeneous cells. On the other hand, HMM is perfectly suitable for integrating Linkage Disequilibrium information, which is the major source of haplotype inference. We present a method based on Hidden Markov Model to detect parent specific copy number variation with fractional copy number estimation. Two haplotype trees are constructed for the two chromosomes and the states inferred are the copy numbers of the two alleles under consideration. Each allele is modeled with three states, the normal, plus and minus copy numbers. The transition of states helps to construct the haplotype for either chromosome as well as the copy number status. The CNV regions thus inferred will be pooled together to make the copy number estimation. The enormous computation load can be greatly reduced with the help of localized haplotype clustering proposed by Browning et al. (2006 AJHG). A few iterations are required to reach the convergence. The proposed method is evaluated on the known regions of copy number variation on 270 HapMap individuals as well as an oral cancer cohort of 112 samples. We also compare the other four popular methods including PenCNV, genoCN, COKGEN, and QuantiSNP. Although they do not provide parent specific haplotypes, we compare the overlapping rates of the detected regions, and consistency of the copy number estimation. Our observation found that PenCNV gives the best overlapping rates of the known detection regions and is the most efficient package with the shortest running time. Our method is more sensitive to short regions while the long regions can also be correctly detected.

2907/T

Towards a genetic screening test for dyslexia allowing functional regeneration: A strategy for identification and analysis of genetic risk factors. *H. Kirsten[1,2], A. Wilcke[1,2], P. Ahnert[3], J. Boltze[2].* 1) University Leipzig TRM, Leipzig, Germany; 2) IZI Fraunhofer Leipzig, Leipzig, Germany; 3) University Leipzig , IMISE; Leipzig, Germany.

Our aim is to develop a genetic screening test for dyslexia, a severe disorder of reading and frequently of writing, affecting approx. 4 % of all schoolchildren. A significant problem is late diagnosis resulting in a decreased chance of functional regeneration. Our solution is an early genetic test that will initiate existing early training programs. Genetic dyslexia markers necessary for this test are identified in a micro array based fine screen supplemented by polymorphisms of highly relevant candidate genes. Validation of these markers is done by A) genotyping an independent cohort; B) by characterising markers in functional magnet resonance imaging (fMRI) and electroencephalography (EEG); and C) by characterising markers in allele specific mRNA-expression analysis or allele-specific chromatin immunoprecipitation (ChIP). The final test will neither include fMRI/EEG nor expression analysis. It translates genetic findings into a clinical assay. This test would allow early identification of children at risk, enabling early support resulting in functional regeneration.

2908/T

A sex-specific association between a 15q25 variant and upper-aerodigestive tract cancers. *D. Chen, T. Truong, V. Gaborieau, P. Brennan, JD. McKay on behalf of the INHANCE consortium.* International Agency for Research on Cancer, Lyon, France.

Sequence variants located at 15q25 have been associated with lung cancer and propensity to smoke. We recently reported a modest association between rs16969968 and risk of upper aerodigestive tract (UADT) cancers (oral cavity, oropharynx, hypopharynx, larynx and esophagus)(Odds Ratio (OR)=1.08), particularly in women (OR=1.24). We sought to replicate these findings in an additional 4,604 cases and 6,239 controls from 10 independent UADT cancer case-control studies participating in the International Head and Neck Cancer Epidemiology (INHANCE) consortium. The rs16969968 variant was genotyped using the TaqMan genotyping platform. We observed a similar association in the replication series (OR=1.07, 95% confidence interval (CI)=1.00-1.13, *P*=0.03) as well as the sex difference (*P*-heterogeneity=0.01).To further evaluate this genetic effect, we combined the individual level data from the UADT cancer studies in the initial study and the 10 independent studies presented here, making for a total of 8,572 UADT cancer cases and 11,558 controls from 14 studies. In the combined analysis, the association was observed almost uniquely in females (OR=1.22, 95% CI=1.12-1.34, $P=7\times10^{-6}$) but not males (OR=1.02, 95%CI=0.97-1.08, *P*= 0.35) (*P*-heterogeneity=6×10^{-4}). In women the association of rs16969968 with UADT cancer risk was relatively consistent among subgroups stratified by smoking status, cigarettes smoked per day (CPD), alcohol consumption, ever/never smoking-drinking status and age. In contrast, among men there was little evidence for association between rs16969968 and UADT cancer in any stratum. The rs16969968 variant has been consistently associated with propensity to smoke cigarettes (particularly CPD), we therefore examined whether rs16969968 was associated with number of CPD among 11,991 ever smokers included in the combined initial and replication data sets. rs16969968 minor allele was associated with propensity to smoke (*P*= 1×10^{-6}), however, there was little evidence for a sex difference in the effect of the rs16969968 variant on propensity to smoke, with male and female variant carriers smoking approximately the same amount more (male and female rare homozygotes smoked 1.79 and 1.29 cigarettes more than common homozygotes, respectively) (*P*-heterogeneity=0.86). No association was noted with age of smoking initiation or cessation in males or females. Further research is warranted to elucidate the mechanisms underlying these observations.

2909/T

Association Mapping Across the Major Histocompatibility Complex Region in the Risk of Childhood Acute Lymphoblastic Leukemia. *K.Y. Urayama[1,5], A.P. Chokkalingam[1], C. Metayer[1], J.L. Wiemels[2], J.K. Wiencke[2], E. Trachtenberg[3], G.M. Taylor[4], P. Brennan[5], H. Hansen[2], S. May[1], L.F. Barcellos[1], P.A. Buffler[1].* 1) School of Public Health, University of California, Berkeley, CA; 2) Laboratory for Neuro and Molecular Epidemiology, University of California, San Francisco, CA; 3) Children's Hospital and Research Center, Oakland, CA; 4) University of Manchester, Manchester, United Kingdom; 5) Genetic Epidemiology Group, International Agency for Research on Cancer, Lyon, France.

Given the accumulating support for a role of immunologic factors in the etiology of childhood leukemia, the major histocompatibility complex (MHC) is an important candidate region for genetic studies of this disease. The MHC is a gene-dense region populated by a large number of genes that play a role in immune function and response, including the highly polymorphic human leukocyte antigen (HLA) genes involved in antigen presentation to T lymphocytes. We conducted association mapping across an approximately 4 megabase region of the MHC using the Illumina MHC Mapping Panel of single nucleotide polymorphisms (SNP) and specific allelic typing of the previously associated *HLA-DPB1* locus among 634 acute lymphoblastic leukemia (ALL) cases aged 0-14 years and 902 population controls enrolled in the Northern California Childhood Leukemia Study. Single-marker logistic regression analyses assuming log-additive and dominant genetic models of 1,143 SNPs and adjusted for age, sex, and Hispanic ethnicity indicated several potentially associated regions. These include regions tagged by SNPs rs7747023 (*OR2W1*, p=0.002) and rs1233388 (*OR2H2*, p=0.003) of the extended class I region, rs3130785 (p=0.006) of the classical class I region, rs9296068 (*HLA-DOA*, p=0.002) of the classical class II region, and rs213203 (*VPS52*, p=0.0004) of the extended class II region. Separate analysis of five common alleles of the *HLA-DPB1* gene, located adjacent to *HLA-DOA*, did not show associations with childhood ALL risk. However, a sliding window haplotype analysis containing *HLA-DPB1* alleles and nearby SNPs identified an associated 4-marker haplotype comprised of rs399604 (*HLA-DOA*), rs9296068 (*HLA-DOA*), *DPB1*, and rs3762013 (*COL11A2*) (global $p=4.1\times10^{-4}$). A specific haplotypic effect of the common haplotype G-A-DPB1*0401-G was observed ($p=7.2\times10^{-6}$). As the first MHC-wide high resolution SNP association study in childhood ALL, we provide preliminary evidence suggesting that genetic variation within specific regions of the MHC may be involved in genetic susceptibility to this disease. The regions identified in this study warrant further evaluation through confirmation in other populations and subsequent fine-mapping of replicated regions.

2910/T

Host Genetic Factors and Vaccine-Induced Immunity to Salmonella typhi and Streptococcus pneumoniae. *B.J. Hennig[1], L. Ashton[2], D. Goldblatt[2], A.M. Prentice[1,3], A.A. Richards[1,3], S.C. Szu[4], S.E. Moore[1,3].* 1) MRC Int Nutrition Group, London School of Hygiene & Tropical Medicine, London, London, United Kingdom; 2) Institute of Child Health, London, UK; 3) MRC Keneba, Medical Research Council Laboratories, Fajara, The Gambia; 4) National Institutes of Health, Maryland, USA.

Background: Little is known about the role of host genetic variation on vaccine-induced immunity. We assessed the genetics of vaccine responses to *S.typhi* and *S.pneumoniae* immunization using data available from two projects from The Gambia (West Africa): Firstly, antibody responses to two polysaccharide vaccines from a study of early life predictors of immunity (Moore SE *et al* in preparation) and secondly, genotype data from a study on HBV vaccine-induced immunity (Hennig BJ *et al* PLoS ONE. 2008 Mar 26;3(3):e1898). Methods: The combined dataset comprised 130 individuals of Mandinka ethnicity (mean age of 18.0 ±2.3 years), vaccinated with a single dose of a S.tyhpi Vi and a 23-valent capsular polysaccharide pneumococcal vaccine (Typhim Vi and Pneumo23, both Sanofi Pasteur). Anti-Vi antibody and antibodies against pneumococcal serotypes 1, 5, 14 and 23f were measured by ELISA at baseline and 14 days post-vaccination. Genotype data on 715 SNP across 133 genes (typed on Illumina BeadArray platform) were employed. Multiple linear regression models with single SNPs as exposure and post-vaccination geometric mean antibody titre (GMT) as outcome were applied, whilst adjusting for pre-vaccination antibody level, age, sex, village and relatedness (clustering by sibship), but not multiple comparisons. Results: Pre-vaccination antibody level had a strongly affected post-vaccination antibody level ($P<1.0 \times 10^{-5}$), but no effect was seen for age, sex and marginal effects of village. SNPs associated with antibody responses to one or more pneumococcal serotype or S.typhi reaching the cut-off of a ratio of GMT of <0.6 or >1.5 and a P-value of $<1.0 \times 10^{-4}$ are: IL19 (rs3950619, rs2056225, rs2243158, rs2243174); IL1RL1 (rs4988956, rs10192157); CTLA4 (rs3087243); IL7R (rs10213865, rs3194051); HLA-DQA1 (rs9272775); NFKBIL2 (rs4082353); FAS (rs3218614); MS4A1 (rs1051756); AICDA (rs2580876); IRAK3 (rs1152918); IL26 (rs3782554); ITGAL (rs2230433); and MAP2K4 (rs2108496). Discussion: These data should be interpreted with caution due to the small sample size and require replication; nevertheless, the observed effect sizes are large (>1.5-fold in/decrease in GMT of vaccine-induced antibody) and the p-values small. Genetic variants shown to associate with immunity induced by *S.typhi* and *S.pneumoniae* vaccination here do not appear to have been reported previously. These data will help understand the mechanisms underlying vaccine-induced immunity and thus to improve future vaccines.

2911/T

Pathway analysis of height, genetic variants and adult systolic blood pressure in the Northern Finland Birth Cohort 1966. *M. Kaakinen[1,2], U. Sovio[3,4], A-L. Hartikainen[1], A. Pouta[5], J. Laitinen[6], M. Savolainen[1,2], K-H. Herzig[1,2], T. Lajunen[1,2], M. Perola[7], N. Freimer[8], B. De Stavola[4], E. Läärä[1], M-R. Järvelin[1,2,3,5].* 1) University of Oulu, Oulu, Finland; 2) Biocenter Oulu, Oulu, Finland; 3) Imperial College London, London, UK; 4) London School of Hygiene and Tropical Medicine, London, UK; 5) National Institute for Health and Welfare, Oulu, Finland; 6) Finnish Institute of Occupational Health, Oulu, Finland; 7) National Institute for Health and Welfare, Helsinki, Finland; 8) Neurobehavioral Genetics and Semel Institute for Neuroscience and Human Behavior, UCLA, Los Angeles, CA.

Height is positively associated with hypertension but the underlying mechanisms behind the phenomenon are unclear. We investigated how birth length (BL), accelerated height growth in infancy, which is often observed in children born small and is a known risk factor for adverse metabolic profile in adulthood, adult height and four candidate single-nucleotide polymorphisms (SNPs) associate with adult systolic blood pressure (SBP). In addition, we studied if these genetic variants associate with height. The study population consisted of 5851 singletons belonging to the Northern Finland Birth Cohort 1966. We used pathway analysis to estimate the associations between BL, peak height velocity (PHV) in infancy and height and SBP at age 31 years. Four SNPs identified via genome-wide association studies and meta-analysis for SBP were included into the model. The analysis also accounted for gender, gestational age, maternal height, age, smoking, parity, socioeconomic status (SES) and subject's own smoking, drinking, SES and BMI at 31 years. Frequent height measurements at 0-2 years were used to estimate individual height curves, and PHV was derived as the maximum value of the growth velocity curve, typically occurring soon after birth. An inverse association between BL and SBP was observed (beta=-0.40 mmHg/cm, SE=0.09, p<0.001) after controlling for all the assumed relationships. Low BL was also associated with increased PHV in infancy (p<0.001), which in turn was associated with increased adult height (p<0.001) but not with SBP adjusted for adult height (p=0.47). Mediation of the BL association on SBP was observed via PHV and height at 31 years (total indirect effects beta=0.23 mmHg/cm, SE=0.04, p<0.001). The sum of the direct and indirect effects, i.e. the total effect of BL on SBP, remained negative (beta=-0.17 mmHg/cm, SE=0.08, p=0.03). SNP rs1378942 in *CSK* associated negatively with SBP (beta=-0.64 mmHg/each copy of A-allele, SE=0.25, p=0.009) and also showed a negative borderline-significant association with PHV (p=0.05). SNP rs932764 in *PLCE1* (for SBP beta=-0.73 mmHg/each copy of A-allele, SE=0.25, p=0.004) showed weak evidence for negative association with adult height (p=0.09). About third of the low BL effect on high adult SBP was mediated via increased PHV in infancy and current height. The weak associations of two of the SBP-associated SNPs with height suggest a possible underlying shared genetic component; however, replication studies are needed.

2912/T

Risk of tuberculosis among close contacts to TB patients: the role of Vitamin D receptor gene polymorphisms. *C. Luo[1], M. Reichler[1], B. Chen[1], E. Sigman[1], F. Maruri[2], T. Sterling[3].* 1) CDC, Atlanta, GA; 2) Tenn. Dept. of Health, Nashville, TN; 3) Vanderbilt Univ., Nashville, TN.

Background: Single nucleotide polymorphisms (SNPs) in the Vitamin D receptor (VDR) gene have been implicated in susceptibility to TB among persons of Black race/ethnicity, but the contribution of VDR gene SNPs to TB susceptibility among persons of other race/ethnicities has not been well characterized. **Methods:** We enrolled U.S.-born contacts with latent TB infection or active TB disease with >180 hours of exposure to smear+ TB patients at 9 sites. Blood was collected and genotyped for VDR gene SNPs BSM1and FOK1 and epidemiologic data collected by contact interview. Pairwise analysis was conducted using χ^2 tests with homozygous wild genotype as the referent group. This analysis is limited to contacts of white (n=69), Hispanic (n=39), and American Indian (n=22) race/ethnicity. **Results:** Among 130 contacts, 9 had active TB and 121 had a positive tuberculin skin test (TST+) but not active disease. Compared with TST+ contacts, contacts with active TB of Hispanic race/ethnicity were more likely to have the homozygous mutant genotype for VDR BSM1 (OR=undefined, P<.001). There was no association for VDR BSM1 among contacts of white or American Indian race/ethnicity, and no association for VDR FOK among contacts of any of the three race/ethnicities. **Conclusions:** In this U.S. population, active TB among Hispanic contacts after exposure to *M. tuberculosis* was associated with homozygous mutant genotype for VDR BSM1. This finding strengthens the association between VDR BSM and TB susceptibility (previously observed for contacts of Black race/ethnicity) by demonstrating the association in a second race/ethnicity. **Funding:** Centers for Disease Control.

2913/T

Evaluating 20 Autoimmune Disease Associated Loci with Rheumatoid Arthritis in a Colombian Population: Evidence for Replication and Gene-Gene Interaction. *A.K. Maiti[1], H. Deshmukh[1], X. Kim-Howard[1], P. Viswanathan[1], A. Rojas-Villarraga[2], J.M. Guthridge1[1], JM. Anaya[2], S.K. Nath[1].* 1) Gen Epidemiology Unit, A&I, OMRF, Oklahoma city, OK; 2) Center for Autoimmune Diseases Research (CREA), Universidad del Rosario-Corporación para Investigaciones Biológicas, Bogota, Colombia.

Objective: Recently, genome-wide and high-density candidate gene association studies have identified multiple SNPs which are associated with rheumatoid arthritis (RA), especially in individuals with European Ancestry. Additionally, many of these associated SNPs are also associated with multiple autoimmune diseases, supporting the hypothesis of shared or general autoimmunity genes/loci. However, due to cultural, nutritional or environmental differences between populations, both ethnic-specific single variant association and gene-gene interaction within these SNPs should also be evaluated. The main objective of this study is to assess and replicate the genotype-phenotype correlation between recently reported associated 20 variants from multiple autoimmune disease and RA genes/loci in an ethnically homogenous non-Caucasian Colombian population from Paisa community. Methods: 20 SNPs from 17 genes/loci were genotyped in 353 RA cases and 368 controls in individuals from Columbia. For each SNP, allelic and genotype based association tests were applied to evaluate genotype-phenotype correlation. Permutation based tests were used to validate the statistical significance. Gene-gene interactions were assessed by logistic regression and multiple testing was adjusted with both Bonferroni corrections and false discovery rate (FDR). To evaluate the veracity of genetic association, we used RA data from GAW16 either from genotypes or by imputation. Results: We replicated the genetic association with rs6822844, (p = 0.008, OR = 0.86) from IL21, rs13277113 (p=0.0009, OR=1.46) and rs2736340 (p= 0.0001, OR=1.63) from C8orf13-BLK, and rs763361 (p=0.03, OR=1.21) from CD226 in the Colombian population. The population attributable risks were estimated as10%, 27%, 34%, and 16% for rs6822844, rs13277113, rs2736340, and rs763361 respectively. We also detected evidence of gene-gene interaction between MMEL1 and C80rf13-BLK (p=0.0002) in Colombian population. 6 of these 20 SNPs were replicated in GAW16 data but no gene-gene interaction is detected. Conclusion: We replicated association with 4 autoimmune predisposing loci in a non-Caucasian population. Contributions of these genes to disease predisposition in RA suggest that there are common disease mechanisms between RA and clinically different autoimmune diseases. Identification of gene-gene interaction in Colombian population is important, and this should be confirmed in another independent homogenous population.

2914/T

Characterization of the *LILR* complex across populations and association analysis of the region with systemic lupus erythematosus. *C.J. Gallant[1], A.M. Delgado-Vega[1], E. Sánchez[2], S. Chung[3], J.A. Kelly[2], K.M. Kaufman[2,4,5], G.S. Gilkeson[6], C.O. Jacob[7], J.A. James[2,4,5], R. Kimberly[8], J. Merrill[2], B.P. Tsao[9], T.J. Vyse[10], C. Langefeld[11], J.B. Harley[2,4,5], R.H. Scofield[2,4,5], P.M. Gaffney[2,5], L.A. Criswell[3], M.E. Alarcón-Riquelme[1,2,12].* 1) Department of Genetics and Pathology, Rudbeck Laboratory, Uppsala University, Uppsala, Sweden; 2) Arthritis and Immunology Program, Oklahoma Medical Research Foundation, Oklahoma City, OK; 3) Rosalind Russell Medical Research Center for Arthritis, Department of Medicine, University of California San Francisco, San Francisco, CA; 4) University of Oklahoma Health Sciences Center, Oklahoma City, OK; 5) US Department of Veterans Affairs Medical Center, Oklahoma City, OK; 6) Division of Rheumatology, Medical University of South Carolina, Charleston, SC; 7) The Lupus Genetic Group, Department of Medicine, University of Southern California, Los Angeles, CA; 8) Comprehensive Arthritis, Musculoskeletal, and Autoimmunity Center, Division of Clinical Immunology and Rheumatology, Department of Medicine, University of Alabama at Birmingham, AL; 9) Division of Rheumatology-Medicine, University of California-Los Angeles, CA; 10) Section of Rheumatology, Faculty of Medicine, Imperial College London, London, UK; 11) Section on Statistical Genetics and Bioinformatics, Division of Public Health Sciences, Department of Biostatistical Sciences and Center for Public Health Genomics, Wake Forest University Health Sciences, Winston-Salem, NC; 12) Pfizer-Universidad de Granada-Junta de Andalucia, Granada, Spain.

The leukocyte immunoglobulin-like receptors (*LILRS*) are a family of 13 genes on chromosome 19q13 that are mainly expressed on myeloid and lymphoid cells. LILRs function as inhibitory, stimulatory or soluble receptors and interact with FCRγ and HLA class I molecules. To date, different *LILRs* in the region have demonstrated extensive polymorphism (CNVs), population differentiation, evidence for balancing selection and association or linkage with disease susceptibility, including the autoimmune disease systemic lupus erythematosus (SLE). Given their importance in immune regulation, we propose that *LILRs* may contribute to SLE susceptibility and that population-specific polymorphisms may help explain differences in disease severity or susceptibility. To test this hypothesis, we combined dense genotyping, sequencing and quantification of copy number variable regions in European, South American and American Hispanic, Asian and African-American SLE cases and controls. We will present the results of our characterization of the *LILR* region across populations and report the findings of the association analysis between SLE and LILR polymorphisms.

2915/T

Evidence that Variants in the Clock Gene PER3 Confer Susceptibility to Type 2 Diabetes in Mexican Americans and to Diabetic Complications in Type 1 Diabetics. *J. Below[1], E. Gamazon[2], G. Bell[1], D. Nicolae[3], N. Cox[2], C. Hanis[4].* 1) Dept of Human Gen, Univ Chicago, Chicago, IL; 2) Dept of Genetic Medicine, Univ Chicago, Chicago, IL; 3) Dept of Statistics, Univ Chicago, Chicago, IL; 4) Dept of Human Gen, Univ Texas Health Science Center, Houston, TX.

Results derived from a GWAS (Affymetrix 6.0) in 837 unrelated cases with type 2 diabetes and 781 controls all of whom were Mexican Americans from Starr County, TX, and a GWAS (Affymetrix 5.0) in 1651 subjects of recent European ancestry with type 1 diabetes studied through the Genetics of Kidneys in Diabetes (GoKinD) in whom a variety of complications of diabetes were measured, both identified functional SNPs controlling expression of PER3. Functional Genome Wide Association (FGWA) utilizes a priori functional information for markers combined with set-level analysis to calculate a single p-value at the level of the gene. The gene-level p-value for PER3 approached genome-wide significance at 2.67E-05 (given that we have at least one functional SNP in 13,000 genes) for type 2 diabetes, and was the second most significant gene-based signal observed for the type 2 diabetes study. The gene-level p-value for kidney failure (the primary complication phenotype examined in GoKinD) was 9.5E-04 and was the 13th most significant signal. The GoKinD signal is driven by 3 missense mutations: rs10462020, rs228697, and rs10462021. rs10462021 and rs10462020, also contribute to the significance in Starr County as well as an additional missense mutation: rs2640909. For all of these missense variants, the minor allele was associated with increased risk for type 2 diabetes and kidney failure. PER3 is a key player in the transcriptionaltranslational feedback loop of the circadian system, which is responsible for regulation of numerous physiological and behavioral systems. Recent papers have implicated circadian clock function and metabolic diseases, showing that glucose intolerance is connected to expression of PER3 in humans. Our results support these findings, and are consistent with the hypothesis that missense mutations in PER3 may degrade glucose homeostasis sufficiently to increase risk of type 2 diabetes and lead to increased risk of diabetic kidney failure.

2916/T

Pre-Diabetic Ascertainment and the Relationship between BMI and Diabetes/Glucose Genetic Variants. *J.C. Engert[1,4], S.D. Bailey[1], C. Xie[2], G. Paré[2], A. Montpetit[3], S. Anand[2], The EpiDream investigators.* 1) Dept of Human Genetics, McGill University, Montreal, Quebec, Canada; 2) Population Health Research Institute, McMaster University, Hamilton, Ontario, Canada; 3) McGill University and Genome Quebec Innovation Centre, Montreal, Quebec, Canada; 4) Dept of Medicine, Royal Victoria Hospital, McGill Univ, Montreal, PQ, Canada.

Recent genome-wide association studies (GWAS) have convincingly associated several genetic loci with type-2 diabetes (T2D) and obesity. However, there is little overlap in the susceptibility loci identified for both diseases. In fact, only the FTO gene has been identified as contributing to both traits. We tested approximately 2000 inflammatory, metabolic, and cardiovascular candidate genes in 9451 European EpiDREAM participants (individuals screened for the DREAM trial) for an association with body mass index (BMI) and fasting plasma glucose (FPG). As expected, the SNPs most significantly associated with BMI are within the FTO locus (rs7193144, β= 0.63, p=3.30x10-14) and those most significantly associated with FPG are within the TCF7L2 locus (rs7903146, β=0.10, p=4.564x10-10). Interestingly, SNPs within both of these genes were significantly associated with both traits (p<0.05). Previous reports from ascertained populations have identified an association of TCF7L2 SNPs with BMI, but this result has not been replicated in prospective studies. In EpiDREAM, we observed that the TCF7L2 allele that increased BMI lowered FPG (β=-0.38, p=1.591x10-5). A significant number of the SNPs tested (n=175) affect both BMI and FPG (p<0.05) and the direction of the effect for the majority of these SNPs (n= 137) are the same. 38 SNPs, from 26 genes, (including TCF7L2 appeared to have an effect on both traits, but in opposite directions. We created a genotype score of 13 previously reported loci convincingly associated with T2D or glucose-related traits, excluding the FTO locus. We observed a graded relationship between the allele score and BMI in EpiDREAM individuals. Specifically, individuals with a low T2D genetic risk score (£8) had an average BMI that was more than two full points higher than individuals with a high T2D genetic risk score (≥19)(average BMI = 31.1 and 29.0, respectively, p=3.18x10-3). These results can be explained if the sum of T2D and FPG risk alleles, leads to diabetes in an additive model with BMI. Thus, more risk alleles are required on average to cause diabetes in lean individuals than in obese individuals, and a negative correlation between these loci and BMI is observed in a sample ascertained for glucose-related traits such as EpiDREAM. We believe that risk allele associations with BMI may be the result of ascertaining pre-diabetic individuals.

2917/T

***MTNR1B* is Associated with Glycemia During Pregnancy in Mexican-American Populations.** *M.G. Hayes[1], H. Lee[1], D. Levine[2], C. McHugh[2], L.P. Lowe[1], J. Morrison[3], A. Pluzhnikov[3], M. Urbanek[1], L. Armstrong[1], C. Ackerman[1], D. Mirel[4], A.R. Dyer[1], B.E. Metzger[1], N.J. Cox[3], W.L. Lowe[1] for the HAPO Study Cooperative Research Group.* 1) Northwestern University, Chicago, IL; 2) University of Washington, Seattle, WA; 3) University of Chicago, Chicago, IL; 4) The Broad Institute, Cambridge, MA.

The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study, a multicenter international study, examined the association of maternal glucose levels with fetal growth and outcome in >25,000 pregnant women from multiple ethnic groups. Results showed a continuous relationship between maternal glucose measures and birth size throughout the range of glucose concentrations less than those diagnostic of diabetes. We conducted a genomewide association study in a subset of HAPO populations (European-ancestry, Mexican-American, and Afro-Caribbean) to determine genetic associations with maternal glycemia and insulin secretion (fasting, 1-hr, and 2-hr glucose and fasting and 1-hr C-peptide from the OGTT) at 27.8±1.8 weeks gestation. Genotyping results are currently completed for 840 Mexican-American mothers. Associations were assessed through linear regressions with the single trait/outcome under an additive genetic model adjusting for known confounders (mother's age, BMI, height, and mean arterial pressure at the OGTT; gestational age of the baby at OGTT; parity; and ancestry [the first two principal components of variation calculated from a LD trimmed set of 95K SNPs spanning the genome]). Among our strongest signals was rs10830963, a C>G transversion occurring within an intron of *MTNR1B* on chromosome 11 which encodes the melatonin receptor 1B, which was recently identified in a GWAS meta-analysis as a variant associated with increased fasting glucose levels in non-pregnant women and men of European ancestry. In the HAPO study, this SNP was strongly associated with increased fasting glucose (2.1 mg/dL [95%CI: 1.4-2.8] per maternal minor allele; P=3.4x10-8), as well as associated with 1-hour glucose (5.5 mg/dL [95%CI: 2.0-9.0]; P=0.0024), and 2-hour glucose (3.4 mg/dL [95%CI: 0.9-5.9]; P=0.0086) during the OGTT. This same minor allele was also associated with the new diagnostic criteria for hyperglycemia during pregnancy (OR=1.73 [95%CI: 1.30-2.29]; P=0.0001). These results suggest that genetic variation in *MTNR1B* is not only associated with increased fasting glucose levels in non-pregnant women and men of European ancestry, but also associated with glycemia during pregnancy in Mexican-Americans.

2918/T

Variants in *PFKFB2* are Associated with Body Mass Index and Insulin Secretion in Pima Indians. *Y. Muller, J. Fleming, S. Bhutta, R. Hanson, L. Bian, W.C. Knowler, C. Bogardus, L. Baier.* NIDDK, NIH, Phoenix, AZ.

A prior genome-wide association study (GWAS) in Pima Indians using the Affymetrix 1-million SNP chip identified variants within *PFKFB2* that were associated with quantitative traits that predict obesity. *PFKFB2*, located on chr.1:207226620-207254368bp, encodes 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase, a bifunctional enzyme involved in the synthesis and degradation of fructose 2,6-bisphosphate. PFKFB2 has a role in regulation of insulin secretion in pancreatic β-cells *in vitro*, and high fructose 2,6-bisphosphate levels in transgenic mice result in weight gain. To follow-up on our GWAS findings, the putative promoter, exons and exon-intron boundaries of *PFKFB2* were sequenced in 24 Pima Indians, and 13 single nucleotide polymorphisms (SNPs) were identified. Fourteen SNPs were genotyped in a population based sample of 3501 full heritage Pima Indians informative for body mass index (BMI). A 3'-UTR SNP (rs17258746) with a risk allele frequency of 0.10, was associated with BMI (mean BMI 38.3 kg/m^2 vs. 36.8 kg/m^2, p=0.00007, adjusted for age, sex and family membership). Among 415 non-diabetic Pimas studied for quantitative traits, the obesity risk allele for rs17258746 was associated with higher percent body fat (p= 9.9 x 10^{-9}, adjusted for age, sex and family membership), higher fat mass (p=0.0001, adjusted for age, sex, % body fat and family membership), and higher waist and thigh circumferences (p=0.001, 0.0005, respectively, adjusted for age, sex, % body fat and family membership). Among 268 subjects with normal glucose tolerance, the risk allele of rs17258746 was further associated with lower acute insulin response to an intravenous glucose bolus infusion (p=0.005, adjusted for age, sex, % body fat, family membership and glucose disposal), as well as lower 30-min plasma insulin level during an oral glucose tolerance test (adjusted p=0.0002). These results suggest that PFKFB2 has roles in metabolic pathways that affect acute/early insulin secretion and BMI in humans.

2919/T

A genome-wide search for non-synonymous SNPs affecting rate of progression from proteinuria to end-stage renal disease (ESRD) in patients with type 1 diabetes (T1D). *J. Skupien, G.D. Poznik, A.S. Smiles, M.G. Pezzolesi, J.H. Warram, A.S. Krolewski.* Section on Genetics and Epidemiology, Joslin Diabetes Center, Boston, MA.

Objective: We carried out a genome-wide association analysis to identify non-synonymous genetic variants underlying susceptibility to ESRD in patients with T1D and proteinuria. As a precise indicator of the disease process underlying progression to ESRD, we used the rate of decline in renal function from an estimated glomerular filtration rate (eGFR) of 75 down to 30 ml/min/1.73 m^2, as we have previously demonstrated this decline to be linear. Methods: A cohort of 334 European-descent T1D patients presenting with proteinuria was genotyped with the 500K Affymetrix microarray. Imputation was performed with IMPUTE2 software using reference haplotypes from HapMap Phase 3 and 1,000 Genomes Project to yield data for a total of 16,934 non-synonymous SNPs. Serial measurements of serum creatinine were used to estimate eGFR using the MDRD formula. Subjects were followed for up to 18 years, during which time 153 developed ESRD. We used a random effects model to test the impact of each SNP on the average individual rate of GFR decline within the range of 75-30 ml/min/1.73 m^2. Results from this analysis were compared to Cox proportional hazards models both with and without adjustment for baseline eGFR. Results: We identified 32 non-synonymous SNPs that were significant at a $p<10^{-3}$ level. Only 2 SNPs were confirmed by Cox model at the same significance level. The SNPs meeting genome-wide significance criterion of $p<10^{-6}$ included rs2298624 on chromosome 18 in the *MYO5B* gene (myosin V B, $p=8.5\cdot10^{-8}$) and rs16844152 on chromosome 4 in the *RGS12* gene (regulator of G-protein signaling 12, $p=2.7\cdot10^{-7}$). Only the latter SNP was significant in our Cox model analysis ($p=3.2\cdot10^{-4}$). Survival analysis did not identify any SNPs with genome-wide significance. Conclusions: We analyzed non-synonymous SNPs with a statistical model that uses subject's rate of eGFR decline as a measure of the risk of progression to ESRD. We postulate that this analysis is the most appropriate for studying the genetics of ESRD risk. Time-to-event analysis suffers from biases caused by differing lead-in times before onset of renal function decline, as well as late follow-up entry. Adjusting for baseline eGFR may not properly correct for these. Despite intuitive equivalence between progression rate and time-to-event phenotypes, the results were not concordant. This interesting issue requires further careful research. Our findings will be tested in an independent case-control study.

2920/T

Identification of novel type 2 diabetes loci through systematic evaluation of linked regions 1q21-23 and 20q13 in North Indians. *R. Tabassum[1], A. Mahajan[1], O.P. Dwivedi[1], G. Chauhan[1], V. Sharma[1], S. Ghosh[2], N. Tandon[3], D. Bharawaj[1].* 1) Genomics and Molecular Medicine Unit, Institute of Genomics and Integrative Biology, CSIR, Delhi, India; 2) Human Genetics Unit, Indian Statistical Institute, Kolkata 700 108, India; 3) Department of Endocrinology, All India Institute of Medical Sciences, New Delhi 110 029, India.

The robust replication of 1q21-23 and 20q13 as strongly linked genomic regions in multiple ethnic groups warrants their systematic exploration for the discovery of type 2 diabetes susceptibility genes. Here, we performed gene-centric candidate region analysis to examine association of variants in putative regulatory regions of the genes on 1q21-23 and 20q13 with type 2 diabetes. We genotyped 207 SNPs in 2,115 Indo-European participants (1,073 cases and 1,042 controls) and replicated the associations detected in an independent sample set of 2,079 Indo-European subjects (1,041 cases and 1,038 controls), recruited from North India. Association of 12 SNPs with type 2 diabetes at nominal significance was observed with strongest association at variants in two genes [OR=1.31, P=1.4×10-3 and OR=1.25, P=1.1×10-3] Allele dosage analysis revealed 2.89 fold risk of type 2 diabetes in individuals carrying >10 risk alleles than those having <6 risk alleles [P= 1.4×10-9], with 1.21 times increase in risk with every increase in effective allele score [PTrend=1.5×10-10]. In the replication analysis, we confirmed the association of these variants with type 2 diabetes that was further corroborated through meta-analysis [OR=1.29, P=3.9×10-5 and OR=1.19, P= 1.8×10-4 respectively]. Moreover, we identified chromosomal segments on 1q21-23 and 20q13 influencing type 2 diabetes related traits. Variants of two genes were found to influence the measures of glucose homeostasis [β=2.39 mg/dl, P=3.3×10-4] and obesity (β= -1.13 kg/m2, P=3.4×10-5] respectively. Interestingly, all the four strongly associated genes have probable roles in inflammatory processes. In conclusion, we identified novel genes, both with probable role in inflammation as novel type 2 diabetes susceptibility genes and hence, further reinforced the contribution of inflammation in the development of type 2 diabetes.

2921/T

A large-scale candidate-gene association study of age at menarche and age at natural menopause. *C. He[1,2], P. Kraft[3,4], J.E. Buring[4,5,6], C. Chen[3,4], G. Paré[5,6,7], S.E. Hankinson[4,8], S. Chanock[9], P.M. Ridker[4,5,6,7], D.I. Chasman[5,6,7], D.J. Hunter[3,4,8,10].* 1) Department of Public Health, Indiana University School of Medicine, Indianapolis, IN; 2) Melvin and Bren Simon Cancer Center, Indiana University, Indianapolis, IN; 3) Program of Molecular and Genetic Epidemiology, Harvard School of Public Health, Boston,; 4) Department of Epidemiology, Harvard School of Public Health, Boston, MA; 5) Donald W. Reynolds Center for Cardiovascular Research, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; 6) Division of Preventive Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; 7) Center for Cardiovascular Disease Prevention, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; 8) Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA; 9) Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, MD; 10) Program in Medical and Population Genetics, Broad Institute of Harvard and MIT, Cambridge, MA.

Recent genome-wide association (GWA) studies have identified several novel genetic loci associated with age at menarche and age at natural menopause. However, the stringent significance threshold used in GWA studies potentially lead to false negatives and true associations may have been overlooked. Incorporating biologically relevant information, we examined whether common genetic polymorphisms in candidate genes of 9 groups of biologically plausible pathways and related phenotypes are associated with age at menarche and age at natural menopause. A total of 18,862 genotyped and imputed single nucleotide polymorphisms (SNPs) in 278 genes were assessed for their associations with these two traits among a total of 24,341 women from the Nurses' Health Study (NHS, N=2,287) and the Women's Genome Health Study (WGHS, N=22,054). Linear regression was used to assess the marginal association of each SNP with each phenotype. We adjusted for multiple testing within each gene to identify statistically significant SNP associations at the gene level. To evaluate the overall evidence for an excess of statistically significant gene associations over the proportion expected by chance, we applied a one-sample test of proportion to each group of candidate genes. The steroid-hormone metabolism and biosynthesis pathway was found significantly associated with both age at menarche and age at natural menopause (nominal p=0.040 and 0.011, respectively). Additionally, the group of genes associated with precocious or delayed puberty was found significantly associated with age at menarche (nominal p=0.013), and the group of genes involved in premature ovarian failure with age at menopause (nominal p=0.025). Our results suggest polymorphic variants in candidate genes of biologically-relevant pathways and related phenotypes are associated with age at menarche and age at natural menopause.

2922/T

Variability in heritability estimates as a confounding effect for association studies in nonsyndromic cleft lip and palate in Brazilian populations. *M.R.S. Passos-Bueno[1,2], L.A. Brito[1,2], L.A. Cruz[1,2], M. Aguena[1,2], L.B. Kobayashi[1,2], D.F. Bueno[1,2], D. Franco[4], A. Mendonça[5], N. Alonso[3], D. Bertola[3], P.A. Otto[1,2].* 1) Dept Gen & Evol Biol, Univ De Sao Paulo, Sao Paulo SP, Brazil; 2) Human Genome Research Center, University of São Paulo, São Paulo-SP, Brazil; 3) School of Medicine, University of São Paulo, São Paulo-SP, Brazil; 4) School of Medicine, Federal University Of Rio de Janeiro-RJ, Brazil; 5) School of Medicine, Federal University Of Rio de Alagoas-AL, Brazil.

Nonsyndromic cleft lip and palate (NSCLP) is a complex multifactorial disorder with average prevalence of 1:1000 live births, varying according to ethnicity, geographic origin and socioeconomic level. A wide range of heritability (30%-85%) for NSCLP has also been estimated in different populations. Several candidate genes have been emerged, but few have been replicated. These controversial results can be partly attributed to differences of the genetic contribution to NSCLP in each population, heterogeneity or its ethnic constitution. Considering the high level of ethnic admixture and the lack of heritability estimates in Brazilian population, we have evaluated these aspects in a large sample of NSCLP patients, from 1042 families ascertained in 5 regions of Brazil (Fortaleza-n=331; Barbalha-n=86, Santarem-n=121, Maceio-n=188, Rio de Janeiro-n=316). Analyzing 40 insertion-deletion ancestry informative markers, we observed that except for the patients from Santarém, the European (~70%), African (~20%) and Amerindian (~10%) contributions were similar among the studied groups of patients, which in turn did not differ from the local control population. In contrast, in Santarem, a larger contribution of Amerindians (42%) was identified and it is apparently higher compared to controls (~20%). Our heritability estimates, based on number of affected relatives of proband, varied among the 5 ascertained samples, with the highest value in Barbalha (85%). Santarém (71%), Rio de Janeiro (70%) and Fortaleza (64%) presented intermediate genetic contributions, whereas NSCLP in Maceio had the lowest heritability (45%). Rate of consanguinity among parents of the probands was significantly different among the 5 studied regions (p=0.001),with higher values in Fortaleza and Barbalha. These results suggest that the genetic contribution varies according to geographic region and inbreeding is one potential explanation for this finding. Ethnicity does not seem to have a major contribution for variability of the heritability estimates; as except for the sample of Santarem, the studied patients' groups were constituted with similar contribution of European, African and Ameridian ancestries. Our results suggest that the proportion of genetic factors contributing to the etiology of NSCLP might vary according to the studied population, a factor that can mask positive associations in case-control studies. Availability of this data in a population is important to interpret GWAS studies.

2923/T

Follow-up of regions linked to tuberculosis in a Ugandan genome scan. *C.M. Stein[1,2], A.R. Baker[1], S. Zalwango[4], R.P. Igo[1], L.L. Malone[2], M.D. Adams[2,3], H. Mayanja-Kizza[4], W.H. Boom[2].* 1) Dept of Epidemiology & Biostatistics, Case Western Reserve Univ, Cleveland, OH; 2) Tuberculosis Research Unit, Case Western Reserve Univ, Cleveland, OH; 3) Dept of Genetics, Case Western Reserve Univ, Cleveland, OH; 4) Uganda - CWRU Research Collaboration, Kampala, Uganda.

Tuberculosis (TB) is a significant public health problem globally, particularly in the face of the HIV epidemic. Several studies, including genome-wide linkage scans, have suggested a role for human genetics in susceptibility to developing TB. Previously, we conducted a genome-wide linkage scan for TB as part of our ongoing household contact study in Kampala, Uganda; in this study, we identified regions on chromosomes 7 and 20 linked to TB. We have followed up those regions by analyzing specific candidate genes - IL6 and CARD11 on chromosome 7, and CTSZ and MC3R on chromosome 20 - and fine mapping a 1-LOD drop region on chromosome 7p22-7p21. We analyzed 564 individuals from 243 households identified through an index case with culture-confirmed TB, and we genotyped 1536 SNPs on the Illumina platform. In addition to these genotyped SNPs, we also imputed SNPs using MaCH, using three African HapMap populations as our reference sample (Yoruba, Maasai, and Luhya). Though we did not observe association with any single SNPs in CTSZ or MC3R, we detected association between two CTSZ haplotypes and TB (p=0.047 and p=0.0964, respectively); this suggests that there may be an untyped risk variant in the region, or the sequence of SNPs may be associated with TB risk. This association with CTSZ replicates a finding in a South African population. We did not observe association with IL6 or CARD11. In addition, preliminary analysis of imputed markers suggests association with numerous SNPs, including the SDK1 gene (p-values between 0.0063 and 0.044), which has been associated with HIV nephropathy, and FOXK1 (p=0.031), which is associated with lung function; these results warrant confirmation with newly genotyped markers.

2924/T

Domain-dependent clustering and phenotype association of LGI1 gene mutations in autosomal dominant partial epilepsy with auditory features (ADPEAF). *Y. Ho[1,2,3], I. Ionita-Laza[1], R. Ottman[4,5].* 1) Division of Statistical Genetics; 2) Department of Biostatistics; 3) Department of Psychiatry; 4) Department of Epidemiology; 5) G.H. Sergievsky Center, Columbia University, New York, NY 10032, USA.

Introduction: The leucine-rich, glioma inactivated 1 gene (LGI1) encodes a secretory protein with two major functional domains: the N-terminal leucine-rich repeats (LRR) and the C-terminal epitempin repeats (EPTP). Mutations in LGI1 cause autosomal dominant partial epilepsy with auditory symptoms (ADPEAF) (OMIM600512), a focal epilepsy syndrome with auditory symptoms as prominent ictal manifestations, with an estimated penetrance of 67%. Hypotheses: 1. The distribution of ADPEAF-causing mutations is not uniform within the LGI1 gene. 2. Phenotypic features in affected individuals differ depending on the coding domain in which mutations are found. Materials and Methods: Clustering of mutations within the gene was analyzed for all 30 previously reported ADPEAF-causing mutations using a sliding window approach. Phenotypic and genetic information were analyzed on 52 patients with idiopathic focal unprovoked seizures from 11 ADPEAF families with LGI1 mutations. Bivariate analysis was performed using general estimating equations to test for association between mutation site and auditory symptoms. 95% confidence intervals (CI) for the odds ratios (OR) were calculated by the logit method. Results: ADPEAF-causing mutations clustered significantly in the LRR domain (exons 3-5) of LGI1 ($p = 0.001$). The coding domain in which mutations were located was not associated with disease penetrance. Auditory symptoms were present in 91% of patients with mutations in the LRR domain and 73% of patients with mutations in the EPTP domain (OR=3.7; 95% CI=1.1-12.5, $p = 0.038$). Conclusion: ADPEAF-causing mutations cluster significantly within the LRR region of the LGI1 gene, and mutations in this region are also associated with an increased likelihood of manifesting auditory symptoms in ADPEAF. Acknowledgement: This project is supported by grants no. T32-MH065213 and R01 NS036319.

2925/T

Large-scale meta-analysis identifies more than 40 ulcerative colitis risk loci. *C.A. Anderson[1], G. Boucher[2], R.H. Duerr[3], C.W. Lees[4], G. Radford-Smith[5], J.D. Rioux[2], S. Vermeire[6], R.K. Weersma[7], The International IBD Genetics Consortium.* 1) Statistical Genetics, Wellcome Trust Sanger Institute, Hinxton, United Kingdom; 2) Department of Medicine, Université de Montréal and Montreal Heart Institute, Research Center, Montreal, Québec, Canada; 3) University of Pittsburgh, School of Medicine, Department of Medicine, Division of Gastroenterology, Hepatology and Nutrition, University of Pittsburgh Medical Center, Presbyterian, Pittsburgh, USA; 4) Gastrointestinal Unit, Molecular Medicine Centre, University of Edinburgh, Western General Hospital, Edinburgh, UK; 5) Inflammatory Bowel Diseases Laboratory, Royal Brisbane and Women's Research Foundation, Brisbane, Queensland, Australia; 6) Department of Nephrology, University Hospital Gasthuisberg, Leuven, Belgium; 7) Department of Gastroenterology and Hepatology, University Medical Centre Groningen, University of Groningen, Groningen, The Netherlands.

Several genome-wide association studies (GWAS) of ulcerative colitis (UC), a common form of inflammatory bowel disease (IBD), have been published to date. These studies identified 18 UC susceptibility loci, and highlighted the importance of the epithelial barrier, cell-specific innate response, adaptive immunity and resolution of inflammation in disease pathogenesis. Interestingly, 8 of these loci were previously associated with Crohn's disease, the other common form of IBD, indicating that T-cell differentiation and the innate immune response are broadly involved in IBD pathogenesis. In the current study, data from 6,433 patients with UC and 20,099 population controls, collected from 6 GWAS of North American and European samples, were combined across 1.2 million SNPs using genotype imputation. In total, 75 independent genomic regions were associated at $P<1\times10^{-5}$, including all previously confirmed UC associations. We selected 52 potentially novel UC risk loci for follow-up in an additional set of around 10,000 UC cases and 10,000 population controls of European descent. Of these, 24 loci had a $P<0.05$ in the replication study and $P<5\times10^{-8}$ in the combined analysis and thus represent novel UC loci. Interestingly, 10 of these loci have previously been associated with CD (including *IL12B*, *PTGER4*, *CARD9*, *STAT3* and *JAK2*). Loci associated with other autoimmune diseases (such as multiple sclerosis, celiac disease and systemic lupus erythematosus) were also identified (e.g. *IL7R* and *ETS1*). The association with *ETS1*, which is expressed in all Th subsets and is required for optimal production of inflammatory cytokines (and the suppression of IL10), further implicates the Th17 pathway in disease pathogenesis. The central role of the epithelial barrier in disease risk is further highlighted by the novel, UC specific, association with *GNA12*, which regulates protein interactions within (and inhibits the assembly of) tight junctions. In an expression quantitative trait meta-analysis of 1,469 whole blood samples, 17 of 42 UC associated loci were correlated ($P<0.0028$, FDR 5%) with cis gene expression. In aggregate, known UC risk loci now account for around 10% of phenotypic variance in disease risk. This work has greatly increased the number of known UC loci and has significantly increased our understanding of disease pathogenesis relating to both shared and UC-specific inflammatory pathways.

2926/T

Estimating genetic effects under multistage sampling design of family data. *Y. Choi[1], L. Briollais[2].* 1) Epidemiology and Biostatistics, Univ Western Ontario, London, ON, Canada; 2) Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada.

In family-based genetic studies, multistage sampling permits the allocation of resources to families that are most informative for a given objective while allowing population-based inference. The objective of this study is to provide population-based estimates of the relative risk of both major gene and unknown gene mutations and the age-specific cumulative risk of a disease associated with a mutated gene based on the family data collected from a multistage sampling design. A major problem in this study design is to deal with partially or completely missing genetic information among family members. To analyze multistage family designs, we propose a composite likelihood approach and further extend it to account for missing genetic information using an Expectation-Maximization (EM) algorithm. We infer the missing genetic information by its conditional expectation given their phenotype information and known mutation statuses of the other family members. Our approach is developed for time-to-event data to handle missing genetic covariates by using an EM algorithm. We also investigate the role of an unknown major genetic effect that could segregate within families. Assuming the presence of a second gene associated with disease but its genotypes are unknown i.e., all missing, the EM algorithm is used to infer the unknown genetic effect besides the segregation of major gene muations. Our simulation studies have demonstrated the good properties of our approach in terms of consistency and efficiency of the genetic relative risk estimate under different multistage sampling designs in the presence of missing genotypes. In addition, an application to a family study of early-onset breast cancer demonstrates the interest of our approach. The family data are collected from several population-based breast cancer family registries (Ontario, California and Australia), as a part of the NCI-funded Cancer Families Registries initiatives based on a two-stage sampling design. It confirms the important effects of the genes BRCA1 and BRCA2 in these families, while it shows that a wrong inference can be made about this effect if the sampling design is not properly taken into account.

2927/T

A critical assessment of *CIITA* variation and risk for SLE, lupus nephritis and other related clinical outcomes.

P.G. Bronson[1], B.A. Goldstein[2], S.L. May[1], P.P. Ramsay[1], K.B. Beckman[3,4], J.A. Noble[4], J.A. Lane[4], M.F. Seldin[5], J.A. Kelly[6], J.B. Harley[6], K.L. Moser[6], P.M. Gaffney[6], T.W. Behrens[7], L.A. Criswell[8], L.F. Barcellos[1]. 1) Division of Epidemiology, School of Public Health, University of California, Berkeley, CA; 2) Division of Biostatistics, School of Public Health, University of California, Berkeley, CA; 3) Biomedical Genomics Center, University of Minnesota, Minneapolis, MN; 4) Children's Hospital Oakland Research Institute, Oakland, CA; 5) Department of Biochemistry and Molecular Medicine, School of Medicine, University of California, Davis, CA; 6) Oklahoma Medical Research Foundation, Oklahoma City, OK; 7) Immunology Diagnostics and Biomarkers, Genentech, South San Francisco, CA; 8) Rosalind Russell Medical Research Center for Arthritis, Department of Medicine, University of California, San Francisco, CA).

The major histocompatibility complex (MHC) class II transactivator gene (*CIITA*) is an important transcription factor regulating gene required for human leukocyte antigen (HLA) class II MHC-restricted antigen presentation. Association with HLA class II variation, particularly *DRB1*1501* and **0301*, has been well-established for systemic lupus erythematosus (SLE). However, MHC genes only account for a portion of the genetic risk. Several non-MHC genes have recently been associated with risk for SLE, including *PTPN22*, *STAT4* and *TNFAIP3*. In addition, studies suggest that genes regulating initiation of the most serious SLE complication, lupus nephritis (LN), may differ from genes involved in SLE risk. Thus, we investigated *CIITA* variation, *DRB1*1501*, **0301* and important primary and secondary outcomes in SLE. We tested 18 *CIITA* SNPs in 637 SLE trio families and 826 unrelated SLE cases (n=2,737) of European ancestry. Family-based association tests were conducted to compare transmitted vs. nontransmitted alleles. Case-control, case-only and novel genic association tests were conducted in the combined trios and unrelated cases, utilizing nontransmitted parental alleles as controls. Analyses were stratified by *DRB1*1501*, **0301*, LN, arthritis, serositis, neurological involvement, Sm and Ro autoantibody production in cases. No evidence for association was observed between *CIITA* and SLE in family-based and case-control analyses after correcting for multiple testing. Modest evidence for association between the intronic rs11074938**G* variant and the presence of either *DRB1*1501* or **0301* (OR= 1.27, 95% CI=1.11-1.47, *P*=2x10-3), and the UTR-3' rs45617532**C* variant and neurological involvement (OR=4.07, 95% CI=1.83-9.06, *P*=1.2x10-3) were observed in case-only analyses. The genic test did not reveal evidence for association. This is the first comprehensive and well-powered study of *CIITA* and SLE, including examination of clinical phenotypes and influence of known HLA risk alleles. Recent studies have reported evidence for association between *CIITA* and multiple sclerosis, ulcerative colitis and celiac disease. Although the majority of common genetic variation (MAF $\geq$ 0.01) in *CIITA* and its promoter region was fully captured in the current study, results do not provide evidence to support a role for this locus in SLE.

2928/T

Genetic Data Suggest a Novel Association Between the Vitamin D Pathway and Development of Age-related Macular Degeneration.

M. DeAngelis[1], M.A. Morrison[1], A.C. Silveira[1], S.M. Adams[1], M.G. Kotoula[2], E.E. Tsironi[2], F. Zacharaki[2], I.K. Kim[3], J.W. Miller[3], N.B. Haider[4], A. Jelcick[4], B.W. Hollis[5], R. Chen[6], G.S. Hageman[7], D.A. Schaumberg[8]. 1) Ocular Molecular Genetics Institute, Harvard Medical School, Massachusetts Eye and Ear, Boston, MA; 2) Department of Ophthalmology, University of Thessaly School of Medicine, Larissa, Greece; 3) Retina Service and Ophthalmology, Harvard Medical School, Massachusetts Eye and Ear, Boston, MA; 4) Department of Genetics, Cell Biology, and Anatomy, University of Nebraska Medical Center, Omaha, NE; 5) Medical University of South Carolina, Charleston, SC; 6) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 7) Ophthalmology and Visual Sciences, John A. Moran Eye Center, University of Utah, Salt Lake City, UT; 8) Division of Preventive Medicine, Brigham and Women's Hospital, Boston, MA.

Data from our laboratory suggest a protective role for lifetime UV exposure in susceptibility to age-related macular degeneration (AMD). Thus, we evaluated the genetic contribution of variation in vitamin D pathway genes and serum vitamin D levels to AMD. Using 135 extremely phenotypically discordant sibling pairs, we evaluated 59 SNPs within the vitamin D pathway genes VDR, CYP27B1, CYP24A1, and CYP27A1. Additionally, 50 pairs from this cohort were ascertained for serum vitamin D levels. Single SNPs, haplotypes, and gene-gene interaction were tested for association using the model for sibships in UNPHASED. Initial findings were then evaluated in an extended family cohort, an unrelated case control cohort from central Greece, and a prospective based cohort derived from the Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS), which included patients with various types of AMD (2,528 individuals). Sera analyses showed a slight trend of higher vitamin D levels in unaffected siblings. After controlling for covariates, only variation in CYP24A1 influenced AMD risk. Specifically, rs2181874 was modestly associated with decreased risk of all types of AMD in both the extended sibpair cohort and the Greeks (p= .04 for both). In Greek and NHS/HPFS cohorts, this SNP was significant as part of a haplotype with rs1570669 (overall p = .007 and .009 respectively). Meta-analysis showed that rs1570669, rs1570670, rs2274130, and rs2296239 were more significant for risk of all AMD subtypes in meta-analysis than in any one cohort alone ($p < .01$ for all). While the VDR SNP rs2189480 was significant in the extended family based cohort, it was not significantly associated with AMD in the other cohorts. However, significant gene-gene interaction was shown between CYP24A1 and VDR in all cohorts examined ($p < .05$). Haplotypes and SNPs in CYP24A1 were demonstrated to influence AMD risk after controlling for smoking, sex, and age in all populations both separately and in meta-analysis. This is the first report demonstrating a genetic association between vitamin D metabolism and AMD risk. These data not only extend previous epidemiological and biological studies in AMD, but further relate to common antecedents among several diseases with an inflammatory/immunogenic component including cardiovascular disease and AMD and cancer and AMD.

2929/T

Platelet endothelial cell adhesion molecule-1 gene polymorphisms are associated with coronary artery lesions in chronic stage of Kawasaki Disease.

L.P. Ger[1, 2], S.C. Huang[1, 2], W.H. Lu[3, 4], K.P. Weng[3], T.J. Lai[1, 4], K.S. Hsieh[3]. 1) Department of Medical Education and Research, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan; 2) Institute of Biomedical Sciences, National SunYat-Sen University, Kaohsiung, Taiwan; 3) Department of Paediatrics, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan; 4) Institute of Clinical Medicine, National Yang-Ming University, Taipei, Taiwan.

Kawasaki disease (KD) is the most common cause of pediatric acquired heart disease. Platelet endothelial cell adhesion molecule-1 (PECAM-1) plays an important role in platelet activation modulation, which may be associated with coronary artery lesions (CALs) of KD. We tested the hypothesis that three common single nucleotide polymorphisms (SNPs), Leu125Val, Ser563Asn, and Arg670Gly, of PECAM-1 gene may be associated with coronary artery lesions (CALs) of KD in the acute and chronic stage. The genotyping of SNPs in PECAM-1 using the TaqMan assay was conducted in a case-control study of 299 KD patients with or without CALs. We found that no association between genotypic and allelic types of these three SNPs and CALs of KD. As compared with non-Leu-Ser-Arg haplotype, Leu-Ser-Arg haplotype was with a significantly increased risk for CALs in chronic stage (AOR 2.42, 95% CI 1.02-5.75, P=0.045), but not for CALs in acute stage. Analyses based on the diplotypes of PECAM-1 also showed that KD patients with one or two alleles of Leu-Ser-Arg had a significantly increased risk of CALs in chronic stage (AOR 2.85, 95% CI 1.12-7.30, P=0.029) and increased counts of platelet (×1000/Cumm) (672.6±207.6 VS. 563.1±196.8; P=0.027), as compared to those with other diplotypes. In conclusion, haplotype and diplotype of PECAM-1 might be associated with the increased counts of platelet and the following risk of chronic CALs, which needs further validation in other independent cohort.

2930/T

Replication and generalization of genetic risk factors for depression, anxiety and panic attack in European, African, and Mexican Americans from the National Health and Nutrition Examination Surveys. *K. Glenn, K. Gentry-Brown, M. Allen, P. Mayo, N. Schnetz-Boutaud, D.C. Crawford, T.A. Thornton-Wells.* Ctr Hum Genet Res, Vanderbilt Univ, Nashville, TN.

Anxiety and mood disorders are two of the most common psychiatric disorders, with lifetime prevalence estimates above 20% and heritability estimates of 35-50%, despite clear environmental stressors and risk factors. Many functional candidate genes have been implicated in anxiety, stress and mood; however, investigation within non-European populations is incomplete. As part of the Epidemiologic Architecture for Genes Linked to Environment (EAGLE), we conducted an association study of previously-reported genetic risk variants for anxiety or depression in the ethnically diverse population-based cohort, the National Health and Nutrition Examination Survey (NHANES) ascertained and maintained by the Centers for Disease Control and Prevention (CDC). For this study, we accessed NHANES 1999-2002, which includes psychological survey data for 596 non-Hispanic whites (NHWs), 231 non-Hispanic blacks (NHBs), and 354 Mexican Americans (MAs). We selected 35 SNPs in 23 candidate genes previously implicated in anxiety, mood or personality disorders or the hypothalamic-pituitary-adrenal (HPA) axis. We performed genotyping using the Sequenom and Taqman OpenArray platforms and tested for association using logistic regression with an additive allelic model. The G allele of rs6269 in COMT was associated with decreased anxiety in MAs (OR=0.447, CI=[0.262 - 0.763], p=0.0031), consistent with previous reports that the G allele is associated with higher COMT activity and lower pain sensitivity. The T allele of rs6999100 in TRIM55, downstream of CRH, was associated with decreased rates of anxiety in non-Hispanic whites (OR=0.633, CI=[0.426 - 0.941], p= 0.0236), consistent with a previous report that the T allele is under-transmitted to individuals with behavioral inhibition to the unfamiliar, with is an intermediate phenotype and risk factor for panic disorder and social phobia. The G allele of rs4570625 in the upstream regulatory region of TPH was associated with decreased rates of depression in non-Hispanic blacks (OR = 0.453, CI = [0.234 - 0.876], p = 0.0187), consistent with previous reports that the T allele is associated with amygdala responsivity, panic disorder, anxiety-related disorders and affective disorders. Despite being underpowered, we were able to replicate and characterize genetic associations for anxiety, depression and panic attack. Larger, diverse studies are required to characterize other associations with small genetic effect sizes.

2931/T

Life-span influences of Apolipoprotein E and association with longevity in a large sample. *G.J. McKay, AMD APOE Consortia.* Centre for Public Health, Queen's University Belfast, Belfast, United Kingdom.

The human Apolipoprotein E (*APOE*, MIM 107741) locus located on chromosome 19q13.2 is central to the metabolism of low-density lipoprotein cholesterol and triglycerides and has been associated with increased risk of a variety of complex and age-related disorders. Several reports on human longevity have shown that *APOE E4* allele frequency is lower in older age groups such as octogenarians, nonagenarians and centenarians than younger or middle-aged subjects and that absence of *E4* seems to be a favorable survival factor. In populations of Caucasian descent, an elevated mortality risk has been reported with the *E3/E4* genotype relative to the *E3/E3* genotype with a slightly decreased risk associated with *E2/E3* genotypes. Previously increased frequency of *E2* with advancing age in males only was reported, although follow-up studies have shown inconsistent findings, so whether this effect is specific to males or females is as yet unclear.

This study sought to assess the frequency of *APOE* isoforms in pooled samples from investigations in Caucasian populations. The initial aim was to study the influence of this gene on Age-related Macular Degeneration (AMD) but in this report we examine allele frequency as a function of age and gender in individuals free from the severe form of the disease.

We assessed allelic distribution of *APOE* isoforms, *E2*, *E3* and *E4* in 17,292 non-AMD Caucasian samples from 15 study populations. Following adjustment for center ascertainment, our data demonstrates a significant decreasing frequency of the *E4* isoform from 16.3% to 10.7% between ages of <60 and >85 years (Chi^2= 20.6; *df*=6; P=0.002) with the *E3* isoform increasing from 75.1% to 81.9% as a consequence (Chi^2=17.9; *df*=6; P= 0.006). Assessment for gender bias was found not to be significant following adjustment for center. The age-related effect observed was most prominent in individuals homozygous for the *E4* isoform (ca. 1.84% of sample), the frequency of which decreased by 73% between the ages of 60 and 85 years while the proportion with the *E3/E4* haplotype decreased by 28%. Individuals heterozygous for the *E2/E4* haplotype showed no significant change in frequency within this age range. This study supports the role of *APOE* isoforms in human longevity.

2932/T

Association of SNPs of the IGF Axis with developmental growth and blood pressure. *P. Parmar[1,2], J.A Marsh[1], N.M. Warrington[2], R. Taal[3], S. Louise[1], J.P. Newnham[2], V.W.V. Jaddoe[3], G.D. Smith[4], L. Briollais[5], S.J. Lye[5,6], L.J. Beilin[7], L.J. Palmer[1], C.E. Pennell[2].* 1) Centre for Genetic Epidemiology and Biostatistics, The University of Western Australia, Perth, Australia; 2) School of Women's and Infants' Health, The University of Western Australia, Perth, Australia; 3) Department of Epidemiology, Erasmus Medical Centre, Rotterdam, the Netherlands; 4) Department of Social Medicine, University of Bristol, Bristol, United Kingdom; 5) Samuel Lunenfeld Research Institute, Mount Sinai Hospital, University of Toronto, Canada; 6) Canadian Institutes of Health Research, Canada; 7) School of Medicine and Pharmacology, Royal Perth Hospital Unit, The University of Western Australia, Perth, Australia.

Purpose: The aim of this study was to investigate the role of polymorphisms in genes within the IGF axis in the relationship between fetal growth trajectories and blood pressure from 1-year to late adolescence. **Methods:** Participants in The Western Australian Pregnancy Cohort (Raine) Study were recruited at 16-18 weeks gestation and followed throughout childhood. Analyses focused on 1,162 singleton-birth Caucasians with complete data in antenatal life and childhood. Genes in the IGF axis (including IGF-1, IGF-2, IGF-receptor-1, IGF-receptor-2 and IGF-binding proteins 1-5) were tagged with 145 single nucleotide polymorphisms (SNPs). Associations were investigated between these SNPs and fetal growth trajectories, anthropometric measures at birth and both BMI and blood pressure trajectories throughout childhood. All analyses were stratified by sex. Replication analyses are currently underway and will be presented for GenR (The Generation R Study) and ALSPAC (Avon Longitudinal Study of Parents and Children) for antenatal and postnatal findings respectively. **Results:** All effects described here were significant to p<0.05 ***Males:*** RS4966015 (IGF1R) was found to be significantly associated with increased head circumference (HC), reduced ponderal index (PI) and increased average SBP throughout childhood. Conversely RS8041224 (IGF1R) was associated with reduced antenatal growth (abdominal circumference (AC) and HC) and reduced SBP. ***Females:*** Both RS3213221 (IGF2) and RS2670504 (IGF1R) were associated with increased fetal growth (AC and PI; AC respectively). During postnatal life RS3213221 (IGF2) was associated with reduced SBP whereas RS2670504 (IGF1R) was associated with increased BMI. Conversely RS1003737 (IGF2R) was associated with reduced birth weight and PI; increased weight change over the first year of life and increased SBP in childhood. **Conclusion:** Multiple SNPs in genes within the IGF pathways are associated with fetal growth trajectories and adolescent SBP trajectories. These data provide evidence to suggest that the relationship between antenatal growth and postnatal blood pressure measures is in part mediated by genetic variation. Patterns of associations were different in males and females and require detailed evaluation and replication in other pregnancy cohorts. These data support the concept that complex gene-environment interactions underlie the developmental origins of hypertension.

2933/T

Genome-wide Association Studies (GWAS) of Uric Acid in African Americans: the CARe Renal Consortium. *A. Tin[1], W.H. Kao[1], C.T. Liu[2], X. Lu[2], E.L. Akylbekova[3], S. Wyatt[4,5], S.J. Hwang[6], Q. Yang[7], M.G. Shlipak[8], M. Reilly[9], D.S. Siscovick[10], I.H. de Boer[11], M.F. Flessner[5], L.A. Cupples[2], C.S. Fox[6], A. Kottgen[1,12] on behalf of the CARe Renal Consortium.* 1) Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205; 2) Department of Biostatistics, Boston University School of Public Health, 801 Massachusetts Ave, CT3, Boston MA 02117; 3) Jackson State University, 350 West Woodrow Wilson Drive, Suite 701, Jackson, MS 39213; 4) School of Nursing, University of Mississippi Medical Center, 2500 North State Street, Jackson, MS 39216-4505; 5) Department of Medicine, University of Mississippi Medical Center, 2500 North State Street, Jackson, MS 39216-4505; 6) NHLBI's Framingham Heart Study and the Center for Population Studies, 73 Mt Wayte Ave Suite #2, Framingham MA 01702; 7) Department of Biostatistics, Boston University School of Public Health, 715 Albany Street, Boston, MA 02118; 8) General Internal Medicine, University of California, San Francisco, 4150 Clement St. , San Francisco, CA 94121; 9) University of Pennsylvania Division of Cardiology, Perelman Center for Advanced Medicine, East Pavilion, 2nd Floor; 3400 Civic Center Boulevard, Philadelphia PA 19104; 10) Departments of Epidemiology and Medicine, University of Washington, Seattle, WA, USA, 1730 Minor Ave, Suite 1360, Seattle, WA, 98101; 11) Departments of Medicine and Epidemiology, University of Washington, Box 357183, 1959 NE Pacific St, Seattle, WA 98195; 12) Renal Division, University Hospital of Freiburg, Freiburg, Germany.

Few genome-wide association studies (GWAS) have been conducted in populations of non-European ancestry, where differences in linkage disequilibrium (LD) and potential allelic heterogeneity exist. We conducted a genome-wide association analysis of serum uric acid levels of 5820 Africans Americans (AA) from the Atherosclerosis Risk in Communities, Coronary Artery Risk Development in Young Adults, and Jackson Heart Study, as part of the CARe Consortium, to 1) identify potentially novel loci in African Americans and 2) validate lead SNPs and interrogate regions around each of the loci that were previously reported in studies of European ancestry (EA). A meta-analysis was conducted. For the interrogation of previously discovered loci, alpha level was set at 0.05 for the lead EA SNP and corrected for the number of independent SNPs at the locus for the interrogation of each region. In the GWAS of serum uric acid, we identified SNPs at three loci achieving genome-wide significance: a novel locus on chromosome 6 (rs9321453, p=1.4E-8) and two loci previously identified in EA (SLC2A9 and SLC22A12). Of the previously identified loci among individuals of EA (PDZK1, GCKR, SLC2A9, ABCG2, LRRC16, SLC17A1/ SLC17A3, SLC16A9, SLC22A11, and SLC22A12), 8 of 9 beta coefficients of the lead EA SNP showed direction-consistent association for the same modeled allele in AA. Further, four lead EA SNPs at three distinct loci were statistically significant in our AA cohorts (rs12129861 at PDZK1, p=8.7E-5; rs734553 and rs6449213 at SLC2A9, p=2.7E-24 and 2.8E-14; rs505802 at SLC22A12, p=5.2E-6). Next, we interrogated 50 kb flanking regions around the remaining six EA loci and identified the SNP with the lowest pvalue within each region. This resulted in three loci that contained SNPs with Bonferroni-corrected p<0.05 (rs2869736 near ABCG2, p=1.1E-3; rs9467527 at LRRC16, p=2.7E-4; and rs12294207 at SLC22A11, p=4.5E-5). Finally, two independent SNPs (r2 of 0.16 in AA) in SLC2A9 accounted for 2.4% and 2.1% (combined 3.2%) of the variance in uric acid. In summary, we have identified one novel locus in association with serum uric acid in AA and observed similar genetic associations among African Americans for six loci previously reported in studies of EA. Our data support the importance of multi-ethnic GWAS in fine-mapping and the identification of new loci and potential allelic heterogeneity at known loci. With respect to uric acid, the genetic architecture appears similar in EA and AA.

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Validating Risk Prediction Models using Family Registries. *W. Wang[1,2], AP. Klein[3], B. Caffo[4], G. Parmigiani[5].* 1) Stanford Genome Tech Ctr, Stanford Univ, Palo Alto, CA; 2) UC Berkeley Dept of Statistics, Berkeley, CA; 3) Johns Hopkins Univ School of Medicine, Dept of Oncology and Pathology, Baltimore, MD; 4) Johns Hopkins Bloomberg School of Public Health, Dept of Biostatistics; 5) Harvard School of Public Health, Dept of Biostatistics, Boston, MA.

We report a novel design and analysis framework for utilizing family data to validate personalized risk assessment models. It is motivated by applications to genetic diseases, and illustrated in the context of pancreatic cancer risk prediction. It has long been recognized that individuals with a family history of cancer are at higher risk of developing the disease in future. Risk prediction models for cancer are important for guiding decision about prevention. A subset of risk prediction models focus on evaluating the risk of developing cancers for which a known genetic effect or familial segregation exist, but specific genes remain unknown. Validation in independent data is an essential component for the selection, improvement and application of these models. Having realized the importance of family history for diseases such as cancer, researchers have established family registries. We describe a design strategy for defining a prospective cohort of individuals from an existing familial cancer registry, and performing the associated validation analysis. Complicating matters is the fact that it is important to consider multiple prospectively followed individuals in one family. In order to account for the genetic correlation among individuals from the same family, we propose to consider the joint risks for correlated individuals, based on the risk assessment model of interest, and use these to calculate evaluation statistics including the observed versus expected ratio, the receiver operating characteristic curve, the concordance index and the Brier's score, both at the family level and the individual level. We implement this strategy by comparing two different Markov chain Monte Carlo methods and choose the method that is much more computationally efficient. We illustrate our methods using PancPRO, a Mendelian risk prediction model of pancreatic cancer, based on data from the National Familial Pancreatic Tumor Registry.

2935/T

Cumulative effect of lipids-related variants on hypercholesterolemia in the adult U.S. population: the Third National Health and Nutrition Examination Survey (NHANES III). *A. Yesupriya, M. Chang, R.M. Ned, Q. Yang, N.F. Dowling.* National Office of Public Health Genomics, Centers for Disease Control and Prevention, Atlanta, GA.

Genome-wide association studies (GWAS) have identified a number of SNPs associated with serum lipids level. The contributions of these SNPs on hypercholesterolemia are largely unclear for the U.S. adult population. This study evaluated the individual and joint SNP effects on prevalent hypercholesterolemia [total cholesterol ≥ 5.2 mmol/L (200 mg/dL) or use of cholesterol-lowering medications] in U.S. adults using a large population-based study. We examined associations between prevalent hypercholesterolemia and 22 genetic loci previously found to be associated with lipid levels using data from the second phase (1991-1994) of the Third National Health and Nutrition Examination Survey (NHANES III). Individuals aged 17 years and older that self-reported as non-Hispanic white (NHW, n=2323), non-Hispanic blacks (NHB, n=1742), or Mexican Americans (MA, n=1765) were included in the analysis. Within each race/ethnic group, we used logistic regression to examine single SNP associations, as well as the cumulative effect of multiple SNPs (using a genotype score) on risk of prevalent hypercholesterolemia. These analyses assumed an additive genetic model and were adjusted for a set of conventional risk factors. Allele frequencies for all SNPs varied significantly by race/ethnicity (p<0.02). APOE (rs7412 and rs429358) was significantly associated (p<0.05) with hypercholesterolemia in all three race-ethnic groups. An additional nine GWAS-validated SNPs were significantly associated with hypercholesterolemia in at least one race/ethnic group. A 22-SNP genetic score was associated [OR=1.17, 1.14, and 1.13 in NHW, NHB, and MA, respectively. p< 0.0001 in all three race/ethnic groups) with the prevalence of hypercholesterolemia in all three ethnic groups. The area under the curve (AUC) increased 2-3% when comparing models with and without the genetic score in addition to the set of conventional risk factors. Roughly 10% to 15% of the total variation in hypercholesterolemia could be explained by the genetic variants and conventional risk factors. We conclude that the prevalence of these lipids-associated variants differs significantly across the three U.S. race/ethnic groups. However, the distribution of genetic risk score was similar across the race/ethnicities. The combined genetic variants are associated with the prevalence of hypercholesterolemia.

2936/T

Estimating the known-unknowns from known-knowns in the search for missing heritability. *N. Chatterjee, J.-H. Park.* Division of Cancer Epidemiology and Genetics, NCI, Rockville, MD.

Recent genome-wide association (GWAS) studies have discovered a large number of susceptibility loci for many complex traits, yet for any given trait, much of its heritability remains unexplained. We have rencently developed a set of tools to estimate the number of undiscovered susceptibility loci and the distribution of their effect sizes for a trait based on discoveries from existing studies and then to project statistical power and risk prediction utility for future studies integrating over estimated distributions of effect sizes (Park et al., Nature Genetics, In press). In this report, we will use reported GWAS findings for more than 10 complex traits to illustrate the utility of our methodology in planning future GWAS of common variants. We will present data on validation of these methods and related projections using results from GIANT consortium that have used meta-analysis of more than 100,000 subjects to detect a large number of common susceptibility loci for a number of anthropometric traits such as height and BMI. In addition, we will investigate an approach to association scan for future studies incorporating the estimated effect size distribution as prior for Bayesian analysis.

2937/T

Analysis of Multiple Phenotype Data in Genome-Wide Association Studies. *G. Clarke, A.P. Morris.* Wellcome Trust Centre for Human Genetics, University of Oxford, Roosevelt Drive, Oxford, OX3 7BN, UK.

Traditionally, statistical methods for genome wide association studies have mainly concentrated on univariate trait analyses for association mapping of quantitative trait loci (QTL). However, correlated and uncorrelated phenotypes that may or may not share common genetic or environmental factors are often available. Here we show how the power to detect association between a causal genetic locus and a given phenotype of interest can be affected by the inclusion of additional correlated or uncorrelated phenotypes as covariates in simple multivariate regression analyses. We denote two phenotypes as *dissimilar* if their expected values are not proportional. Using simulations we show that if a phenotype is influenced by a QTL, then, in comparison to separate univariate analyses, the combined analysis of that phenotype and any correlated dissimilar phenotype will increase the power to detect the QTL. This boost in power occurs even if the additional phenotype has a null association with the QTL. If the additional phenotype is uncorrelated, then it is only a useful additional covariate if it has a non-null association with the QTL. Results indicate that the multivariate analysis of correlated traits is a powerful screening tool for the preliminary identification of QTLs. Further steps would then be required to make precise estimates of effect sizes.

2938/T

Genome-wide association identifies more than ten novel primary biliary cirrhosis risk loci. *J.A.B. Floyd[1], G. Mells[2], K.I. Morley[1], H.J. Cordell[3], P. Donaldson[4], M. Heneghan[5], D. Jones[4], J. Neuberger[6], G. Alexander[7], R. Sandford[2], C.A. Anderson[1], The UK PBC Genetics Consortium, The Wellcome Trust Case Control Consortium 3.* 1) Wellcome Trust Sanger Institute, Cambridge, United Kingdom; 2) Department of Medical Genetics, Cambridge Institute of Medical Research, Addenbrooke's Hospital, Cambridge, United Kingdom; 3) Institute of Human Genetics, Newcastle University, International Centre for Life, Newcastle-upon-Tyne, United Kingdom; 4) Institute of Cellular Medicine, Medical School, Newcastle University, Newcastle upon Tyne, United Kingdom; 5) Institute of Liver Studies, Kings' College Hospital, London, United Kingdom; 6) Liver Unit, Queen Elizabeth Hospital, Birmingham, United Kingdom; 7) Department of Hepatology, Addenbrooke's Hospital, Cambridge, United Kingdom.

Primary biliary cirrhosis (PBC) is a chronic, cholestatic liver disease characterized by progressive, autoimmune (AI) destruction of the small intrahepatic bile ducts. PBC primarily affects women ≥40 years of age and in this high-risk cohort the prevalence rate is approximately one in 1,000. The cause of PBC is unknown. Approximately 5% of PBC patients have a positive family history and the sibling relative risk (λs) is ~10. Over the last 20 years, a succession of candidate gene association studies has established that PBC is associated with the class II HLA haplotype, DRB1*0801-DQA1*0401-DQB1*0402, in Europeans. More recently, a genome-wide association study (GWAS) of PBC identified two additional, non-HLA susceptibility loci, *IL12A* and *IL12RB2*. Here we report a GWAS of 1,840 PBC cases and 5,163 controls of UK origin genotyped across 507,467 SNPs using the Illumina HumanHap670. We identified ten novel PBC loci ($P < 5\times10^{-8}$), in addition to replicating the two previously reported non-HLA PBC associations (*IL12RB2*: $P = 9.5\times10^{-20}$, OR = 1.52; *IL12A*: $P = 2.3\times10^{-16}$, OR = 1.38) and the HLA association. The most strongly supported novel PBC-associated locus was at 7q32, where seven SNPs exceed genome-wide significance (minimum $P = 8.91\times10^{-19}$, OR = 1.58). This locus contains the *IRF5* gene, which has previously been implicated in both systemic lupus erythematosus and rheumatoid arthritis. Of the ten novel PBC-associated loci found, seven have previously been implicated in other autoimmune diseases (including Crohn's disease, psoriasis and multiple sclerosis). Of the nine loci showing nominal evidence of association (three or more SNPs with $P < 1\times10^{-5}$), five overlap with regions previously found to be associated with another autoimmune disease. Three of the novel PBC-associated loci, on chromosomes 12, 14 and 19, do not overlap with any currently known loci involved in autoimmune disease, and thus may represent PBC specific risk loci. More than 20 loci were selected for follow-up in an additional 1,800 cases and 3,300 controls from North America and the UK. This work has significantly increased the number of known PBC loci and has increased our understanding of disease pathogenesis, in particular how PBC clusters biologically with other autoimmune diseases.

2939/T

Genome-wide meta-analysis for serum calcium identifies significantly associated SNPs near the calcium-sensing receptor (CASR) gene. *K. Kapur[1,2], T. Johnson[1,2,3], N. Beckmann[1], J. Sehmi[4], T. Tanaka[5,6], Z. Kutalik[1,2], U. Styrkarsdottir[7], W. Zhang[8], D. Marek[1,2], D. Gudbjartsson[7], Y. Milaneschi[9], H. Holm[7], A. Dilorio[10], D. Waterworth[11], Y. Li[12], A. Singleton[13], U. Bjornsdottir[14], G. Sigurdsson[15,16], D. Hernandez[13], R. DeSilva[4], P. Elliott[8], G. Eyjolfsson[14], J. Guralnik[17], J. Scott[4], U. Thorsteinsdottir[7,15], S. Bandinelli[9], J. Chambers[8], K. Stefansson[7,15], G. Waeber[18], L. Ferrucci[6].* 1) Department of Medical Genetics, University of Lausanne, Lausanne, Vaud, Switzerland; 2) Swiss Institute of Bioinformatics, Lausanne, Switzerland; 3) Institute of Social and Preventive Medicine, Centre Hospitalier Universitaire Vaudois and University of Lausanne, Bugnon 17, Lausanne, Switzerland; 4) National Heart and Lung Institute, Imperial College London, UK; 5) Medstar Research Institute, Baltimore, MD; 6) Clinical Research Branch, National Institute on Aging, Baltimore MD; 7) deCODE Genetics, Sturlugata 8, 101 Reykjavik, Iceland; 8) Department of Epidemiology and Public Health, Imperial College London, UK; 9) Geriatric Rehabilitation Unit, Azienda Sanitaria Firenze (ASF), Florence, Italy; 10) Department of Medicine and Sciences of Aging, Laboratory of Clinical Epidemiology, University G. D.Annunzio, Chieti, Italy; 11) Division of Genetics, R&D, GlaxoSmithKline; 12) Department of Genetics, Department of Biostatistics, University of North Carolina, Chapel Hill, NC 27599; 13) Laboratory of Neurogenetics, National Institute on Aging, Bethesda, MD 20892; 14) The Laboratory in Mjodd, RAM, Reykjavik, Iceland; 15) Faculty of Medicine, University of Iceland, Reykjavik, Iceland; 16) Department of Endocrinology and Metabolism, University Hospital, IS-108 Reykjavik, Iceland; 17) Laboratory of Epidemiology, Demography and Biometry, National Institute on Aging, Bethesda, MD 20892; 18) Department of Medicine, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland.

Calcium has a pivotal role in biological functions, and serum calcium levels have been associated with numerous disorders of bone and mineral metabolism as well as with cardiovascular mortality. Here we report results from a genome-wide association study of serum calcium, integrating data from four independent cohorts including a total of 12,865 individuals of European and Indian Asian descent. Our meta-analysis shows that serum calcium is associated with SNPs in or near the calcium-sensing receptor (CASR) gene on 3q13. The top hit with a p-value of 6.3x10-37 is rs1801725, a missense variant, explaining 1.26% of the variance in serum calcium. This SNP had the strongest association in individuals of European descent, while for individuals of Indian Asian descent the top hit was rs17251221 (p = 1.1x10-21), a SNP in strong linkage disequilibrium with rs1801725. The strongest locus in CASR was shown to replicate in an independent Icelandic cohort of 4,126 individuals (p =1.02 x 10-4). This genome-wide meta-analysis shows that common CASR variants modulate serum calcium levels in the adult general population, which confirms previous results in some candidate gene studies of the CASR locus. This study highlights the key role of CASR in calcium regulation.

2940/T

Genome wide association study of lung function measures in the Korean population. *K. Min[1], J. Min[2], S. Cho[3], J. Sung[3].* 1) Ajou University School of Medicine, Suwon, Korea; 2) Institute of Health and Environment, Seoul National University, Seoul, Korea; 3) School of Public Health, Seoul National University,Seoul, Korea.

Objective: Lung function is of major importance in public health. Genetic influence on lung-related phenotypes has highlighted from family, twin, and adoption studies to candidate gene studies. However, most studies have focused on lung disorders or diseases with multifactorial background. Such outcomes are likely to have genetic heterogeneity, when searching for genetic links to lung disease or functional variability in the general population. We investigated genetic contributions to lung function in the Korean population, using a population-based genome-wide association study (GWAS). Methods: Affymetrix 5.0 (~550K) SNP arrays were genotyped in 8842 subjects (4183 men and 4659 women) participating in the Korean Genome Epidemiology Study (KoGES). After applying genetic quality-control (genotypic call rates≥95%, MAF≥10%, HWE P≥1.0x10-6), a total of 352,228 markers were included for this analysis. Lung function is focused on three spirpometric outcomes: % predicted FEV1 (forced expiratory volume in 1 second), % predicted FVC (forced vital capacity), and % predicted FEV1/FVC. All procedures were approved by an institutional review committee. Results: Six previously implicated genes in chromosomes 1, 2, 3, 6, 7, and 9 were significantly associated with % predicted forced expiratory volume in 1 second (FEV1) and % predicted forced vital capacity (FVC): cyclin-dependent kinase 5 (CDK5); fragile histidine triad (FHIT); FYN oncogene related to SRC, FGR, YES (FYN); histamine N-methyltransferase (HNMT); protein tyrosine phosphatase, receptor type, D (PTPRD), and vascular cell adhesion molecule 1 (VCAM1). Of the genes, as the number of minor alleles in PTPRD increased, two parameters, % predicted FEV1 and % predicted FVC, also increased, particularly in men. Conclusion: We replicated six potential lung function genes in a Korean population-based association study. The effect of these genes on lung function might be independent of ethnic origin. Additionally, the PTPRD gene region may be of importance in understanding the pathophysiology of lung function.

2941/T

G6PC2 associated with fasting glucose levels in pregnant women of European ancestry. *J. Morrison[1], A. Pluzhnikov[1], M.G. Hayes[2], H. Lee[2], D. Levine[3], C. McHugh[3], L.P. Lowe[2], M. Urbanek[2], L. Armstrong[2], C. Ackerman[2], D. Mirel[4], A.R. Dyer[2], B.E. Metzger[2], N.J. Cox[1], W.L. Lowe[2], HAPO Study Cooperative Research Group.* 1) University of Chicago, Chicago, IL; 2) Northwestern University, Chicago, IL; 3) University of Washington, Seattle, WA; 4) The Broad Institute, Cambridge, MA.

The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study is an international epidemiologic study which recruited ~25,000 women to identify levels of maternal glycemia, as determined by an oral glucose tolerance test (OGTT), associated with risk of adverse pregnancy outcome. OGTTs were performed at 28 weeks gestation with determination of fasting, 1 hr, and 2 hr glucose and fasting and 1 hr C-peptide. We have conducted a genome wide association study of 1360 HAPO European mothers genotyped on the Illumina 610 quad platform to identify genetic factors influencing glucose tolerance and insulin secretion during pregnancy. We used a linear regression model adjusting for age, body mass index, height, parity and gestational age as well as the first two eigenvectors calculated on an LD pruned SNP set. For fasting glucose we saw a strong association with G6PC2 which encodes a glucose-6-phosphate catalytic protein expressed only in pancreatic islets. G6PC2 has been previously associated with fasting plasma glucose levels in non-diabetic European and Asian adults as well as having been associated in a recent GWAS with development of type 2 diabetes. Our strongest association with fasting glucose was with rs560887 - a SNP located in an intron of G6PC2. Minor alleles for this SNP resulted in a reduction of maternal fasting glucose of 1.39 mg/dl [95%CI 0.85-1.92] with a p-value of 3.97e-7. This finding suggests that maternal glucose intolerance is mediated in part by genetic factors including G6PC2.

2942/T

Genome-wide association study on Anorectal Malformations in the Chinese Population. *E.H.M. WONG[1], C.S.M. TANG[1,2], M.G. BARCELO[2], X.J. ZHANG[4], J.J. LIU[5], S.S. CHERNY[1], P.C. SHAM[1,3], P.K. TAM[2].* 1) Department of Psychiatry, University of Hong Kong, Hong Kong SAR, China, Hong Kong; 2) Department of Surgery, University of Hong Kong, Hong Kong SAR, China; 3) Genome Research Centre, University of Hong Kong, Hong Kong SAR, China; 4) 4Department of Dermatology and Venereology, Anhui Medical University, Hefei, Anhui 230032, P.R. China; 5) 5Department of Human Genetics, Genome Institute of Singapore, Singapore, Singapore.

Anorectal malformations (ARM) represent a complex group of congenital diseases characterized by the obstruction of anal opening. Occurring in 1 out of every 4,000 to 5,000 individuals, ARMs are one of the most common pediatric surgical problems. The spectrum of ARMs ranges from anal stenosis to anal atresia/imperforated anus with/without fistula to persistent cloaca. The etiology of ARMs remains unknown, although there is strong evidence for a genetic component. This is indicated by the very early developmental disruption, its recurrence in families and the high concordance of its existence with some abnormalities. Though several candidate genes e.g. endothelin-β receptor (EDNRB) and sonic hedgehog (Shh) were proposed for their potential roles in the development of ARMs, the causes of ARMs still remain unknown. To explore the genetic contribution to the pathogenesis of ARM, we performed systematic analyses of genetic markers, in 176 Chinese patients and 2986 ethnically matched individuals as controls. The genome-wide association study (GWAS) was performed using the Illumina Human 610-Quad BeadChips with 488, 394 SNPs successfully genotyped. An association peak encompassing two gene members of the solute carrier (SLC) family was observed, with p-value= (odds ratio 1.94, 95% CI: 1.54-2.45 for allele C) for the most associated SNP (rs11045422). To confirm the observed association, those statistically significant SNPs will be genotyped in an independent set of cases and controls. Bioinformatics and experimental analysis will be used to study the biological relevance of SLC genes in ARMs.

2943/T

Substantial risk factor of myeloperoxidase gene Polymorphism with Myopia. *c. Hemabindu.* Biotech, GRIET, Hyderabad, India.

Purpose : Myopia or short sightedness is the most common human eye disorder affecting 30% of world population. Genetic and environmental factors are implicated in the onset of myopia. Environmental causes include mechanical factors, oxidative stress, nutritional factors etc. Oxidative stress can arise due to intrinsic or extrinsic factors. myleoperoxidase is one of the antioxidant enzyme. It catalysis the reaction which produces hypochlorous acid. The lack of enzyme activity might result in accumulation of free radicals contributing to oxidative stress .To find out the association of myeloperoxidase gene polymorphism with myopia by genotyping. Methods : In the present study 320 cases of myopia were analyzed for myeloperoxidase gene polymorphism using PCR.for amplification of the gene and genotyping was done. Results : Results were compared with 320 cases of age and sex matched controls. There is a significant difference in the genotype distribution of AA genotype of myeloperoxidase gene polymorphism between disease(0.94%) and controls(5.63%). When the data on myopia was sub grouped with respect to different parameters, interesting results were obtained. Patients with age at onset in between 41-50 years had very high frequency GG Genotype (83.3%) as compared to other ranges of age at onset. Further myopia patients having refractive error 4-6 diopter have high frequency of GG genotype (95.45.6%) when compared with other with lower than 4 diopter and higher than 6 diopter. There is significant difference in the genotype distribution of GG genotype has been observed between consangunity (85.71%) and Non consangunity(66.89%) in myopia patients. Conclusion : These results indicates that there is an elevation of GG genotype in myopia patients which indicate that inefficient detoxification system to combat the accumulation of free radicals might be responsible for the onset of myopia . There was no association of Myleoperoxidase gene polymorphism with , familial incidence, male and female.

2944/T

The FGF/FGFR gene family and risk of cleft lip with/without cleft palate. *H. Wang[1,9], J. Hetmanski[2], T. Wu[1,2,9], I. Ruczinski[2], H. Schwender[2], K. Liang[2], T. Murray[2], R. Redett[3], G. Raymond[3], S. Jin[2], Y. Wu-Chou[4], P. Chen[4], V. Yeow[5], S. Chong[6], F. Cheah[6], S. Jee[7], R. Ingersoll[3], E. Jabs[3,8], A. Scott[3], T. Beaty[2].* 1) Epidemiology and Biostatistics, Peking University School of Publich Health, Beijing, China; 2) Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 3) Johns Hopkins University, School of Medicine, Baltimore, MD; 4) Chang Gung Memorial Hospital, Taoyuan, Taiwan; 5) KK Women's & Children's Hospital, Singapore; 6) National University of Singapore, Singapore; 7) Yonsei University, Graduate School of Epidemiology & Health Promotion, Seoul, Korea; 8) Mount. Sinai School of Medicine, New York, NY; 9) Key Laboratory of Epidemiology, Ministry of Education, China.

Isolated, nonsyndromic cleft lip with or without cleft palate (CL/P) is a common human congenital malformation with a complex and heterogeneous etiology. Both genes, regulatory elements outside genes and environmental risk factors may influence risk to clefts. Genes coding for fibroblast growth factors and their receptors (FGF/FGFR genes) are legitimate candidates for CL/P. As part of an international study, we collected DNA, and environmental exposures on 297 CL/P case-parent trios recruited through treatment centers from four sites (Maryland, Taiwan, Singapore, and Korea) to test the importance of FGF/FGFR genes in controlling risk to CL/P. Ten FGF/FGFR genes included: FGFBP1, FGF2, FGF10, FGF18, FGFR1, FGFR2, FGF19, FGF4, FGF3, FGF9, and 122 single nucleotide polymorphic (SNP) markers were genotyped at the Center of Inherited Disease Research (CIDR) of which 112 SNPs were suitable for analysis. Because Asian trios showed very similar LD patterns, all trios from the three Asian sites were combined into one group. The transmission disequilibrium test (TDT) of 112 SNPs using FBAT confirmed previous evidence of linkage and association to markers in FGFR1. Both individual SNPs and haplotypes of multiple markers in FGF19 also showed suggestive evidence of linkage and association with CL/P, giving odds ratios (OR) between 1.31 and 1.87. Haplotypes of three SNPs in the FGFR1 gene (rs6987534, rs6474354 and rs10958700) gave suggestive significance (p=0.04) among Asian trios and one particular haplotype appeared strongly protective, yielding an estimated OR=0.51 (p= 0.0058). We also found suggestive evidence of maternal effects for markers in FGF2 and FGF10 among Asian trios. We used PBAT to scan for gene-environment (GxE) interaction and statistical significance was seen between certain markers in FGFR2 gene and environmental maternal exposures, particularly multivitamin supplementation. Tests of gene-gene (GxG) interaction using Cordell's method yielded significant interaction between SNPs in FGF9 and FGF18. These results raise the possibility that several genes in the FGF/FGFR family may influence risk to oral clefts through distinct biological mechanisms.

2945/T

Evidence of gene-environment interaction for the IRF6 gene and maternal multivitamin supplementation in controlling the risk of cleft lip with/without cleft palate. *T. Wu[1,2,9], K. Liang[1], J. Hetmanski[1], I. Ruczinski[1], M. Fallin[1], R. Ingersoll[1,3], H. Wang[2,9], S. Huang[4], X. Ye[5,6], Y. Wu-Chou[7], P. Chen[7], E. Jabs[3,5], B. Shi[8], R. Redett[3], A. Scott[3], T. Beaty[1].* 1) Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 2) Beijing University School of Public Health, Beijing, China; 3) Johns Hopkins School of Medicine, Baltimore, MD; 4) Peking Union Medical College, Beijing, China; 5) Mount. Sinai School of Medicine, New York, NY; 6) Key Laboratory for Oral Biomedical Engineering of Ministry of Education, Hospital and School of Stomatology, Wuhan University, Wuhan, China; 7) Chang Gung Memorial Hospital, Taoyuan, Taiwan; 8) West China College of Stomatology, Sichuan University, Sichuan, China; 9) Key Laboratory of Epidemiology, Ministry of Education, China.

Although multiple genes have been identified as genetic risk factors for isolated, non-syndromic cleft lip with/without cleft palate (CL/P), a complex and heterogeneous birth defect, interferon regulatory factor 6 gene (*IRF6*) is one of the best documented genetic risk factors. In this study, we tested for association between markers in *IRF6* and CL/P in 326 Chinese case-parent trios, considering gene-environment (GxE) interaction for two common maternal exposures, and for parent-of-origin effects. CL/P case-parent trios from three sites in mainland China and Taiwan were genotyped for 22 single nucleotide polymorphisms (SNPs) in *IRF6*. The transmission disequilibrium test (TDT) was used to test for marginal effects of individual SNPs using Clayton's extension in Stata 10. We used PBAT to screen the SNPs and haplotypes for GxE interaction and conditional logistic regression models to quantify effect sizes for SNP-environment interaction. After Bonferroni correction, fourteen SNPs showed statistically significant association with CL/P ($0.018 < \text{corrected } P < 2.1 \times 10^{-5}$). Evidence of GxE interaction was found for both maternal exposures, multivitamin supplementation and environmental tobacco smoke (ETS). Two SNPs showed evidence of interaction with multivitamin supplementation in conditional logistic regression models [*rs2076153*: odds ratio (OR) =1.28, 95%CI =1.01-1.63, nominal P =0.020; *rs17015218*: OR=0.13, 95%CI=0.02-0.99, nominal P =0.012]. In addition, *rs1044516* yielded evidence for interaction with maternal ETS (OR=1.96, 95%CI =1.38-2.78, nominal P =0.041). Haplotype analysis using PBAT also suggested interaction between SNPs in *IRF6* and both multivitamin supplementation and ETS. However, no evidence for maternal genotypic effects or significant parent-of-origin effects was seen in these data. These results suggest *IRF6* gene may influence risk of CL/P through interaction with multivitamin supplementation and ETS in the Chinese population.

2946/T

Heritability of Ocular Phenotypes in the Genetic Isolate of Norfolk Island. *E. Matovinovic[1], R. Lea[1], H. Cox[1], J. Herruer[1, 3], M. Hanna[1], J.C. Sherwin[4], A.W. Hewitt[4], L.S. Kearns[4], J. Kelly[5], Y. Ma[4], D.A. Mackey[2,4,6], L. Griffiths[1].* 1) Genomics Research Centre, Griffith Institute for Health and Medical Research, Griffith University, Gold Coast, Queensland, Australia; 2) Lions Eye Institute Centre for Ophthalmology and Visual Science, University of Western Australia, Nedlands, WA; 3) Vrije Universiteit, 1081 HV, Amsterdam, The Netherlands; 4) Centre for Eye Research Australia, Dept of Ophthalmology, University of Melbourne, East Melbourne, Victoria, Australia; 5) Norfolk Optical, Norfolk Island, South Pacific; 6) Dept Ophthalmology, Royal Hobart Hospital, University of Tasmania, Hobart, Tasmania, Australia.

Glaucoma is the leading cause of irreversible blindness worldwide. Chronic glaucoma prevalence in the US population 40 years and older is 1.86%. With an aging population, this prevalence is projected to increase by 50% in 2020. We derived heritability estimates for various ocular phenotypes in the Norfolk Island "Mutiny on the Bounty" population. This well documented pedigree has mean European and Polynesian ancestry of 88% and 12% respectively. Glaucoma incidence on Norfolk Island is high despite uncommon prevalence in Polynesian populations suggesting that one of the European founders may carry a glaucoma related genotype. Eight hundred Norfolk Island participants received exams consisting of several ocular measurements. Of these, 153 participants were identified within the core Norfolk Island pedigree. Ocular phenotype heritability estimates for the Norfolk Island pedigree were generated in this subset using SOLAR. Heritability estimates were determined for several quantitative metrics in both eyes. In the right eye, corneal thickness was the most heritable measure (h2 0.66 p=0.001) followed by intraocular pressure (h2 0.61 p=0.021) and anterior chamber depth (h2 0.54 p=0.008). Similar results were obtained for left eye. Age was a significant covariate for axial length and cup: disc area ratio measures. Hence, several ocular phenotypes are highly heritable in the Norfolk Island population providing opportunities for further genetic research. We are currently conducting linkage studies using microsatellite markers in order to identify genetic loci for these heritable metrics which may be involved in glaucoma and other eye diseases.

2947/T

Multiple sclerosis susceptibility alleles modulate levels of RNA expression involved in lymphocyte activation pathways. *P. De Jager[1], L. Ottoboni[1], F. Esposito[2], N. Patsopoulos[1], F. Martinelli-Boneschi[2], G. Comi[2], P. de Bakker[1], D. Hafler[3].* 1) Brigham & Women's Hospital, Boston, MA; 2) Institute San Raffaele, Milan, Italy; 3) Yale University, New Haven CT.

Background and goals: Multiple Sclerosis (MS) is an inflammatory disease for which 17 susceptibility loci have been identified. The goal of the project is to explore the functional consequences of these susceptibility loci in genome-wide RNA expression data generated from peripheral Blood mononuclear cells (PBMCs) of subjects with MS. Methods and results: We assessed our data for Cis and Trans effects on RNA expression by each of 17 validated MS loci variants. The dataset consists of genome wide RNA expression data on PBMCs from 255 MS subjects. They were either untreated (n=83) or treated with immunomodulatory drugs (n=105 interferon-beta; n=67 glatiramer acetate). Analyses were performed using linear regression and additive model for each SNP as an independent variable and adjusting for SNP-treatment interactions. We have identified: (1) a robust Cis association of MPHOSPH9 RNA expression with the MPHOSPH9 variant rs1790100 (p=1.04x10-6), (2) using pathway analyses (IPA, Ingenuity Systems), we find that 7 different loci (CD6, TNFR1, IL12A, CD226, CLEC16A, IRF8, IL2RA, and RGS1) affect RNA expression of multiple genes in the same 4 signaling pathways: CD28 signaling, TCR signaling, iCOS_iCOS-Ligand and IL2 signature. For example, rs2760524 on RGS1 affects the expression of multiple genes in the CD28 pathway (P=1.479x10-9). Conclusion: We show a remarkable level of interconnectedness among a subset of MS susceptibility loci: some of these loci affect RNA expression within other susceptibility loci and, more striking, multiple different loci have a broad effect on signaling pathways that are critical for T cell function and proliferation. These observations provide a strong bridge between the recent genetic discoveries in MS and the wealth of immunological data that has been accumulated in this disease.

2948/T

Common Genetic Polymorphisms and Risk of Serious Neurological Complications of West Nile Virus Infection. *M. Loeb[1], S. Eskandarian[1], M. Rupp[2], N. Fishman[3], L. Gasink[4], J. Patterson[5], J. Bramson[1], T. Hudson[6], M. Lemire[6].* 1) Dept Pathology & Molec Med, McMaster Univ, Hamilton, ON, Canada; 2) University of Nebraska Medical Center, Omaha, NE; 3) University of Pennsylvania Medical Center, Philadelphia, PA; 4) Hospital of the University of Pennsylvania, Philadelphia, PA; 5) University of Texas Health Science Center San Antonio and South Texas VA, San Antonia, TX; 6) Ontario Institute for Cancer Research, Toronto, ON, Canada.

Background: The relationship between common genetic polymorphisms and susceptibility to neurological complications in West Nile virus (WNV) infected individuals is not well understood. Methods: From 2003 to 2008 patients meeting case definitions for severe WNV disease (meningitis, encephalitis, or acute flaccid paralysis) from Canada and U.S were compared to WNV infected patients without severe disease. Whole blood samples were collected for genotyping using whole genome screening with the Illumina HumanNS-12 Infinium assay. This included 13,371 single nucleotide polymorphisms (SNPs) which were mostly non-synonymous variants but also included synonymous, UTR, and tag-SNPs. To validate association results from the primary analysis, a panel of 34 SNPs was designed using Sequenom MassARRAY IPLEX Gold including tag SNPs from the primary gene of interest, top 12 SNPs from the primary analysis. Results: 445 neuroinvasive cases and 813 controls were compared in the primary analysis. SNPs of importance based on statistical significance and biological plausibility included RFC1 (replication factor) (rs2066786) p= 1.67 x 10-6, OR 1.5 (95%CI 1.21 to 1.86); SCN1A (sodium channel, neuronal type 1 alpha subunit) (rs2298771), p= 1.73 x 10-6, OR 1.50 (1.21 to 1.86); and ANPEP (ananyl aminopeptidase) (rs25651) p = 1.73 x 10-4. Replication samples from 617 patients (277 cases and 340 controls) were obtained. Although there was an overall lack of significance in the replication cohort (p > 0.5 for all three SNPs), RFC1- rs2066786 did replicate in the cohort from one state (Nebraska). Joint analysis showed for RFC1 -rs2066786 p= 3.0 x 10 10-5; SCN1A- rs2298771 p= 1.4 x 10-3; and ANPEP- rs25651 p= 3.1 x 10-3. SNPs in other candidate genes (OAS, TLR3) were not significant. Conclusion: Novel genetic variants may play a role in susceptibility to severe WNV disease.

2949/T

GRACE Network of Excellence: Genetic Susceptibility to Lower Respiratory Tract Infections. *A. Rautanen, T.C. Mills, S.J. Chapman, A.V.S. Hill, GRACE study group.* Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom.

GRACE (Genomics to combat Resistance against Antibiotics in Community-acquired LRTI in Europe) is a Network of Excellence focusing on community-acquired Lower Respiratory Tract Infections (LRTI); one of the leading reasons for seeking medical care. The overall objective of GRACE is to combat antimicrobial resistance by integrating microbial and human genetics with health sciences research. 3000 patients with LRTI and 3000 matched controls were recruited by 14 primary care research networks in 12 European countries, making this the largest prospective study of LRTI ever undertaken in primary medical care. Adult patients with acute cough or clinically suspected LRTI as the main presenting symptom were eligible. Based on our earlier candidate gene and genome-wide association findings with severe LRTI cases suffering from invasive pneumococcal disease, we have selected and genotyped 27 SNPs in 19 genes with Sequenom's iPLEX technology. In the initial case-control analysis where half of the GRACE individuals were genotyped, we were able to replicate associations in the genes PTPN22 (Arg620Trp:p=0.037, OR 2.0) and NFKBIZ (rs616597:p=0.022, OR 0.87; rs600718: p=0.028, OR 0.87). PTPN22 belongs to a family of lymphoid specific protein tyrosine phosphatases and regulates the immune response through the T-cell signaling. NFKBIZ is one of the NFκB inhibitors and thereby affects the transcription of pro-inflammatory genes. For more informative analyses, we will subdivide our patients based on the microbiological cause of their LRTI, namely between bacterial and viral infection, and further to specific pathogens. To enable this subdivision, interpretation of a vast amount of microbiological and clinical data is ongoing. Surprisingly it seems that the majority of the GRACE LRTIs have a viral cause instead of bacterial. Therefore, more candidate SNPs will be genotyped based on their possible involvement in the host immune response to viral infections. The second set of SNPs to be genotyped, however, will be mainly selected based on our currently ongoing meta-analysis of genome-wide screens of invasive pneumococcal disease and community-acquired pneumonia with sepsis. Although the identification of host genetic factors cannot be directly applied to combat antimicrobial resistance, it will provide valuable information about disease pathogenesis and host defence mechanisms. This information could be used to detect, treat, and prevent LRTI in a more individualised manner.

2950/T

Admixture mapping analysis of blood pressure and BMI in Family Blood Pressure Program (FBPP). *F. Zakharia[1], K. Sunjung[2], Y. SongGon[2], T. Bamidele[3], A. Morrison[4], C. Hanis[4], D.C. Rao[5], R.S. Cooper[3], H. Tang[1], X. Zhu[2], N. Risch[6].* 1) Department of Genetics, Stanford University School of Medicine, 300 Pasteur Drive, Stanford, CA; 2) Department of Biostatistics and Epidemiology , Case Western Reserve University, Cleveland, OH; 3) Department of Preventive Medicine and Epidemiology, Loyola University Chicago Stritch School of Medicine, Maywood, IL; 4) Division of Epidemiology and Disease Control, School of Public Health, University of Texas at Houston Health Science Center, Houston, TX; 5) Division of Biostatistics, Washington University in St. Louis, MO; 6) Institute for Human Genetics, University of California, San Francisco, 513 Parnassus Ave., San Francisco, CA.

African Americans are, on average, at higher risk for hypertension and obesity, compared to European Americans. Previously we have used admixture mapping to identify candidate regions, which both contribute to disease risk and to disparity between populations. Using genotype data at 2,593 ancestry informative SNPs in 2,005 African-American subjects from three networks in the FBPP project, we performed admixture mapping analysis for hypertension related traits and BMI. We observed 5 genomic regions showing strong evidence (P<0.01) of potentially harboring genetic variants contributing to the variation in hypertension related phenotypes, including chromosome 6 and 21 genomic regions reported previously. We were able to refine these two regions by typing additional SNPs. The association evidence in admixture mapping analysis was improved for all hypertension related traits (P<0.001). In single marker analysis we observed SNP rs9490191 is significantly associated with diastolic blood pressure after adjusting for multiple comparisons (P=5.64E-06). For BMI, the most significant region in admixture mapping analysis is at SNP rs8134809 (P=2.3E-04). We did not observe any SNPs significantly associated with BMI after adjusting for multiple comparisons. In summary our analysis suggests that admixture mapping can be a useful method to detect genetic variants underlying complex diseases.

2951/T

Quantification of DNA instability in the inherited neuromuscular disease myotonic dystrophy using a hierarchical Bayesian approach. *C.F. Higham, C. Cobbold, D.T. Haydon, D.G. Monckton.* University of Glasgow, Glasgow, Scotland, United Kingdom.

Myotonic dystrophy type 1 is caused by inheriting an unstable expanded CTG repeat on chromosome 19 in the 3' untranslated region of the DMPK gene. Expansion of the disease allele during the lifetime of patients and variation in the level of mutation between somatic tissues of an individual are thought to account for the tissue specificity and progressive nature of the symptoms. Clinical diagnosis is currently based on a measure of repeat length from blood cells, but variance in length only accounts for about 25% of the variance in age of onset and, therefore, is not predictive. This low correlation arises because modal length is dependent on age and so confounds the analysis. But also, age of onset is linked to tissues other than blood, such as muscle, which can have greater levels of instability. A more reliable measure for patients would therefore be an indication of the length of their inherited disease allele, otherwise known as the progenitor allele length.

We have developed a new mathematical model to describe the evolution of repeat length in individuals over their lifetime. The model is based on the assumption that the repeat length in each cell changes independently according to a stochastic process that has biologically relevant parameters. By calibrating the parameters in this mathematical model to recent data which quantify, at a fine resolution, the variation in blood DNA from myotonic dystrophy patients, we are able to infer an individual's progenitor allele length and level of mutation. However, applying the model to each patient does not provide a basis for inference about the population, so we will present new work that investigates the distribution of DNA instability within the population using a hierarchical Bayesian approach. This rich statistical framework allows us to provide robust prognostic information for patients. DNA instability is also a quantitative trait that could be assessed in terms of its heritability and used as a biomarker to identify any trans-acting genetic, epigenetic or environmental effects. Our expectation is that these trans-acting genetic modifiers will also apply in the general population where they will affect ageing, cancer, inherited disease and human genetic variation.

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Comparison of Methods for Evaluating the Ability of Genetic and Clinical Information to Predict Prostate Cancer Risk. *B.H. Reck[1], P.J. Newcombe[2], J. Sun[4,5], G.T. Platek[1], A.K. Kader[4,6], S-T. Kim[4,5], T. Jin[4,5], Z. Zhang[4,5], S.L. Zheng[4,5], L.D. Condreay[1], J.C. Whittaker[2], C.F. Spraggs[2], V.E. Mooser[3], R.S. Rittmaster[1], J. Xu[4,5,6].* 1) GlaxoSmithKline Research and Development, Research Triangle Park, NC, USA; 2) GlaxoSmithKline Research and Development, Harlow, UK; 3) GlaxoSmithKline Research and Development, King of Prussia, PA, USA; 4) Center for Cancer Genomics, Wake Forest University School of Medicine, Winston-Salem, NC, USA; 5) Center for Human Genomics, Wake Forest University School of Medicine, Winston-Salem, NC, USA; 6) Departments of Urology, Wake Forest University School of Medicine, Winston-Salem, NC, USA.

Prostate cancer (PCa) is the most common solid organ malignancy affecting American men and the second leading cause of cancer related death in men. The ability of clinical parameters to predict PCa is limited with only a quarter of biopsies resulting in a diagnosis of PCa. Recently, more than 30 PCa risk-associated single nucleotide polymorphisms (SNPs) have been discovered in genome-wide association studies and replicated in multiple populations of European descent. We compared three statistical methods for constructing predictors of PCa based on 33 risk SNPs and clinical variables using genetic samples obtained from 1608 Caucasian men who consented to genetic analyses for prostatic indications and were randomized to receive placebo in the 4 year PCa risk reduction study, The REduction by DUtasteride of prostate Cancer Events (REDUCE®), evaluating the safety and efficacy of Avodart®. Patients participating in this study were required to have a prostate biopsy showing no evidence of PCa at recruitment, elevated PSA levels (2.5 - 10 ng/mL), and agree to having 10-core biopsies after 2 and 4 years of study. The first method was a standard logistic regression model which included separate terms for the clinical covariates and 33 genetic markers. In the second method, PCa effect estimates for the 33 SNPs obtained from external meta-analyses were used to construct a PCa risk "score" for each subject. The score and clinical covariates were subsequently evaluated in a logistic regression model. The third method utilized a Bayesian approach to incorporate PCa effect estimates from the external meta-analysis into a logistic regression model via informative priors. PCa prediction was evaluated using 10-fold cross-validation for all 3 scores. For all three scores, genetic information incorporated alongside the clinical variables significantly improved model fit, leading to a marginal improvement in cross-validated predictive ability. Incorporation of external information, whether via a weighted score (method 2) or informative priors (method 3), led to an apparent but modest improvement in PCa prediction.

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ASSOCIATION STUDY OF THE 5HTT-LPR AND VNTR-5HTT POLYMORPHISMS OF THE *SLC6A4* GENE AND BIPOLAR DISORDER. *V. Peralta[1], E. Leal[1], JP. Meza[1], J. Duran[2], VL. Lozano[1], M. Gutierrez[3], IP. Davalos[4], CT. Hernandez[5], A. Garcia[5], LJ. Rodriguez[5], HG. Torres[1], M. Partida[6], JG. Perales[1], JC. Moterrubio[1], V. Garcia[1], LA. Cruz[1], KG. Martinez[1].* 1) Genetica Humana, Universidad Autonoma de Tamaulipas, Matamoros, Tamaulipas, Mexico; 2) Biology dept, University of Texas at Brownsville, TX 78520; 3) CU-ALTOS UdeG; 4) CIBO/ CUCS UdeG; 5) Hosp. Gral. Subzona No. 33, Deleg.Ver-Norte del IMSS; 6) Instituto de Genética Humana CUCS.

Polymorphism in the serotonin transporter gene (SLC6A4) has been associated with depressive disorders in some populations in recent publications (1). The aim of this study was analyze the presence of the polymorphism in the SLC6A4 gene and risk of suffering bipolar disorder. The SLC6A4 gene its located at 17q11.2 and shows two common allele variations, one at the promoter region (a 44 base pair deletion/insertion or 5HTT-LPR) related with low mRNA and other inside the second intron (a 9,10 or 12 tandem repeats of 16-17 base pairs or 5HTT-VNTR) (2,3). Methods: From Mexican-Mestizo population, 163 healthy controls and 17 patients with bipolar disorder were analyzed. Bipolar disorder diagnose was made using the DSM-IV criteria. Genomic DNA was extracted from peripheral blood by the phenol-chloroform method. Genotyping was performed by PCR with primers previously described (3). Visualization and determination of the genotypes was made by electrophoresis using polyacrylamide and agarose gels stained in silver nitrate and sybr-safe respectively. Results: The studied polymorphisms are in Hardy-Weinberg equilibrium, the VNTR-5HTT allele shows an X2=0.80, p=0.36974, and the LPR-5HTT an X2=2.98, p=0.08429. This preliminary result shows non statistical association between the presence of the polymorphism and bipolar disorder risk, however, the number of patients was small, and future analysis in Mexican-mestizo population with a bigger number of patients can give results with more statistical accuracy. References: 1.-Luddington NS, Mandadapu A, Husk M, El-Mallakh RS. Clinical implications of genetic variation in the serotonin transporter promoter region: a review. Prim Care Companion J Clin Psychiatry, 2009: 11(3): 93-102. 2.-Ferreira Ade A, Neves FS, da Rocha FF, Silva GS, Romano-Silva MA, Miranda DM, De Marco L, Correa H. The role of 5-HTTLPR polymorphism in antidepressant-associated mania in bipolar disorder. J Affect Disord, 2009: 112: 267-272. 3.-Cook EH Jr, Courchesne R, Lord C, Cox NJ, Yan S, Lincoln A, Haas R, Courchesne E, Leventhal BL. Evidence of linkage between the serotonin transporter and autistic disorder. Mol Psychiatry, 1997: 2(3): 247-250.

2954/T

Association study between polymorphisms in PPARγ, CRP, HSD11B1and ADIPOQ genes and obesity in a young Mexican-American population from S.Texas. *J. Duran[1], I. Ortiz[1], N. Ruiz[1], B.N. Newton[1], G. Zavala[1], M. Ortiz[1], A. Gonzalez[1], E.K. Sanchez[1], S. Fisher-Hoch[2], A. Rentrefo[1], S. Nair[1].* 1) Biology, University of Texas at Brownsville, Brownsville, TX; 2) Division of Epidemiology, University of Texas School of Public Health, Brownsville Campus, Brownsville, TX.

In US, the prevalence of obesity in people 20 years or older is 33.9%, but this statistic varies according to the ethnicity. Mexican-American populations in the US have increased risk for the development of obesity with a prevalence of 39.3% in the same age group. This suggests that in addition to environmental influences, susceptibility to obesity may have a strong genetic component in the Mexican-American population. In this study we analyzed single nucleotide polymorphisms (SNP) rs1205(G>A), rs1801282(C>G), rs846910(A>G) and rs1501299(G>T) of the CRP, PPARγ, HSD11B1 and ADIPOQ genes respectively because of their well documented roles in regulation of insulin sensitivity and homeostatic levels of glucose. The study population (n= 446) including 323 adolescent children from Brownsville schools and 123 college going students from University of Texas at Brownsville (UTB) at South Texas consented to participate. First degree familiars were excluded from statistical analysis. Height, weight, body mass index (BMI) (kg/m2) and waist circumference (WC) were obtained using standard procedures. WHO and CDC classification values were applied for obesity diagnosis. Genomic DNA was extracted with QIAamp DNA kit (Qiagen, Alameda, CA). Genotyping was performed with Assays on demand allelic discrimination assays (Applied Biosystems, Carlsbad, CA). Hardy Weinberg equilibrium and $\chi 2$ test was used to examine differences among categorical variables. The allelic and genotypic frequencies are show in table 1. All the alleles were found in Hardy Weinberg equilibrium. No statistical differences were found in the association of the alleles for any of the analyzed SNPs with obesity (table-2). Study population has an average age of 17.5 years old (range from 14 to 30 years, 283 less than 17 years and 165 between18 and 30) and the obese population represents 22.4% of the individuals. Although NHANES III revealed a 11.5% frequency in the prevalence of obesity in adolescents, a more recent report shows that the frequency of obesity in this age group was 22.1% in South Texas. A similar frequency is reported in the present study in at young Mexican American population from S.Texas. Although we did not observe associations between the investigated polymorphisms and obesity measures, we cannot rule out that these associations do not exist in larger samples of the population.

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Guidelines to Strengthen the Reporting of Genetic Risk Prediction Studies (GRIPS). *A.C.J.W. Janssens[1], J.P.A. Ioannidis[2,3], P. Boffetta[4], S.M. Dolan[5], N.F. Dowling[6], I. Fortier[7], A.N. Freedman[8], J.M. Grimshaw[9], J. Gulcher[10], M. Gwinn[6], M. Hlatky[11], H. Janes[12], P. Kraft[13], S. Melillo[6], C.J. O'Donnell[14], M.J. Pencina[15], D. Ransohoff[16], S. Schully[8], D. Seminara[8], D. Winn[8], C.F. Wright[17], C.M. van Duijn[1], J. Little[9], M.J. Khoury[6].* 1) Dept Epidemiology, Erasmus University Medical Center, Rotterdam, Netherlands; 2) Dept Hygiene and Epidemiology, University of Ioannina, Ioannina, Greece; 3) Dept Medicine, Tufts University School of Medicine, Boston, USA; 4) Mount Sinai School of Medicine, New York, USA; 5) Dept Obstetrics & Gynecology and Women's Health, Albert Einstein College of Medicine, Bronx, USA; 6) National Office of Public Health Genomics, Centers for Disease Control and Prevention, Atlanta, USA; 7) Public Population Project in Genomics (P3G), Montreal, Canada; 8) Division of Cancer Control and Population Sciences, National Cancer Institute, Bethesda, USA; 9) Dept of Epidemiology and Community Medicine, University of Ottawa, Ottawa, Canada; 10) Decode Genetics, Reykjavik, Iceland; 11) Dept Health Research and Policy, Stanford University, Palo Alto, USA; 12) Fred Hutchinson Cancer Research Center, Seattle, USA; 13) Dept Epidemiology, Harvard School of Public Health, Boston, USA; 14) The National Heart, Lung, and Blood Institute's Framingham Heart Study, Framingham, USA; 15) Dept Biostatistics, Boston University, Boston, USA; 16) Division of Digestive Diseases and Nutrition, University of North Carolina, Chapel Hill, USA; 17) PHG Foundation, Cambridge, UK.

The rapid and continuing progress in gene discovery for complex diseases is fuelling interest in the potential implications of this knowledge for clinical and public health practice. An essential prerequisite for many genome-based health care applications is the predictive ability of the genetic risk model. The number of studies assessing the predictive performance of genetic risk models is steadily increasing, with widely variable completeness of reporting and apparent quality. Transparent reporting of the strengths and weaknesses of empirical studies is important to facilitate the accumulation of evidence on genetic risk prediction. A multidisciplinary panel developed a checklist of 25 items recommended for strengthening the reporting of Genetic RIsk Prediction Studies (GRIPS), building on the STREGA, REMARK and STARD reporting guidelines. The recommendations do not prescribe or dictate how genetic risk prediction studies should be conducted, but offer consensus guidelines to enhance the transparency of their reporting, and thereby to improve the process of synthesizing information from multiple studies with different strategies regarding design, conduct or analysis.

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Harnessing hidden relatedness to incorporate missing heritability into genetic risk prediction. *H.M. Kang[1], S. Sanna[2], C. Sidore[2], A. Mulas[2], R. Nagaraja[3], M. Uda[2], D. Schlessinger[3], G.R. Abecasis[1].* 1) Department of Biostatistics, Univ Michigan, Ann Arbor, Ann Arbor, MI; 2) Istituto di Neurogenetica e Neurofarmacologia del CNR, Cagliari, Italy; 3) Laboratory of Genetics, National Institute on Aging, Baltimore, MD, USA.

Genome-wide association studies have identified a number of markers robustly associated with various complex traits. Even in aggregate, these markers account for only small changes in individual genetic risk, and only account for a fraction of heritability in most complex traits. Recently, several studies have demonstrated improved genetic risk prediction utilizing large numbers of markers that are only tentatively associated with a trait of interest, but it remains unclear how to interpret the underlying prediction model. Here we propose a model-based approach for predicting complex trait outcomes using a variance component model. Our model explicitly models robustly associated loci as fixed effects, while cumulative effects from additional small effect loci are modeled as polygenic random effects by leveraging hidden relatedness. Our approach uses available genotype data to estimate this "hidden" kinship matrix. For nearly all complex traits, individual trait values can be better predicted when phenotypes of close family members are available, in addition to genotype of the sample individual of interest. Our variance component model extends this observation to settings where a large number of distantly related individuals are studied and can be used to aid prediction in lieu of closer relatives can substantially improve the predictions. Our approach in effect combines Common-Disease-Common-Variants (CDCV) hypothesis and Multiple-Rare-Variants (MRV) hypothesis in the context of phenotype prediction. We applied our method to 6,000 Sardinian individuals genotyped at >150,000 SNPs. With a set of known loci explaining 2.8% of variance in height under linear model when cross-predicted across different families, our prediction results explain 10.7% of phenotypic variance, and it increases up to 28.6% when close relatives are included in the reference sample. We demonstrate that our method substantially increases the predictive power across multiple complex traits such as serum lipid levels and body mass index.

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Genetic Risk Modeling using Genome-wide Association Studies for Psoriasis. *D.I. Perera[1], J.J Liu[1], X.J. Zhang[2,3,4], G.T.H. Keong[1], A. Thalamuthu[1].* 1) Human Genetics, Genome Institute of Singapore, Singapore, Singapore; 2) 2. Institute of Dermatology and Department of Dermatology at No.1 Hospital, Anhui Medical University, Hefei, Anhui 230022, China; 3) 3. The Key Laboratory of Gene Resource Utilization for Severe Diseases, Ministry of Education and Anhui Province, Hefei, Anhui 230032,China; 4) 4. Department of Dermatology and Venereology , Anhui Medical University, Hefei, Anhui 230032, China.

Medical and bio-statistical researchers have used clinically measurable markers for disease classification and prediction for a long time. The successful completion of the HapMap project and development of new and efficient genotyping technologies have given rise to numerous genome-wide association studies which in turn have infused several new markers that bring in a genetic component to the disease classification and risk prediction models. Using Genome-wide Association Studies (GWAS) we attempt to identify a set of Single Nucleotide Polymorphisms (SNPs) for predicting the risk of psoriasis. The data set comprise of 2505 controls and 2488 cases from Caucasian and Chinese populations. We use simple logistic regression and Support Vector Machine (SVM) to obtain Receiver Operating Characteristic (ROC) curve and the estimate of area under the ROC curve (AUC) to measure the predictive power of the SNPs. The set of SNPs for the joint risk modeling is selected based on the predictive power of individual SNPs. Individual SNPs can be selected based on its association p-value. But to have a good predictive power, say AUC, a SNP should have high Odds Ratio (OR), be highly polymorphic and the allele frequency difference between cases and controls should be high. Therefore instead of choosing SNPs based on p-values, we directly choose the SNPs based on its AUC value. Many SNPs in the major histocompatibility (MHC) locus are associated with psoriasis. Therefore we perform the analysis focusing separately on MHC and non-MHC SNPs. To estimate the AUC we perform a 5-fold cross validation based on the combined data set of the two cohorts. Using the top SNPs that give 80% AUC in each cross validation, we obtain 115 common SNPs for the non-MHC region with mean AUC estimates of 0.79 and 0.76 for the training set and a test set respectively. Similarly for the MHC region, we find 30 common SNPs with mean AUC values of 0.79 and 0.78 for the training and test set respectively. We will also be extending the study to identify a set of SNPs common for other auto-immune diseases and to multiple cohorts.

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Association of IL-23 receptor pathway genes with rheumatoid arthritis. *M. Seddighzadeh[1], B. Ding[2], L. Klareskog[1], L. Alfredsson[2], K. Dunussi-Joannopoupos[3], JD. Clark[3], L. Padyukov[1].* 1) Dept Medicine, Karolinska Inst & Hosp, Stockholm, Sweden; 2) Institute for Environmental Medicine, Karolinska Institutet, Stockholm, Sweden; 3) Inflammation and Immunology, Pfizer Research, 200 CambridgePark Drive, Cambridge, MA 02140.

Background: The activation of IL-23 receptor launches, directed by TYK2 (non-receptor tyrosine-protein kinase), the phosphorylation of STAT (signal transducer and activator of transcription) 3 and 4. These molecules are critical in development of the Th17-dependent autoimmune disorders. No previous association study has investigated these genes in relation to RA subgroups with different autoantibody production, although previous data demonstrated divergence in genetic background for these subgroups for other genetic risk factors. Objectives: We investigated the contribution to association with rheumatoid arthritis (RA) of the TYK2, STAT3 and STAT4 gene polymorphisms with regards to the serologic characteristics of the disease. Methods: Genome-wide association studies (GWAS) data for 2260 RA cases and 898 healthy controls included in the Swedish Epidemiological Investigation of Rheumatoid Arthritis (EIRA) study population was examined for the variants within TYK2, STAT3 and STAT4 genes and their flanking regions (*RAVER1*, *ICAM3* and *STAT1*). The regions of interest were additionally fine-mapped with 64 SNPs using the iPLEX Gold platform and/or TaqMan to define LD-blocks in each gene that have a higher association with RA subgroups. Results: We could demonstrate that *TYK2* (rs12720253), *RAVER1* (rs3181049) and *ICAM3* (rs2304237) are in association with RA and these associations are not RA subgroup specific (OR 1.38 [95% CI 1.11-1.71], OR 1.22 [95% CI 1.07-1.38] and OR 1.25 [95% CI 1.10-1.42] respectively). Furthermore, *STAT3* (rs9891119), *STAT4* (rs3024935) and *STAT1* (rs12693591) demonstrated associations that are stronger in the anti-citrullinated peptide antibody (ACPA) negative group of patients compared to the ACPA positive group of patients (OR 1.27 [95% CI 1.11-1.47],OR 1.48 [95% CI 1.15-1.92] and OR 1.40 [95% CI 1.15-1.71] respectively). Conclusion: Our study confirmed the importance of IL-23 pathway for RA susceptibility. We identified new genetic risk factors for RA with an indication that some of them are more characteristic for autoantibody negative subgroup of patients. The latter one is of particular interest since up till now very few genetic risk genes have been found for this subgroup of RA.

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Prospective Assessment of Different Genetic Effects on Progression to Intermediate or Large Drusen and Advanced Stages of Age-related Macular Degeneration. *Y. Yu[1], R. Reynolds[1], J. Fagerness[2], MJ. Daly[2], JM. Seddon[1,3].* 1) Ophthalmic Epidemiology and Genetics Service, Department of Ophthalmology, Tufts Medical Center, Boston, MA; 2) Center for Human Genetic Research, Massachusetts General Hospital, Program in Medical and Population Genetics, 185 Cambridge St. 6th Floor, Boston, MA; 3) Tufts University School of Medicine, Boston, MA.

Accumulation of drusen is seen in early and intermediate stages of age-related macular degeneration (AMD) and can progress to advanced AMD. Genetic variants in the complement, lipid and other pathways are associated with advanced AMD. Understanding roles of different genes in AMD progression may lead to prevention and treatments of this disease in its early stages. Eyes of 3,006 subjects in the Age-Related Eye Disease Study were graded as no drusen or small drusen (<63μm), intermediate drusen (63-124μm), large drusen (≥125μm), GA, or NV based on data from longitudinal ocular examinations and fundus photography. SNPs in the CFH, C2, C3, CFB, CFI, ARMS2/HTRA1, and novel LIPC, CETP, ABCA1 genes/regions were genotyped. Effects of SNPs on progression of each eye to intermediate drusen, large drusen, or advanced AMD, were assessed by Cox proportional hazards models with robust estimates of variance controlling for baseline status including age, gender, smoking, body mass index (BMI), education, antioxidant treatment, baseline drusen size in each eye, and SNPs of interest. The T allele of rs10490924 (ARMS2/HTRA1) increased hazard of intermediate drusen (hazard ratio [HR] = 1.2 [1.05-1.36], p=6.7E-03), large drusen (HR=1.48 [1.28-1.71], p=1.6E-07) and advanced AMD (HR = 1.34 [1.18-1.52], p=4.3E-06). The T allele of rs2230199 in C3 was associated with increased incidence of intermediate drusen (hazard ratio [HR] = 1.21 [1.06-1.37], p=4.2E-03), large drusen (HR=1.33 [1.14-1.55], p=2.8E-04) and advanced AMD (HR = 1.24 [1.09-1.42], p=1.1E-03). Two independent SNPs in CFH were also associated with progression to advanced AMD and large drusen (p<0.05). Decreased incidence of advanced AMD was associated with the TT genotype of LIPC (rs10468017, p=0.01, HR=0.66[0.47-0.92]). The T allele of rs1883025 in ABCA1 was associated with decreased risk of development of intermediate drusen (HR= 0.84[0.73-0.96], p=0.01) and large drusen (HR=0.85[0.73-1.0], p=0.05). Genes in different pathways influence AMD progression in different stages.

2960/T

CYP2R1 is a Potential Candidate for Predicting Serum 25(OH)D Variation as Suggested by Genetic and Epigenetic Studies. *Y. Zhou, F. Bu, J. Lappe, L. Armas, R. Recker, L. Zhao.* Creighton University Medical Center, Omaha, NE.

Achieving and maintaining an optimal level of serum 25-hydroxyvitmain D [25(OH)D] are important for preventing rickets, osteoporosis and osteoporotic fracture. Oral vitamin D3 supplementation is the best approach to increase serum 25(OH)D levels. However, high variability of serum 25(OH)D in response to a given dose of vitamin D supplementation is widely observed. Factors contributing to the wide variability in serum 25(OH)D are largely unknown. We first conducted a genetic epidemiology study to identify genes important for prevalent serum 25(OH)D variation. Nine candidate genes (ALPL, CYP24A1, CYP27A1, CYP27B1, CYP2R1, CYP3A4, GC, VDR, and PTH) important for vitamin D metabolism were comprehensively screened using 49 tag SNPs in 156 unrelated healthy Caucasian subjects. Evidence of association was observed at six SNPs in the CYP2R1 and GC gene. We further conducted a replication study for these six SNPs in an independent cohort with 340 unrelated healthy Caucasian subjects. Two SNPs in the promoter region of the CYP2R1 gene, rs10766197 (Pdiscovery = 0.007, Preplication = 0.019) and rs12794714 (Pdiscovery = 0.001, Preplication = 0.016), were confirmed to be significantly associated with prevalent serum 25(OH)D levels. CYP2R1 was first identified in 2003 as a key vitamin D 25-hydroxylase that converts vitamin D into 25(OH)D. In order to test whether the gene contributes to serum 25(OH)D variability in response to vitamin D3 supplementation, we conducted an epigenetic study. In total, 446 non-Hispanic white postmenopausal women were treated with calcium (1500 mg/day) and vitamin D3 (1100 IU/day) for 12-months. We selected 18 responders and 18 non-responders from the 446 subjects, at the two extreme tails of the distribution of dose-adjusted 12-month increase of serum 25(OH)D. For each subject, genomic DNA before and after 12-month vitamin D3 intervention was extracted from frozen serum. The average methylation ratio of the CYP2R1 promoter in the non-responder group (30%) was significantly higher than that in the responder group (8%) (p=0.004). In both groups, the DNA methylation ratio was unaffected by vitamin D3 intervention. The hypermetylation in the non-responder group may contribute to gene silencing of CYP2R1, leading to lower increase of serum 25(OH)D levels. These data suggest that CYP2R1 is a strong candidate for the variability in prevalent serum 25(OH)D, and variability in its response to vitamin D supplementation.

2961/F

Assessing Batch effects for BRLMM-P using the Affymetrix Axiom Platform. *R.W. Davies[1], A.F.R. Stewart[2], S. Shah[3], W.E. Kraus[3], E.R. Hauser[3], C.B. Granger[3], C.H. Haynes[3], R. McPherson[4], R. Roberts[3], G.A. Wells[1].* 1) Cardiovascular Research Methods Centre, University of Ottawa Heart Institute, Ottawa ON, Canada; 2) John and Jennifer Ruddy Canadian Cardiovascular Genetics Centre, University of Ottawa Heart Institute, Ottawa ON, Canada; 3) Department of Medicine, Duke University Medical Center, Durham NC, USA; 4) Atherogenomics Laboratory, University of Ottawa Heart Institute, Ottawa ON, Canada.

Introduction: The generation of genotypes from DNA microarrays involves the processing of batches of samples using genotype calling algorithms (GCA). These algorithms often borrow methodology from the field of machine learning, such that inference of genotypes depends on the samples included in the same batch. The extent to which various configurations of batches affect genotypes for the new Affymetrix Axiom genotyping platform using the default BRLMM-P GCA is as of yet unknown. Objective: To determine whether batch configuration plays a significant role in samples genotyped using BRLMM-P on the Axiom array. Methods: We genotyped 2081 samples from the Duke CATHGEN study, a coronary artery disease GWAS, for which 1942 CEL files which passed QC were generated. Splitting the data into four batches of equal size, we genotyped the samples and used principal-components analysis to remove samples of non-Caucasian ancestry. For the subsequent 1858 samples, we considered 5 different batch configurations, under the premise that while processing by plate, a common practice is to wait until at least X samples are available before genotyping (where X was 100, 200, 500, 1000 and all samples), genotype these samples, and then to wait until at least X samples are available before genotyping the next batch. We considered a dual-pass genotyping method, where on the second pass any sample which failed a 95% call rate on the first pass was excluded and genotyping re-performed. SNPs were deemed to pass QC if (HWE>1e-6 & ((CR>99% & 0.5%< MAF <5%) or (CR>95% & 5%< MAF <50%))). Analyses were conducted using apt-1.12.0 on a 4xE7440 computer with 16GB of RAM running RHEL5. Results: Call rates and sample exclusion by call rate in the first pass of genotyping were relatively consistent across batch configurations, so the number of samples available for each configuration was quite similar (>1843). The number of SNPs passing SNP-wise QC was also consistent at about 500,000. However, the genome-wide inflation factor lambda (logistic regression case versus control adjusted for sex only) was 1.040, 1.033, 1.026, 1.024, and 1.023 across the 5 methods as listed above, respectively. Run times were naturally longer for the configuration with all samples, but all analyses took less than 24 hours. Conclusions: The decrease in lambda with only a marginal increase in computational requirements and negligible loss of call rate suggests running BRLMM-P with as many samples as possible.

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The distribution of signal and noise in high throughput SNP arrays: application for IBD mapping. *B. Markus[1], O.S. Birk[1,2].* 1) The Morris Kahn Laboratory of Human Genetics at the National Institute for Biotechnology in the Negev, Beer-Sheva, Israel; 2) Institute of Genetics at Soroka Medical Center, Ben-Gurion University, Beer-Sheva, Israel.

High throughput SNP arrays have become the standard platform in genetic mapping of Mendelian and more complex traits. These dense arrays provide a convenient and practical way to compare the genomes of individuals and search for common regions indicating candidate loci for common phenotypes. Traditionally, methods have been developed to detect Identical By Descent (IBD) regions using low coverage markers and assuming no errors in the data. However, errors are intrinsic to high throughput technologies and have been shown to seriously hinder the ability to detect relatedness signals. A common practice is to try and eliminate as much as possible the errors prior to downstream statistical analyses. While this approach works very well within pedigrees, between pedigrees there is little information that facilitates error detection. Hence, IBD methods for unrelated individuals should incorporate a model for errors within the calculation of IBD probabilities. In this study we examined the distribution of mistyping in Affymetrix 250K arrays and suggested a model for incorporating this information in pair-wise IBD methods that use the Lander-Green algorithm. We demonstrate using real data the efficacy of incorporating errors within pair-wise IBD analyses which allows the recovery of IBD signals even in very low quality and noisy measurements. We discuss implication for IBD mapping in isolated population under unknown or hidden relatedness with examples of recessive traits from the Bedouin population.

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Quality Control Pipeline for Genome-Wide Association Studies in the eMERGE Network: Comparing Single Site QC to a Merged QC Approach. *M. Ritchie[1], L. Armstrong[2], Y. Bradford[1], C. Carlson[3,4], D. Crawford[1], A. Crenshaw[5], M. de Andrade[6], K. Doheny[7], J. Haines[1], G. Hayes[2], G. Jarvik[3,4], L. Jiang[1], H. Ling[7], I. Kullo[6], R. Li[8], T. Manolio[8], M. Matsumoto[6], C. McCarty[9], A. McDavid[3,4], D. Mirel[5], L. Olson[1], J. Paschall[10], E. Pugh[7], L. Rasmussen[9], R. Wilke[1], R. Zuvich[1], S. Turner[1].* 1) Molec Physiology & Biophysics, Vanderbilt Univ, Nashville, TN; 2) Northwestern University, Chicago, IL; 3) University of Washington, Seattle, WA; 4) Group Health Cooperative, Seattle, WA; 5) Broad Institute of MIT and Harvard, Cambridge, MA; 6) Mayo Clinic, Rochester, MN; 7) Center for Inherited Disease Research (CIDR), John Hopkins University, Baltimore, MD; 8) National Human Genome Research Institute (NHGRI), Bethesda, MD; 9) Marshfield Clinic, Marshfield, WI; 10) national Center for Biotechnology Information (NCBI).

Genome-wide association studies (GWAS) are being conducted at an unprecedented rate in disease-based cohorts and have increased our understanding of the pathophysiology of complex disease. Regardless of context, the practical utility of this information will ultimately depend upon the quality of the original data. Quality control (QC) procedures for GWAS are computationally intensive, operationally challenging, under constant evolution, and critically important. What has not yet been explored in detail are the challenges that emerge when multiple GWAS datasets, genotyped in different labs, are merged for downstream GWAS analysis; a scenario that is likely to increase in frequency with the advent of dbGaP. The genomics workgroup of the NHGRI funded electronic Medical Records and Genomics (eMERGE) network has spent a considerable amount of effort developing strategies for quality control of these data. eMERGE consists of five sites, each with DNA databanks linked to electronic health information. Approximately 17,000 samples have been genotyped (~15000 European Americans using Illumina 660W half performed at each (Broad and CIDR) and ~2000 African Americans using Illumina 1M performed at Broad), and phenotypes have been enumerated for ~20 diseases and traits. The lessons learned by this group of investigators will be valuable for the genomics community also dealing with the combining of large scale genomic datasets. We compare the characteristics of various quality control measures between each of the five eMERGE sites, and the merged dataset. Here we enumerate some of the challenges in QC of merged GWAS datasets, including population substructure, merging data from 2 genotyping centers, strand orientation, and other errors inherent from merging ~17000 samples GWAS data, and describe the approaches that the eMERGE network uses to guarantee quality assurance in GWAS data, thereby minimizing potential bias and error in GWAS results. Finally, we describe the best practices that we have decided upon, such as having 2 sites duplicate efforts to eliminate errors early on in the process, and discuss areas of ongoing and future research.

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On data normalization and pre-processing of family-based association studies of copy number variations. *J. Chu[1], I. Ionita-Laza[2], A. Rogers[1], K. Darvishi[3], N. Laird[4], C. Lee[3], B.A. Raby[1].* 1) Channing Laboratory, Brigham and Women's Hospital, Boston, MA; 2) Columbia University, New York, NY; 3) Department of Pathology, Brigham and Women's Hospital, Boston MA; 4) Department of Biostatistics, Harvard School of Public Health, Boston MA.

The task of normalizing and preprocessing data from copy-number variation (CNV) experiments is a challenging problem. While many methods have been considered to improve the CNV calling and data quality, none have leveraged family-based data. Here we describe a normalization method for family-based CNV studies which applies a simple linear regression model for continuous CNV measurements from parents and children. This approach is advantageous because the normalization of an individual array is informed by intensity distributions generated from three related, yet semi-independent arrays. The method is computationally efficient. We demonstrate our method by its application to a family-based CNV data set of 385 trios from a CNV genome-wide association study of asthma patients, using a custom 180k Agilent CGH CNV genotyping array which characterizes ~20,000 known CNV regions. We compare the results of FBAT (family-based association test) and eQTL analysis with expression data from peripheral blood CD4+ lymphocytes, using various combinations of preprocessing and normalization methods, including quantile normalization, PCA (principle component analysis) and our new method for intra-family adjustment. We show that the family adjustment improves upon more standard normalization methods. It can facilitate the identification of poor quality arrays, sample mix up, and outliers. Importantly, this approach results in more self-consistent signals within the family in ~95% of the trios, and when used in conjunction with other normalizing methods, can improve identification of disease-relevant CNVs. In our applied example - a genome-wide CNV association study of asthma - implementation of our method resulted in much stronger associations as compared to more traditional normalization methods, resulting in the identification of several novel asthma-susceptibility candidates.

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Description of transmission distortion in two family-based GWAS. *H. Ling[1], JR. Shaffer[2], TH. Beaty[3], ML. Marazita[2], I. Ruczinski[3], E. Feingold[2], CC. Laurie[4], D. Crosslin[4], AF. Scott[4], KF. Doheny[1], EW. Pugh[1].* 1) IGM, Johns Hopkins University SOM, Baltimore, MD; 2) University of Pittsburgh, Pittsburgh, PA; 3) Johns Hopkins University, School of Public Health, Baltimore, MD; 4) University of Washington, Seattle, WA.

Transmission equilibrium, defined as equal transmission probabilities for each of the two alleles at a genetic locus passed from parent to child, is a fundamental assumption for many genetic analyses. However, distortion of the overall transmission ratio has been reported in a variety of studies for both humans and model organisms. Two NIDCR supported GWAS genotyped at CIDR and also part of GENEVA, one of the programs of the trans-NIH Genes, Environment and Health Initiative, included family data (1) a parent-offspring trio study of oral cleft where families were ascertained through an affected child and (2) a nuclear family cohort study of dental caries where ascertainment was independent of phenotype. Both were genotyped on the Illumina 610Quad array. These two studies contain a large number of trios, ~2000 and 752 for cleft and dental caries studies respectively, allowing us to investigate the transmission ratio for high density arrays of SNPs on a genome wide scale. We examined the transmission rate for autosomal SNPs overall, and by direction and degree of difference from expectation in both studies. For the cleft study we also looked at results after excluding regions associated with the trait. Overall, the transmission ratio for the minor alleles was less than 0.5 more often than it was greater. For most SNPs, the deviation from 0.5 was small. SNPs with low minor allele frequency were more likely to have large departures from 0.5 in both directions. Applying SNP quality control (QC) filters developed by the GENEVA coordinating center remarkably reduced the number of SNPs showing significant evidence of transmission distortion. Among these QC filters, the most important one for removing apparent transmission distortion was call rate. Review of the clustering plots for selected SNPs found a variety of clustering problems for many, but not all SNPs showing extreme transmission distortion. Thus, the statistical evidence for true transmission distortion in humans based on genome wide marker panels is tenuous and may be driven by genotyping errors. NIH grants U01DE004425 and U01DE018903, U01DE018993, NIH Contract HHSN268200782096C, U01HG004446.

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DNA Fingerprinting: Utilizing the OpenArray® Platform for Genetic Barcoding of Research Samples. *P.L. Whitehead[1], G.W. Beecham[1], S. Slifer[1], I. Konidari[1], W.F. Hulme[1], K. Hayashibara[2], M.A. Pericak-Vance[1], J.M. Vance[1], J.R. Gilbert[1], D.J. Hedges[1], J.L. McCauley[1].* 1) Hussman Institute for Human Genomics, University of Miami, Miami, FL; 2) Applied Biosystems (a part of Life Technologies), 850 Lincoln Centre Drive, Foster City CA.

We have defined a panel of single nucleotide polymorphisms (SNPs) to obtain a “genetic barcode” or “fingerprint” of DNA samples. The primary purpose of this panel is to aid in the quality control of biological research sample storage and experimental processing. The need for this panel stems from the continued expansion of biological repository collections (so-called Biobanks). Rapid progress in genomic technology has allowed for large numbers of loci to be rapidly assayed within individuals (e.g. GWAS arrays & Next Generation Sequencing). While powerful, these experiments are costly, and handling errors can both confound downstream analysis as well as waste financial resources. As the number of samples stored and processed in the course of biological research expands by orders of magnitude, it becomes imperative that increased rigor is devoted to the tracking and confirmation of sample identity. To this end, we have created a genetic barcode panel with markers selected based on a number of criteria, including: high minor allele frequency in multiple ethnic populations, representation on high throughput genotyping, and robust genotyping performance via the Applied Biosystem’s TaqMan® assays using the OpenArray® platform. A set of 32 markers were selected to allow unique identification of individuals within the context of a large biorepository setting. This panel takes into account as much diversity as possible to provide both current and future utility as sample collections grow. This barcoding system allows us to uniquely identify individuals throughout each stage of the handling and experimental process. It provides both sample identity and gender checks in the event of sample mix-ups or labeling mistakes. Finally, it serves as a thorough and robust internal control for the expensive genome-wide association studies that have become common place in the field of complex disease research. As our panel contains markers from these large arrays as a primary criterion for inclusion, we now have the ability to match sample genotypes directly from the panel to the GWAS array. This in turn reduces overall experimental error and provides increased assurances and reliability of matching the data to the correct sample. Our primary panel of 32 (along with our extended set of an additional 32 markers) has the potential to be incorporated into a host of genotyping and next generation sequencing platforms, serving the needs of current Biobanks and genomics centers.

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Haplotype Phasing from Signal Intensity Data. *Z. Yu, Y. He.* Dept Statistics, Univ California, Irvine, CA.

Genotype calling and imputation of missing genotype data are traditionally conducted in two separated steps. In the genotype calling step, although different statistical strategies have been applied, when the allele signal of a marker is not perfectly separated, missing values are generated. Missing data are then imputed based on called genotypes and underlying linkage disequilibrium (LD) structure of SNPs. Recently we proposed a unified approach that simultaneously makes genotype calls and imputes missing genotypes; using empirical data from Wellcome Trust Case-Control Consortium (WTCCC), we showed that it improves genotype calling of other alternatives. To quantify its performance on haplotype phasing, we first simulated signal data using parameters estimated from WTCCC data, and then compared the new approach and the traditional two-stage approach on phasing. Our results show that the unified approach reduces haplotype phasing error substantially. For simulated trio data, we found that incorporating family structure further reduces genotyping and phasing errors.

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Comparison of genotype calling algorithms for accuracy and missingness at rare SNPs in a genome-wide association study. *C.S. Franklin[1], P. Williams[2], S. Potter[1], H. Blackburn[1], S. Hunt[1], L. Morgan[2,3], R. McGinnis[1], Wellcome Trust Case Control Consortium 3.* 1) Wellcome Trust Sanger Institute, Cambridge, Cambridgeshire, UK; 2) School of Molecular Medical Sciences, University of Nottingham, Nottingham, UK; 3) UK Genetics of Pre-eclampsia Consortium.

Rare SNPs are likely to cause complex human disease but SNPs with minor allele frequency (MAF) below 5% can be difficult to accurately genotype since their genotype classes are small or absent and can be problematic for clustering algorithms. To assess genotype accuracy and missingness of the GenCall (GEN) and Illuminus (ILM) calling algorithms, we compared GEN and ILM genotypes from the Illumina 660 array in 1036 Pre-Eclampsia women who are the first half of expected cases in the Wellcome Trust Case Control Consortium 3 (WTCCC3) Pre-Eclampsia study. To identify SNPs with large MAF differences in genotype calls from the two algorithms, we treated the GEN data as “cases” and the ILM data as “controls” and thereby conducted a pseudo case-control analysis using Fisher’s Exact (FE) test. A subset of SNPs identified as significant (p-value< 0.05) were assessed by visually inspecting cluster plots using EVOKER software to determine which, if either, algorithm was correct. Overall, GEN was more stringent in entirely excluding 31245 out of 580,030 autosomal SNPs which ILM called at a mean call rate of 97.4%. By contrast, ILM entirely excluded only 236 SNPs of which 116 were also excluded by GEN. The pseudo association analysis identified 3033 polymorphic SNPs successfully called by both algorithms and having a FE-test p-value <0.05. The MAF distribution of these SNPs was highly skewed with 63% falling below a MAF of 0.05. These 63% low frequency SNPs were characterised by high call rate in both GEN (0.99) and ILM (0.98) with a mean MAF difference between the two algorithms of 0.022. The 37% higher frequency SNPs had on average lower call rates in one or both algorithms and a larger mean MAF difference of 0.071. Visual inspection of genotype cluster plots from over 1100 SNPs with the largest difference in GEN and ILM MAF found the GEN genotype calls correspond far more accurately to true genotypes especially at low MAF. For SNPs with MAF <0.05, 91.5% were correctly called by GEN, 0.5% by ILM and 8% were poorly called by both algorithms. Our initial results indicate that both ILM and GEN perform well on SNPs with high MAF and, given the higher rate of missingness when using GEN, ILM may be preferable for analysis of common variants. However, our examination of low MAF SNPs with divergent calls for the two algorithms finds that the GEN algorithm is considerably more accurate.

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False positive associations due to batch effects in a genome-wide association study of brain arteriovenous malformation (BAVM). *H. Kim[1,2,3], L. Pawlikowska[1,2], S.M. Weinsheimer[1], N. Bendjilali[1], P.Y. Kwok[2,4], C.E. McCulloch[3], W.L. Young[1,5,6].* 1) Center for Cerebrovascular Research, Dept Anesthesia, UCSF, San Francisco, CA; 2) Institute for Human Genetics, UCSF, San Francisco, CA; 3) Dept Epidemiology & Biostatistics, UCSF, San Francisco, CA; 4) Cardiovascular Research Institute, UCSF, San Francisco, CA; 5) Dept Neurology, UCSF, San Francisco, CA; 6) Dept Neurological Surgery, UCSF, San Francisco, CA.

Background: Genome-wide association (GWAS) studies of complex phenotypes are an established approach for identifying genetic variants, but require stringent quality control to limit false positive findings. While systematic errors can affect genotyping accuracy and call rates, the effect of batch size is not well studied. The optimal batch size for genotype calling would minimize batch to batch effects while providing reliable calls. Thus, we investigated the effect of batch size on genotype calling using GWAS data from a study of sporadic brain arteriovenous malformations (BAVM), a common cause of hemorrhagic stroke in young adults. **Methods:** DNA samples from 401 cases and 1022 controls were genotyped using Affymetrix SNP Array 6.0, and called using Birdseed v2 algorithm (Affymetrix Power Tools). Genotypes were called in 2 batch sizes: large batch (all samples called together), and small batch (≥42 samples in 9 batches ranging from 42-806). Before allelic association analysis, we excluded SNPs with low genotyping call rates (<95%), out of Hardy-Weinberg equilibrium (P<1e-05), and with low minor allele frequency (MAF<1%). Samples were excluded for low genotyping call rates (<95%), sex mismatches, duplicates, or other disease phenotypes (n=313). **Results:** Batch size influenced the number of samples and SNPs passing QC. Large batch calling resulted in exclusion of more samples (848 vs. 933 remaining) and SNPs (717,335 vs. 743,083 remaining), with similar genotyping call rates (99.2% vs. 99.3%). Allelic association analysis yielded 49 SNPs from large batch calling and 212 SNPs from small batch calling (Bonferroni-adjusted P<0.05). The MAF for cases was much higher in small batch vs. large batch, whereas MAF was similar for controls. SNP cluster graphs for 7 SNPs with large MAF batch differences revealed 1 of 5 case batches incorrectly called each time, resulting in falsely elevated MAF. Technical batch effects were not observed, and case batch size did not correlate with calling error. **Conclusions:** Affymetrix recommends ≥44 samples per batch for genotype calling. However, in our study, call reliability was questionable for smaller batches, even with batch sizes of 100. Genotype calling in a single large batch corrected this inflation at the expense of dropping more cases from analysis. Thus, in our study the inaccuracy of calling in smaller batches was a greater concern than controlling for batch to batch variation.

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Genotype concordance between Illumina 1M and Affymetrix 6.0 SNP arrays. *L.C. Kwee[1,2,3], C. Haynes[1,2], J.R. Gibson[1,2,3], A. Stone[4], S.A. Schichman[4], Y. Liu[1,2,3], E.Z. Oddone[2,3], M.A. Hauser[1,2,3], S. Schmidt[1,2,3].* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) Dept. of Medicine, Duke University Medical Center, Durham, NC; 3) Epidemiology Research and Information Center, VA Medical Center, Durham, NC; 4) Pathology and Laboratory Medicine Service and Research Service, Central Arkansas Veterans Healthcare System, Little Rock, AR.

We genotyped 1539 samples from the GENEVA (Genes and Environmental Exposures in Veterans with ALS) study on the Illumina Human1M-Duo and Affymetrix Genome-Wide Human SNP 6.0 arrays. The substantial number of overlapping SNPs between these arrays allowed us to assess genotype concordance and quality for each platform. Samples were included in the analysis if they produced ≥ 98% call rate on both arrays, were not cryptically related to other samples, and showed agreement between self-reported and genotype-inferred gender. We called Illumina genotypes using Illumina's GenomeStudio software, and included SNPs with call frequency ≥98% that passed several QC filters (cluster separation, heterozygote excess, number of replication errors, AB R mean, AB T mean, and T deviation). We analyzed Affymetrix arrays using Birdseed v2 models and included SNPs with call frequency ≥98%. SNPs were excluded from analysis of both arrays if they were monomorphic, showed differential missingness between cases and controls (p<10-5) or failed a test of HWE in controls (p<10-6). After application of these filters, 1462 samples (95%) remained with genotypes on both arrays. Mean genotype reproducibility of duplicate samples was excellent: >99.99% for Illumina and >99.90% for Affymetrix. Of the 294,666 autosomal SNPs present on both arrays, 264,733 SNPs (89.8%) passed the QC filters for both. An additional 25,313 SNPs (8.6%) passed QC criteria for one array, but not the other. In order to assess genotype concordance, we considered 257,202 non-AT/CG SNPs with genotypes on both chips. The median number of discordant Affymetrix-Illumina genotypes per sample was 113 (0.04%), with a range of 43-6309 discordant genotypes (0.02-2.45%). 115,132 markers (44.8%) produced completely concordant genotypes on the two arrays. The remaining SNPs were discordant for a median of 2 samples (range of 1-1264). We propose using an exact test of genotype frequencies between two chips as an additional SNP QC filter when platforms are merged: for the GENEVA data, such a test (p<.001) identified only 422 additional markers to be removed. We also illustrate the results of applying a similar QC strategy to the publicly available genotypes generated on the WTCCC-Phase 2 control samples with the same two platforms. These results show that when appropriate QC filters are applied, the Affymetrix 6.0 and Illumina Human 1M arrays produce highly-concordant genotypes.

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Replication of Genetic Variants associated with Serum Lipids in African Americans. *K. Meilleur, A. Adeyemo, A. Doumatey, D. Shriner, H. Huang, J. Zhou, E. Ramos, G. Chen, C. Rotimi.* Center for Research on Genomics and Global Health, NHGRI, NIH, Bethesda, MD.

Statement of Purpose: Both candidate and genome-wide association studies (GWAS) have identified multiple genetic variants associated with serum lipid parameters including triglycerides, HDL and LDL-cholesterol. However, few of these studies have been conducted in African Americans (AA), who have been reported to display different lipid profiles compared to other US groups. We sought to identify novel susceptibility loci for dyslipidemia in AA and to assess whether observed differences in lipid parameters between AA and other ethnic groups could be explained by differences in genetic background. Methods: In the present study, we tested a set of genetic variants associated with lipid traits in GWAS in a sample of 927 African Americans from the Washington DC metropolitan region. Genotyping was conducted using Affymetric® Genome-wide Human SNP Array 6.0 with genotyping calls determined by Birdseed, v2. Imputation was conducted using MACH and the HapMap reference panels for CEU and YRI. A total of 2,366,856 SNPs were assessed for association with lipid traits using PLINK under the additive model with adjustment for age, sex, BMI, and the first 2 principal components identified in the assessment of population stratification. Results: We identified a significant novel locus (rs1047163; p-value 6.4X10-8) associated with HDL on chromosome 2; this SNP is located in the 3' untranslated region (UTR) of HS1BP3 (hematopoietic-specific protein 1 binding protein 3 gene). An intergenic SNP (rs820042) also on chromosome 2 was identified to be associated with LDL. We replicated previous associations with SNPs in the following genes: LPL, APOB, [APOE, APOC1, APOC4, APOC2], PCSK9, [CELSR2, PSCRC1, SORT1], HMGCR, [NCAN, CILP2, PBX4], CETP, DOCK7, B3GALT4, TRIB1, and TOMM40. Conclusion: Overall, we identified novel susceptibility loci for HDL and LDL cholesterol and replicated several reported GWAS findings in this cohort of African Americans.

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Practical Consideration of Genotype Imputation: Sample Size, Window Size, Reference Choice and Untyped Rate. *N. Liu, B. Zhang.* Dept Biostatistics, Univ Alabama at Birmingham, Birmingham, AL.

Imputation offers a promising way to infer the missing and/or untyped genotypes in genetic studies. In practice, however, many factors may affect imputation results. In this study we evaluated the untyped rate, the sizes of the study sample and reference sample, the window size, and reference choice for admixed study samples as the factors affecting the quality of imputation. The results show that in order to have good imputation quality, it is necessary to have an untyped rate less than 50%, reference sample size greater than 50, and a window size of greater than 200 SNPs (roughly 0.4 Mb in base pairs) on a chromosome. Compared with whole-region imputation, piecewise imputation with large enough chunk size performs better, suggesting that a chunk size smaller than the one recommended by IMPUTE may be possible. For an admixed study sample, in order to have good quality of imputation, the external reference panel should include samples from the source populations of admixture. If possible, internal references are strongly recommended. When internal references are limited, however, augmentation by external references should be used very careful. Surprisingly, augmentation should avoid references from the populations other than the source populations of admixture, especially the major source of admixture.

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Reconstruction of pedigrees from populations using genotype data. *E. Stevens[1], E. Roberson[1,2], G. Heckenberg[3], T.J. Downey[3], J. Pevsner[1,4,5].* 1) Program in Human Genetics, Johns Hopkins School of Medicine, Baltimore, Maryland, USA; 2) Present address: Dept. of Genetics, Washington University, CB8232, 4566 Scott Avenue, St. Louis, Missouri, MO 63110; 3) Partek Inc., St. Louis, Missouri, USA; 4) Department of Neurology, Hugo Moser Institute at the Kennedy Krieger Institute, Baltimore, Maryland, USA; 5) Department of Neuroscience, Johns Hopkins School of Medicine, Baltimore, Maryland, USA.

Patterns of human genetic diversity can be addressed through genotype studies of geographically distinct groups using single nucleotide polymorphism (SNP) data. Methods such as principal components analysis (PCA) are commonly employed to visualize relationships between samples, often matching the geographic distribution of the cases. Identity-by-state (IBS) methods are also useful to describe genetic relatedness of individuals within large samples. We introduce an IBS-based method that is largely independent of population allele frequencies to identify familial relationships, unrelated individuals, and anomalous heterozygosity rates in large-scale SNP data sets. We applied this method to 1,397 Phase 3 HapMap individuals and reconstructed 34 novel pedigrees. In one group having 30 parent/child trios and 94 nominally unrelated individuals (n=184), we reconstructed a single pedigree containing 178 (MKK) individuals; in three separate HapMap groups we reconstructed pedigrees having 32 (ASW), 29 (LWK), and 25 (MXL) individuals. Other Hapmap groups with unexpected relationships were CEU, CHD, GIH, TSI, and YRI. We applied the method to additional data sets: the Human Variation Panel from Coriell, extended CEPH pedigrees, a large gene environment association study, real data from clinical studies and from a large autism study, and synthetic data. This resulted in the identification of many novel, unexpected relationships. The application of this approach could impact genome-wide association studies, linkage, heterozygosity, and other population genomics studies that rely on SNP genotype data.

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They are relatives. *C.H. Chen, M.H. Su, T.H. Lu.* Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan.

Background: Phase III of HapMap project (HapMap3) comprised genome-wide genotypes of 1184 subjects from 11 ethnicity groups. It was documented that 155 trios and 36 parent-offspring duos existed in the sample. However, not all of the relationships between the HapMap3 subjects were fully assessed. We propose a method using two summary statistics, the average identical by state of genome-wide single nucleotide polymorphisms (gIBS) and the count of zero identical by state (IBS0), to determine the kinship type of two persons in question.

Methods: We simulated pedigree data based on population allele frequencies to obtain cutoffs of gIBS for differentiating major types of kinships. We evaluate the cutoffs by the reported kinships in the HapMap3 sample. We also assess relationships between the preassembly unrelated subjects in HapMap3. We further differentiated between full-sibling pairs and parent-offspring pairs by IBS0.

Results: Our results show (1) the gIBS cutoffs for major types of kinships vary but are similar between the 11 HapMap3 ethnicity groups; (2) all of the 346 previously reported parent-offspring pairs in HapMap3 are correctly categorized by the cutoffs; (3) more than 150 cryptic kinships between the HapMap3 subjects, including 26 full sib pairs and 27 parent-offspring pairs, are uncovered using gIBS and IBS0. The results are confirmed by forensic analysis based on likelihood ratios.

Conclusions: Our method using gIBS and IBS0 was able to accurately recognize the major types of kinships between the HapMap3 subjects. The uncovered cryptic kinships between the HapMap3 subjects may guide the use the HapMap3 data and facilitate the on-going sequencing of the 1000 genomes project.

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A quality control algorithm for filtering SNPs in genome-wide association studies. *M. Pongpanich[1], P. Sullivan[2], J. Tzeng[1].* 1) North Carolina State University, Raleigh, NC; 2) University of North Carolina at Chapel Hill, Chapel Hill, NC.

The quality control (QC) filtering of single nucleotide polymorphisms (SNPs) is an important step in genome-wide association studies (GWAS) to minimize potential false findings. SNP QC commonly uses expert-guided filters based on QC variables (e.g., Hardy-Weinberg equilibrium, missing proportion, and minor allele frequency) to remove SNPs with insufficient genotyping quality. The rationale of the expert filters is sensible and concrete, but its implementation requires arbitrary thresholds and does not jointly consider all QC features. We propose an algorithm that is based on principal component analysis and clustering analysis to identify low-quality SNPs. The method minimizes the use of arbitrary cutoff values, allows a collective consideration of the QC features, and provides conditional thresholds contingent on other QC variables (e.g., different missing proportion thresholds for different minor allele frequencies). We apply our method to the seven studies from the Wellcome Trust Case Control Consortium (WTCCC) and the major depressive disorder study from the Genetic Association Information Network (GAIN). We measured the performance of our method compared to the expert filters based on the following criteria: (a) percentage of SNPs excluded due to low quality, (b) inflation factor of the test statistics (λ), (c) number of false associations found in the filtered dataset, and (d) number of true associations missed in the filtered dataset. The results suggest that with the same or fewer SNPs excluded, the proposed algorithm tends to give a similar or lower value of λ, a reduced number of false associations, and retains all true associations.

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Software for Generating Linkage-disequilibrium Aware Genotype Calls From Next Generation Sequence Data. *C. Sidore[1,4,5], G. Abecasis[1], H.M. Kang[1], Y. Li[1,2,3], S. Sanna[4], S. Zollner[1,6], Y. Lo[1].* 1) Department of Biostatistics, Center for Statistical Genetics, University of Michigan, Ann Arbor, Michigan, USA; 2) Department of Biostatistics, University of North Carolina, Chapel Hill, North Carolina, USA; 3) Department of Genetics, University of North Carolina, Chapel Hill, North Carolina, USA; 4) Istituto di Neurogenetica e Neurofarmacologia, CNR, Monserrato, 09042 (CA), Italy; 5) Dipartimento di Scienze Biomediche, Università di Sassari, Sassari, Italy; 6) Department of Psychiatry, University of Michigan, Ann Arbor, Michigan, USA.

The analysis of data from large-scale sequencing efforts poses challenges for management of computational resources. A key goal is to accurately identify and genotype variants using millions of short read sequence reads from many individuals. This process typically involves multiple complex steps and requires substantial amount of computational expertise. We have implemented a variant calling pipeline that identifies a set of high quality variants by combining data across individuals and generates genotypes for each individual, taking into account linkage disequilibrium relationships between markers and previous genotype data for the sequenced samples. Our pipeline facilitates variant calling by connecting procedures such as read mapping, read filtering and recalibration, genotype likelihood computation, initial variant calling and filtering, integration with existing array-based genotypes, and improvement of genotype calls using linkage disequilibrium aware hidden-markov models. Our variant calling pipeline can combine data from multiple sources and accommodate various contexts of types of sequencing project, including traditional deep sequencing of a few samples as well as low-coverage whole genome sequencing or targeted sequencing of many samples. Our pipeline uses Python to integrate multiple independent tools and can execute back-end modules in parallel through a cluster system. The entire pipeline requires minimal computational expertise from end-users but can also be fully parameterized to accommodate different software tools or custom settings for intervening steps. We illustrate performance with results from variant calling in the 1000 Genome Samples, in a an ongoing project that is sequencing the genomes of 1,000 Sardinians, and in targeted sequencing project of 200 genes in 15,000 individuals.

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A genotype imputation algorithm for allelic dropout in microsatellite data. *C. Wang[1], N.A. Rosenberg[1,2,3].* 1) Center for Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI 48109 USA; 2) Department of Human Genetics, University of Michigan, Ann Arbor, MI 48109 USA; 3) Life Sciences Institute, University of Michigan, Ann Arbor, MI 48109 USA.

Allelic dropout is a commonly observed source of missing data in microsatellite genotypes, in which one or both allelic copies at a locus fail to be amplified by the polymerase chain reaction. This problem especially affects studies that use noninvasive and forensic samples, for which DNA quality is often poor. A consequence of allelic dropout is a bias in estimates of heterozygosity, due to mistaken classifications of heterozygotes as homozygotes when one of the two copies drops out. In this study, we propose an imputation strategy to correct for allelic dropout when only one set of nonreplicated genotypes is available. First, we use a maximum likelihood approach together with an EM algorithm to jointly estimate allelic dropout rates and allele frequencies. Next, using the estimated parameters and an assumption of Hardy-Weinberg equilibrium, we correct the bias in the estimation of heterozygosity through the use of multiple imputations of alleles in cases where dropout might have occurred. With simulation data, we show that our method can (1) correctly estimate the dropout rates if they are sufficiently small (<15%), and (2) successfully correct the bias in estimating heterozygosity. Because the datasets imputed under our model can be used in additional subsequent analyses, our method will be useful for a variety of population-genetic studies of degraded or limited DNA samples.

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Ordered subset analysis identifies loci influencing lung cancer risk on chromosomes 6q and 12q. *S. Fang[1], S. Pinney[2], J. Bailey-Wilson[3], M. Andrade[4], Y. Li[1], E. Kupert[2], M. You[5], A. Schwartz[6], P. Yang[4], M. Anderson[2], C. Amos[1].* 1) Epidemiology, UT M.D Anderson Cancer Cente, Houston, TX; 2) University of Cincinnati, Cincinnati, Ohio; 3) National Human Genome Research Institute, Baltimore, Maryland; 4) Mayo Clinic College of Medicine, Rochester, Minnesota; 5) Washington University, St. Louis, Missouri; 6) Karmanos Cancer Institute, Wayne State University, Detroit, Michigan.

Genetic susceptibility for lung cancer can differ substantially across families. Locus heterogeneity in the familial lung cancer linkage study has not been sufficiently evaluated with respect to family-specific susceptibility. We had previously performed a genome-wide linkage screen in 93 families. Samples and data were collected by the familial lung cancer recruitment sites of the Genetic Epidemiology of Lung Cancer Consortium. We estimated linkage scores for each family by the Markov Chain Monte Carlo procedure using SimWalk2 software. We used ordered subset analysis (OSA) to identify genetically more homogenous families by ordering families based on a disease-associated covariate. Permutation testing was used to determine the significance of the linkage in optimal OSA subsets with the maximum linkage score. A genome-wide screen for lung cancer loci identified strong evidence for linkage to 6q23-25 and nearly significant evidence for linkage to 12q24 using OSA, with peak LOD scores of 4.19 and 2.79, respectively. We found other chromosomes also suggestive for linkages, including 5q31-q33, 14q11, and 16q24. Analyses were conducted ranking the family level of risk for several other cancers and identified subsets of families with significantly increased LOD scores on chromosomes 1q23, 2p11, 6q23-25, 13p12, and 17p11 ($p<0.05$). Our OSA results support 6q as a lung cancer susceptibility locus and provide nearly significant evidence for disease linkage on12q24. Validation studies using large sample size are needed to verify the presence of several other chromosomal regions suggestive of an increased risk for lung cancer and/or other cancers.

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Extension of the PPL framework to allow Lander-Green based computation. *S. Seok, Y. Huang, V. Vieland.* BCMM, RINCH, Columbus, OH.

The PPL framework is a flexible set of techniques for linkage and linkage disequilibrium (association) analysis in human genetics. The primary software package in which the PPL is implemented, KELVIN [see Huang et al., this meeting], utilizes the Elston-Stewart (ES) algorithm for pedigree calculations. However, for smaller families with dense SNP maps, it becomes important to have recourse to the Lander-Green (LG) algorithm as well. Because the PPL framework involves numerical integration over moderately high-dimensional trait spaces, it requires large numbers of likelihood ratio or LOD calculations at each position, requiring specifically adapted approaches. With the ultimate goal of providing on-the-fly selection of algorithm type based on data structures, we here demonstrate our underlying approach to LG-based PPL calculations by adapting Merlin [Abacasis, 2002], and formulating a hybird LG-ES approach to handling the trait model. Merlin uses binary tree data structures to represent the likelihoods of all possible inheritance patterns for both trait models (trait tree) and markers (marker tree). The LOD score is a function of the average trait likelihood (via a trait tree), the average marker likelihood (via a marker tree), and the average likelihood under the alternative hypothesis of linkage (via the trait and the marker trees) over all possible inheritance patterns. We take advantage of the fact that at marker positions, marker trees tend to be very sparse. Rather than constructing full trait trees via LG at these positions, we use the ES algorithm to compute the trait likelihood, which is faster (since multipoint marker data are not needed for the trait calculation alone) and which involves negligible memory use. For the alternative likelihood, only the partial trait tree with nodes (inheritance pattern) corresponding to nonzero marker likelihood values is needed. The savings in both memory and compute time are proportional to the sparsity of the marker tree. This technique is also well suited for calculations of LOD scores maximized over the trait model, or MOD scores. Additional features to improve the speed of the calculations while minimizing memory requirements are developed, including implementation of an imprinting model that does not increase the tree size.

2980/F

Using Parametric Multipoint LODs and MODs for Linkage Analysis Requires a Shift in Statistical Thinking. *S.E. Hodge[1], Z. Baskurt[2,3], T. Chiang[2], L.J. Strug[2,3].* 1) NYSPI, Unit 24, Columbia Univ, New York, NY; 2) The Hospital for Sick Children, Toronto, ON; 3) University of Toronto, Toronto, ON.

Multipoint (MP) linkage analysis represents a valuable tool for whole-genome studies but suffers from the disadvantage that its limiting distribution is unknown. Moreover, that limiting distribution varies with marker information and density, genetic model, number and structure of pedigrees, and the affection status distribution [Xing & Elston, 2006; Hodge et al., 2008]. This implies that **the MP significance criterion can differ for each marker and each dataset.** One way to circumvent this difficulty is to use simulations or permutation testing. However, a better theoretical understanding is preferable, which we present here: We show how to use the *evidential statistical paradigm* for planning, conducting and interpreting MP linkage studies for LODs and MODs. This involves a) specifying a simple alternative hypothesis (used for planning only), b) formulating the likelihood ratio (LR) between that alternative and the null hypothesis of no linkage to calculate error rates for planning, and c) maximizing the LR and using the magnitude of the maximized LR as a direct measure of evidence strength for or against linkage, compared to a criterion k (k can be 32, 100, or 1,000, etc.) The evidential analog to type I error rate, M, is calculated for the criterion k. We know from Royall [2000] that there is a large-sample bound for M using MP LODs, also that M®0 as n®¥. Moreover, M is naturally low. So set a reasonable value of k, as opposed to setting a fixed value for Type I error (α) as the criterion; then use sample size to control probabilities of weak evidence (it is weak evidence when 1/k<LR<k). We show: (1) Under the alternative hypothesis, error rates also remain low, and we can similarly control them via sample size (shown via simulations); (2) Although error rates under the null are higher for MODs than for LODs, under the alternative hypothesis the opposite is true where power is higher for MODs than for LODs (shown analytically); and (3) M under both null and alternative is greatest at the midpoint between contiguous markers spaced furthest apart, which provides an obvious simple alternative hypothesis to specify for planning MP linkage studies (shown analytically). In conclusion, with α allowed to vary rather than being fixed, the MP LODs and MODs can, themselves, be used to interpret the evidence strength. Moreover, **a common criterion k can be applied at each marker to represent strong evidence favouring linkage.** .

2981/F

Ordered Subset Analyses of Prostate Cancer Susceptibility in Finland Provides Evidence for Replication of *HPCX1* and *HPC10* Loci and Suggestive Evidence for Several Novel Loci. *C.D. Cropp[1], C.S. Simpson[1], T. Wahlfors[2], A. George[1,3], H. Nati[2], T. Tammela[4], J. Schleutker[2], J.E. Bailey-Wilson[1].* 1) Statistical Gen Branch, IDRB/NHGRI/NIH, Baltimore, MD; 2) Institute of Medical Technology, University of Tampere and Tampere University Hospital, Tampere, Finland; 3) Fox Chase Cancer Center, Philadelphia, Pennsylvania; 4) Department of Urology, Tampere University Hospital, University of Tampere, Tampere, Finland.

Prostate cancer is the most common non-cutaneous cancer in men in America and European industrialized countries. It is a complex disorder, with a strong genetic component. Although better treatments and earlier detection have contributed to decreasing mortality rates for many countries, mortality rates are actually increasing in Asian countries such as Japan and Singapore. We recently reported a genome-wide linkage scan in 69 Finnish Hereditary Prostate Cancer (HPC) families, which replicated the *HPC9* locus on 17q21-q22 and identified a locus on 2q37. Using ordered subset analysis (OSA) and conditioning on non-parametric linkage to these loci, we sought to find other loci linked to HPC in subsets of families not detectable in the overall sample. Significance of the change in LOD scores (ΔLOD) due to OSA analysis was determined using permutation tests (empirical p-value). These analyses revealed a significant linkage peak with an OSA LOD score of 4.876 on Xq26.3-q27 (ΔLOD = 3.193, empirical p = 0.009)in a subset of 41 families weakly linked to 2q37. This region overlaps the *HPCX1* locus. Other linked loci were 12q21.1-q23.3 (OSA LOD = 3.67, ΔLOD = 2.526, p = 0.04) in a subset of 17 families unlinked to 2q37, and 8q24.22-q24.3 (OSA LOD = 3.195, ΔLOD = 2.963, p = 0.02) in a subset of 15 families weakly linked to 2q37, overlapping the *HPC10* locus. Another subset of 41 families most strongly linked to 17q21-q22 revealed a significant linkage to Xq25 with a peak OSA LOD score of 3.542 (ΔLOD = 1.484, p = 0.04). This subset contains many of the same families found in the subset linked to Xq26.3-q27 when conditioning on linkage to 2q37. Thus it is likely that these signals represent the same locus. Other strongly linked loci were found at 3q26.31-q27.1 (OSA LOD = 3.492, ΔLOD = 2.39, p = 0.02) in a subset of 47 families unlinked to 17, and 12q14.2-q21.31 (OSA LOD = 3.23, ΔLOD = 2.326, p = 0.02) in a subset of 34 families unlinked to 17. We also used the maximum of the family NPL scores for 2q37 and 17q21-q22 to condition on linkage to either of these loci. Two novel loci were found in this analysis; 18q12.1-q12.2 (OSA LOD = 2.541, ΔLOD = 1.651, p = 0.03) and 22q11.1-q11.21 (OSA LOD = 2.395, ΔLOD = 2.36, p = 0.006), which is close to *HPC6.* Using ordered subset analysis allows us to find additional loci linked to HPC in subsets of families, which would not otherwise be detectable, thus contributing to the effort to untangle the complex genetic heterogeneity of HPC.

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Genetic Heterogeneity of Myopia Susceptibility in an Ashkenazi Jewish Population. *C. Simpson[1], R. Wojciechowski[1], D. Stambolian[2], J.E. Bailey-Wilson[1].* 1) Inherited Disease Res Branch, NHGRI, NIH, Baltimore, MD; 2) Department of Ophthalmology, University of Pennsylvania.

Myopia affects at least one third of most populations, is a complex disorder, with both genetic and environmental etiological influences. It has a significant impact on the lives of affected individuals and carries high economic costs associated with treatment and with loss of productivity and co-morbidity from vision impairment. Despite many years of research, most of the factors contributing to myopia development remain unknown. Genetic studies have pointed to a strong inherited component, but although many loci have been found, few genes have been positively identified as causal agents. We have previously reported 2 genomewide linkage scans in a population of 49 highly aggregated Ashkenazi Jewish families which identified a locus on chromosome 22. Here we have used ordered subset analysis, conditioned on non-parametric linkage to chromosome 22 to detect other loci which also had evidence of linkage to myopia in subsets of the families, but not the overall sample. Similar analyses using parametric LOD scores in OSA are ongoing and may increase power. Suggestive linkage to a 25-cM linkage interval with a peak OSA nonparametric allele-sharing LOD score of 2.622 on 20p12.1-q11.23 (ΔLOD = 2.193, empirical P = 0.034) was identified in a subset of 15 families with strong evidence of linkage to chromosome 22, which represents 31% of the total dataset (15/49 families). Seven other loci also presented with suggestive LOD scores > 2.0 on chromosome 2q34-q35, 3p11.1-q24, 5q21.1-q23.3, 6q22.31-q24.2, 7p22.3-21.3, 9q31.1-q33.3 and 14q31.3-q32.3. However, none of the ΔLOD scores were significant by permutation testing. Results using model-based parametric LOD scores in OSA will also be presented. The chromosome 20 locus is entirely novel and appears only in a subset of families already known to be strongly linked to chromosome 22. Using ordered subset analysis allows us to find additional loci linked to myopia in subsets of families, and underlines the complex genetic heterogeneity of myopia even in highly aggregated families and genetically isolated populations such as the Ashkenazi Jews.

2983/F

Getting More from Your Dense SNP Data: EAGLET Increases Power and Precision. *E. Drill, W. Stewart.* Biostatistics, Columbia Univ Sch Pub Hlth, New York, NY.

The correlation between genetic markers (a.k.a. linkage disequilibrium (LD)) can be a serious problem for the analysis of dense SNP linkage data, especially when the genotypes of founders are unavailable and families are enriched for affected members (e.g. affected sib-pair (ASP) designs). If completely ignored, LD can bias estimates of trait location, inflate the Type I error, and reduce the power to detect linkage. However, by considering the full spectrum of LD, we show that the power to detect linkage and the precision to localize traits are dramatically improved. In particular, we have extended EAGLET (Efficient Analysis of Genetic Linkage: Estimation and Testing)---our freely available linkage and association software package, in the following ways: (1) unlike most competing methods which force the user to specify unknown tuning parameters, EAGLET finds the optimal value of the LD-tuning parameter for you; (2) our minimum variance, bias-corrected estimates of location yield candidate gene regions with increased precision while maintaining nominal coverage; and (3) the quality of the Monte Carlo sampling is assessed internally, and simple guidelines are given to help optimize program performance. RESULTS: Using dense SNP data simulated with LD and linkage, we show that our estimate of the LD tuning parameter is consistent, irrespective of the underlying pattern of LD, genetic model, or family structure. Furthermore, we show that (on average) the length of our minimum variance, bias-corrected 95% confidence interval (CI) is shorter than or equal to the length of the original EAGLET CI, and that both have approximate 95% coverage. Lastly, we illustrate the utility of the internal Monte Carlo assessments when optimizing program performance. CONCLUSIONS: By increasing both the power to detect, and the precision to localize disease susceptibility genes, our new and improved software package should greatly reduce the cost of sequencing efforts in candidate gene regions. This, in turn, will finally give researchers the ability to realize the full potential of dense SNP linkage analysis. In addition, the algorithmic improvements have significantly increased the speed of all bootstrap-related features, and the user-friendly documentation and tutorials make EAGLET very easy to use.

2984/F

Handling Hierarchical Phenotypes in the PPL Framework. *K.A. Walters, V.J. Vieland.* Battelle Center for Mathematical Medicine, The Research Institute at Nationwide Children's Hospital, Columbus, OH.

The use of hierarchical phenotypes (HPs), such as "strict," "intermediate," and "broad" classifications commonly used in psychiatric genetic diagnosis, pose a dilemma for standard statistical genetic methods. The usual approach is to create a series of increasingly inclusive binary phenotypes, to analyze the data once under each phenotypic model, and to select the strongest linkage or association result across the multiple tests (the maximizing phenotype, or MP, method). This is a form of maximizing over the phenotype, which is known to inflate Type I error rates. The PPL framework offers an alternative approach, in which HPs are taken into consideration within a single analysis using liability classes (LCs). Individuals classified as affected under any level of the HP are coded as affected, but assigned to a LC based on HP level. As with all forms of the PPL, penetrances are then integrated out of the resulting linkage or association statistic separately within each LC, allowing for the possibility of different penetrance vectors in the different classes. In principle, this method should properly allow for increasing "phenocopy" rates as the HP level increases in inclusiveness, while maintaining its robustness even when these rates are the same across classes, all without the need for multiple analyses. Here we compare the sampling behavior of the LC and MP methods in application to linkage data generated under a broad range of HP models. We find that when at least 30% of families contain exclusively cases at the narrowest trait level, the LC model is robust and preferable to the MP approach. Under other circumstances, simply including all HP levels in a single "affectedness" category proves to be equally robust, probably because the technique of integrating over the penetrance vector is intrinsically insensitive to a small amount of diagnostic misclassification. Thus without any modifications to the PPL (and PPLD) framework or software [see Huang et al., this meeting] we are able to make use of hierarchical diagnostic information without the need for multiple testing over HP classes.

2985/F

Sex-Specific Regulation of the mitochondrial DNA (mtDNA) content: Genome-wide Linkage Analysis for Identifying Quantitative Trait Loci. *S. Lopez[1], A. Buil[1], J.C. Souto[2], J. Casademont[3], J. Blangero[4], A. Martinez-Perez[1], L. Rib[1], J. Fontcuberta[2], L. Almasy[4], J.M. Soria[1].* 1) Unit of Genomics of Complex Diseases, Research Institute Hospital de la Santa Creu i Sant Pau, Barcelona, Spain; 2) Department of Haematology, Hospital de la Santa Creu i Sant Pau. Universitat Autònoma de Barcelona, Barcelona, Spain; 3) Internal Medicine Department, Hospital de la Santa Creu i Sant Pau. Universitat Autònoma de Barcelona, Barcelona, Spain; 4) Department of Population Genetics. Southwest Foundation for Biomedical Research. San Antonio, TX, USA.

Mitochondrial DNA (mtDNA) is the key for the maintenance of mitochondrial integrity and function, which indeed influence cell survival. Alteration of the mtDNA quantity has been associated with common diseases and oxidative damage. The aim of the study was to delineate the genomic regions that influence the mtDNA content by a genome-wide linkage analysis in families from the Genetic Analysis of Idiopathic Thrombophilia (GAIT) Project. Mitochondrial DNA levels were measured by quantitative real time PCR in 387 individuals belonging to 21 extended Spanish families. A total of 485 DNA microsatellite markers were genotyped to provide a 7.1 cM genetic map. Genetic heritability (h2) of mtDNA content was estimated in the GAIT sample. A variance component linkage method was conducted to evaluate linkage and to detect quantitative trait loci (QTLs). A first linkage analysis was carried out taking individuals altogether. Further linkage analyses were performed separating the individuals according to the gender. According to our results, mtDNA content is highly heritable (h2=0.334; p=1.82E-05). First linkage analysis showed a QTL suggestive of linkage with mtDNA content on Chromosome 2 (LOD score=2.21; p=7.09E-04). Interestingly, three different strong QTLs influencing mtDNA content were detected when men and women were analysed separated. Women showed a QTL on Chromosome 2 (LOD score=3.09; p=8.11E-05) and another one on Chromosome 3 (LOD score=2.67; p=2.27E-04). Men showed a QTL on Chromosome 1 (LOD score=2.81; p=1.57E-04). Our study clearly reports great evidence of sex specific differences in the genetic determinants involved in the control of the mtDNA content. We report three novel QTLs involved in the quantitative variation of the mtDNA levels. All the regions of linkage contain potential candidate genes that are involved in replication processes, mitochondrial transport, and mitochondrial protein translation. Thus, further genetic and functional analyses are warranted.

2986/F

Complex segregation analysis reveals a major gene effect controlling dental decay resistance in an isolated population from north of Brazil. *M.T. Mira[1], R.I. Werneck[1], F.P. Lazaro[1], A. Cobat[3,4], A.V. Grant[3,4], M.B. Xavier[2], L. Abel[3,4,5], P.C. Trevilatto[1], A. Alcais[3,4,5].* 1) Graduate Program in Health Sciences, Center for Biological and Health Sciences, Pontifical Catholic University of Paraná, Curitiba, Paraná, Brazil; 2) Tropical Medicine Core, Federal University of Pará, Belém, Pará, Brazil; 3) Laboratoire de Génétique Humaine des Maladies Infectieuses, Institut National de la Santé et de la Recherche Médicale, INSERM U550, Paris, France; 4) Université Paris René Descartes, Faculté Médecine Necker, Paris, France; 5) Laboratory of Human Genetics of Infectious Diseases, Rockefeller Branch, Rockefeller University, New York, New York.

Caries is a chronic, complex and one of the most common diseases affecting humans. It is widely accepted that the occurrence of dental decay depends on environmental and host-related factors, such as diet, biofilm composition, host susceptibility and time of exposure. Over the last years, studies using experimental animal models, as well as human observational (familial aggregation and twin studies), linkage and association analysis were conducted aiming to demonstrate the existence of genetic factors influencing dental decay. However, due to the complex characteristic of the trait, questions such as the model of inheritance and parameters as the frequency and penetrance of the deleterious allele are yet to be addressed. We conducted a Complex Segregation Analysis (CSA) using as phenotype both the quantitative Decayed, Missing and Filled Teeth index (DMFT) and the number of Decayed Teeth (DT) in a sample of 11 extended multiplex families (451 individuals) from an isolated population from Amazonic state of Pará, north of Brazil. Complex Segregation Analysis was conducted by using the regressive model, which specifies a regression relationship between each individual phenotype and a set of explanatory variables, including major gene, phenotype of preceding relatives, and other covariates. A major gene effect controlling resistance to both phenotypes was detected, with the best-fit model being co-dominant for DMFT and dominant for DT. For DT, the frequency of the resistance allele "A" was 0.63 and mean DT was 1.53 and 9.53 for genoptypes AA/AB and BB respectively. Our data indicates that the genetic model detected for DMFT is likely the result of a combination of independent genetic mechanisms controlling the components of the index. The CSA is the first step towards a comprehensive description of the exact nature of the genetic risk factors controlling human susceptibility to dental decay. A deeper understanding of the genetic aspects of dental decay pathogenesis will ultimately lead to new strategies for prevention of such prevalent disease worldwide.

2987/F

Comparisons of next-generation sequencing alignment programs. *S. Sun[1,2], X. Yu[2].* 1) Case Cancer Center, Case Western Reserve University, Cleveland, Ohio 44106; 2) Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, Ohio 44106.

Next-generation sequencing (NGS) technology in a single run produces a 2-3 GB's of data, which usually includes millions of sequencing reads. In order to map sequencing reads onto a reference genome, several alignment programs, including ELAND, SOAP, and BOWTIE, have been developed. However, it is unclear how well each existing program performs on real sequencing data that have different low quality issues. In order to determine the strengths and weaknesses of each alignment program, at analyzing data with low quality issues, we have compared different mapping programs using both simulated sequencing data and real NGS data. Our preliminary result shows that SOAP and BOWTIE alignment results have a relatively small percent of agreement, but ELAND and SOAP results have a relatively larger percent of agreement than ELAND and BOWTIE. We also find that the alignment results of using one single alignment program can be significantly different with various parameter settings. These results suggest that significant opportunities for further improving alignment algorithms.

2988/F

How do sequencing quality issues affect SNP calling results? *X. Yu[1], K. Guda[3], J. Willis[5], M. Veigl[2], S. Markowitz[3,4], S. Sun[1,2].* 1) Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, Ohio, 44106; 2) Case Comprehensive Cancer Center, Case Western Reserve University, Cleveland, Ohio, 44106; 3) Department of Medicine, Case Western Reserve University, Cleveland, OH 44106; 4) Howard Hughes Medical Institute, Cleveland, OH 44106; 5) Department of Pathology , Case Western Reserve University, Cleveland, Ohio, 44106.

Next-generation sequencing (NGS) technology provides a powerful way to identify both novel and known single nucleotide polymorphisms (SNPs) in a genome. However, there are different sequencing quality issues in the NGS data: for example, 1) some sequencing reads may be aligned to multiple locations in a genome; 2) a single run may have a very large amount of replicated reads at certain genomic positions; and 3) the 3' end of raw reads may have very low qualities. Currently, how to handle these different quality issues is not clear. We may use different strategies such as trimming reads at 3' end and discarding "problematic" reads. Our preliminary results show that the alignment results vary significantly when different methods are used to handle sequencing quality issues. However, it is unknown how these different alignment strategies and results will affect the downstream SNP calling results. In order to determine the effects of various alignment strategies on downstream SNP calling results, we align NGS data using different methods to handle quality issues and then compare the SNP calling results. In particular, we use known dbSNP to address the false positive and negative rates.

2989/F

A Likelihood Based Flexible Threshold Test for Detecting Associations with Rare Variants. *H. Zhong[1], C. Kooperberg[2].* 1) SAGE Bionetworks, Seattle, WA; 2) Fred Hutch Cancer Research Center, Seattle, WA.

Deep sequencing technology will soon generate comprehensive sequence information in large human samples. However, the potential for discovery of genes associated with a complex disease trait by deep sequencing human exom remains unknown. Although power to detect association with an individual rare variant is limited, pooling variants by gene provides an alternative strategy for identifying susceptibility genes. Here we used a rigorous population-genetic simulation framework to characterize the allele frequency spectrum of rare variants involved in complex traits and to motivate statistical strategies to identify such variants. We developed a likelihood based flexible p-value threshold test that can effectively combine evidence of association over different variants. This method optimizes the gene-specific threshold by an efficient permutation algorithm, making it robust with respect to various properties of individual genes. The proposed methods were extensively evaluated using population genetic simulations and trait simulations inspired by the Women's Health Initiative Sequencing Project. Compared to several existing methods, the proposed test showed higher power and robustness under various trait properties and the presence of mis-genotyping errors.

2990/F

Haplotype Specific Amplification in High Throughput Tumor Sequence Data. *N. Dewal[1], M. Freedman[2,3], T. LaFramboise[4,5], I. Pe'er[6].* 1) Department of Biomedical Informatics, Columbia University, New York, NY; 2) Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA; 3) Medical and Population Genetics Program, The Broad Institute of Harvard and MIT, Cambridge, MA; 4) Department of Genetics, Case Western Reserve University School of Medicine, Cleveland, OH; 5) Genomic Medicine Institute, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, OH; 6) Department of Computer Science, Columbia University, New York, NY.

During tumor progression, culprit genes and variants confer selective advantage to progenitor cancer cells via allowing them to bypass normal growth control mechanisms. Both regions of somatic amplification as well as germline DNA sequence represent variants that are selected for along the tumor genome. High throughput sequencing of tumor and normal tissues identify such somatically amplified regions and hold the potential to reveal the specific gene variants being amplified. We propose a novel Hidden Markov Model-based method -- Haplotype Amplification in Tumor Sequences (HATS) -- that analyzes tumor and normal sequence data to infer amplified alleles and haplotypes in regions of copy number gain. HATS also utilizes existing information from public repositories (e.g. 1000 Genomes Project) in order to infer haplotypes. Our method is designed to handle biases in read data as well as accomodate rare variants. We assess the performance of HATS using simulated amplified regions generated from varying copy number and coverage levels. We demonstrate that HATS infers amplified haplotypes more accurately than naive haplotype construction that is based on allelic read counts alone, especially at lower coverage levels. We thus believe our method will help further the integration of variant types in sequence data and aid the cancer community in identifying causal variants.

2991/F

Detection and Characterization of Novel Sequences in Next-Generation Sequencing Data from Diverse Populations. *M.W. Snyder, G.R. Abecasis, H.M. Kang.* Center for Statistical Genetics, University of Michigan, Ann Arbor, MI.

Ongoing efforts to sequence whole genomes, which are expected to generate hundreds of sequenced genomes this year, are rapidly increasing our catalog of DNA sequence variation, including structural variants, in humans. As the number of sequenced genomes increases, we expect to discover many sequences that are not represented in the current human reference sequence but are present in many or all of these newly sequenced individuals. Some of these newly discovered sequences may include new genes and other functional sequences and could thus contribute to human disease and phenotypic variation. Recent efforts towards novel sequence discovery have focused on de novo genomic assembly, which presents computational challenges when scaled to high-coverage next-generation sequencing data. We evaluate a series of tools designed to identify novel sequences from resequencing data and to describe the presence of such sequences in different populations. We further suggest a method to validate such sequences.

2992/F

Enriching Targeted Sequencing Experiments for Rare Disease Alleles. *T. Edwards[1], Z. Song[2], C. Li[3].* 1) Vanderbilt Epidemiology Center, Division of Epidemiology, Department of Medicine, Vanderbilt University, Nashville, TN; 2) Center for Human Genetics Research, Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN 37212; 3) Center for Human Genetics Research, Department of Biostatistics, Vanderbilt University, Nashville, TN 37212.

Next-generation targeted resequencing of GWAS-associated genomic regions is a standard approach for discovering rare disease alleles, with the intent to genotype candidate SNPs captured by resequencing. This approach is reasonable, but may be inefficient for rare alleles if samples are not carefully selected for the resequencing experiment. We have developed an approach, SampleSeq, to estimate the expected count of captured rare disease alleles for each subject at each considered locus, and to select samples to maximize the chance of detecting rare alleles in a targeted resequencing experiment. SampleSeq requires specification of prevalence and a target rare disease allele frequency range, and assumes genotypes are available at previously associated SNPs. SampleSeq was compared to selecting random cases or controls, or selecting subjects for burden of high-risk alleles at previously associated SNPs. We used the coalescent simulator Cosi, calibrated to produce haplotypes of sequence-level variation with the LD profile of the original 3 HapMap populations (CEU, YRI, JPT/CHB). We simulated 1000 replicates of 2000 cases and 2000 controls for 15 regions with disease allele frequencies 0.001-0.01 and prevalences 0.01-0.2. SampleSeq provided >35% higher yields of rare disease alleles, acquired >35% more samples with at least 1 disease allele, and required >33% less samples to capture the same number of disease alleles in all scenarios compared to the best alternative. This allows for smaller sample sizes in resequencing experiments, or captures rarer risk alleles. SampleSeq can also calculate required sample size to capture rare alleles. If controls are selected for the experiment by SampleSeq, tests of association are also possible.

2993/F

Variant calling from low-pass next generation sequence data in families. *B. Li, W. Chen, G. Abecasis.* Biostatistics, Univ Michigan, Ann Arbor, MI.

Next generation sequencing is currently being employed to uncover rare variants associated with human complex traits after successful genome wide association studies (GWAS). It becomes challenging due to high error rates of next-gen sequencing technologies. Although population based studies are powerful for GWAS to identify common variants, family designs offer an efficient approach for association studies of rare variants. By utilizing transmission constraints within families, accuracy can be greatly increased by jointly calling variants within families. In addition, if families with multiple affected individuals are collected, rare variants are greatly enriched, making variant calling more accurate and association studies more powerful. Family designs also offer an effective approach for follow-up studies by recruiting family members once a significant rare variant is identified in an individual. Here we developed a likelihood framework and implemented it in software for calling variants from low-pass next-gen sequencing data in families, based on likelihood information encoded in Genotype Likelihood Format (GLF) and rigorous population genetics theories. Simulations using different error rates (0.01 ~ 0.001) and sequence coverage (2X ~ 8X) showed that more variants can be called with higher accuracy in families than in unrelated individuals, especially when the error rate is high and coverage is low. For different frequency categories, except for the extremely rare variants, family designs have higher sensitivity and specificity of variant calling. For the situation when only polymorphic sites in a sample are considered, family designs always have better detection rate and accuracy. One additional advantage of this framework is that imputation is inherent in the variant calling which makes it appealing for increasing the power of association studies. We are currently sequencing families in the SardiNIA project and the new software will be applied to the sequencing data for variant calling and association studies.

2994/F

Alternative Grouping Strategies Improve Association Power of Detecting Rare Variants for Complex Diseases. *YF. Pei[1,2], L. Zhang[1,2], C.J. Papasian[1], HW. Deng[1,3,4].* 1) Univ Missouri - Kansas City, Kansas City, MO; 2) Key Laboratory of Biomedical Information Engineering, Ministry of Education and Institute of Molecular Genetics, School of Life Science and Technology, Xi'an Jiaotong University, 710049, P. R. China; 3) Center of System Biomedical Sciences, Shanghai University of Science and Technology, Shanghai 200093, P. R. China; 4) College of Life Sciences and Engineering, Beijing Jiao Tong University, Beijing 100044, P. R. China.

When detecting rare variants for diseases, an efficient analytical approach involves grouping all variants in a genomic region together for examining the association. One factor complicating this approach is that the vast majority of rare variants are actually neutral to diseases, even when only non-synonymous variants in gene expressing regions are considered. Here, we propose two alternative grouping strategies in order to improve the statistical power of tests of association. In the first exhaustive grouping strategy, we group and test all possible groups formed by subsets of rare variants. In the second selective grouping strategy, we group and test variants that are overrepresented in case and control populations separately. When the proportion of neutral variants is moderate to large we show, by simulation, that both of these alternative grouping approaches can improve the statistical power of association compared to the total grouping method. When applied to the sequencing datasets produced by the ENCODE3 project, the proposed grouping strategies clearly outperform their competitors by several fold, demonstrating the feasibility of the proposed methods.

2995/F

Power of selective sequencing strategies in next-generation whole genome sequencing studies. *S. Su, E. Jorgenson.* Ernest Gallo Clinic and Research Center, UCSF, Emeryville, CA.

Next-generation DNA sequencing enables the comprehensive analysis of genomes, including the identification of rare variants that may influence human diseases and traits. Currently, the cost of whole genome sequencing of large numbers of individuals for genetic association studies is prohibitive, necessitating efficient sample selection strategies to maximize statistical power with limited resources. The selective sequencing approach is a cost-efficient way for detecting genetic variants by selecting individuals with extreme phenotypes. While it is clear that selecting subjects with the most extreme values from a quantitative distribution provides the greatest power to detect genetic variants, most large studies measure multiple traits, and optimal selection strategies for multiple phenotypes and multiple causal variants have not been fully investigated. We conducted a simulation study to examine the power to detect disease association between rare genetic variants and multiple quantitative traits after phenotypic selection. The results encompass several selection strategies (various percentages of samples selected from subjects with the most extreme phenotypes) given different genetic models, including models with one QTL (quantitative trait locus) underlying one phenotype, one QTL underlying multiple phenotypes, and multiple QTLs underlying multiple phenotypes. We show the power of detecting disease association under each selection scheme for each genetic model. Our results can be used as a guideline for selecting samples for genotyping to optimize the power of the study given limited research funds.

2996/F

Improved Detection of Rare Genetic Variants for Diseases. *L. Zhang[1,2], YF. Pei[1,2], J. Li[1], C.J. Papasian[1], HW. Deng[1,3,4].* 1) Basic Med Sci, UMKC Sch Med, Kansas city, MO; 2) Key Laboratory of Biomedical Information Engineering, Ministry of Education and Institute of Molecular Genetics, School of Life Science and Technology, Xi'an Jiaotong University, 710049, P. R. China; 3) Center of System Biomedical Sciences, Shanghai University of Science and Technology, Shanghai 200093, P. R. China; 4) College of Life Sciences and Engineering, Beijing Jiao Tong University, Beijing 100044, P. R. China.

Technology advances have promoted gene-based sequencing studies with the aim of identifying rare mutations responsible for complex diseases. A complication in this type of association studies is that the vast majority of non-synonymous mutations are believed to be neutral to phenotypes. It is thus critical to distinguish potential causative variants from neutral variation before performing association tests to increase their power. In this study, we used existing predicting algorithms in order to predict functional amino acid substitutions, and incorporated that information into association tests. Using simulations, we comprehensively studied the effects of several influential factors, including the sensitivity and specificity of functional variant predictions, number of variants, and proportion of causative variants, on the performance of association tests. Our results showed that incorporating information regarding functional variants obtained from existing prediction algorithms improves statistical power under certain conditions, particularly when the proportion of causative variants is moderate. The application of the proposed tests to a real sequencing study confirms our conclusions. Our work may help investigators who are planning to pursue gene-based association studies.

2997/F

Leveraging the added coverage of exome sequencing with the larger sample size of genomewide association data in the Lung Health Study. *R. Mathias, National Heart, Lung, and Blood Institute's Exome Sequencing Program — and Lung Project Team.* Medicine, Johns Hopkins University, Baltimore, MD.

The utility of massively parallel sequencing for identifying causal mutations underlying rare, monogenic diseases has recently achieved much success. We conducted a genomewide association study (GWAS) using the Illumina 660W to identify markers associated with lung function decline on >4,000 subjects with a minimum of 3 time-points where lung function was measured over a 5-year time frame as part of the NHLBI-supported Lung Health Study (LHS). Approximately 1M SNPs were imputed for up to 2.5M SNPs using MACH and tests for association have been performed using a linear model for lung function decline for each SNP. As part of the NHLBI Exome Sequencing Program, a subset of the LHS samples have undergone exome sequencing using in-solution hybridization probes against a CCDS (2008) target, comprising of about 28Mb over 160,000 exons, to an average of 56X. LHS samples were selected from the phenotypic extremes of lung function decline (i.e. from the upper and lower quartiles), a design which optimizes statistical power to detect rare variants controlling lung function decline. Unequal-variance t-tests will be used to test for association against the rare variants identified through sequencing, an approach that may decrease statistical power but prevent inflated type I error for low frequency variants. We will contrast association signals detected throughout the exome via the two data sources and approaches: the lower coverage larger sample GWAS vs. the higher coverage exome sequencing sample of much smaller size. We anticipate that in this population of European ancestry, we will find additional signals and/or narrow the region of association by leveraging both these strategies.

2998/F

Fine-mapping of classical HLA associations in HIV-1 host control and celiac disease reveals key amino acid residues involved in epitope presentation. *X. Jia[1,2], P.J. McLaren[2,3], S. Ripke[3,4], F. Pereyra[2,9], D.W. Haas[5], D. Heckerman[6], M. Carrington[7], C. Wijmenga[8], B.D. Walker[9], P.I.W. de Bakker[2,3], International HIV Controllers Study.* 1) Harvard-MIT Health Sciences and Technology, Boston, MA; 2) Brigham and Women's Hospital, Boston, MA; 3) Broad Institute of Harvard and MIT, Cambridge, MA; 4) Massachusetts General Hospital, Boston, MA; 5) Vanderbilt University School of Medicine, Nashville, TN; 6) Microsoft Research, Los Angeles, CA; 7) National Cancer Institute-SAIC, Frederick, MD; 8) University Medical Center Groningen, The Netherlands; 9) Ragon Institute of MGH, MIT and Harvard, Charlestown, MA.

Background: DNA sequence variation in the major histocompatibilty complex (MHC) influences the heritable risk for autoimmune disease and the host response to pathogenic infections. Many significant associations have been identified within the MHC, but identification of causal variants is confounded due to linkage disequilibrium across the MHC. **Methods**: We developed an automated pipeline to impute classical HLA alleles as well as all polymorphic amino acids in HLA proteins using a reference panel from the Type 1 Diabetes Genetics Consortium with SNP data and 4-digit HLA types at class I and class II loci. We applied this method to two genome-wide SNP data sets in two diseases with MHC associations: host control of HIV-1 (n=1712) and celiac disease (n=2689). Both case-control data sets contain samples of European ancestry. We tested the imputed amino acids for association, and used stepwise regression to identify independent variants. **Results**: In total, we imputed 263 HLA alleles and 372 amino acid positions in class I and II HLA proteins, and validated with experimental HLA types that imputation accuracy is excellent for HLA alleles with frequency >2%. Stepwise regression revealed 7 HLA alleles associated with host control of HIV-1 (B*5701, B*2705, Cw*0802/B*14, B*35, Cw*07, B*52, A*25). Stepwise regression of amino acids identified four independent residues (positions 67, 63, 62, and 97) within the peptide-binding pocket of HLA-B, one residue (77) in HLA-A, and one residue (304) in HLA-C that together explain the classical associations of protective and risk HLA alleles with host control of HIV-1. In celiac disease, we identified HLA alleles associated with risk (DQA1*05, DQB1*02, DQB1*0302), and found that the DQ2.5 heterodimer produced the most significant association (OR=19, P=2E-167), consistent with previous findings. We were able to resolve these associations down to a pair of perfectly correlated residues (74, 71) within HLA-DQB1, one residue (52) in HLA-DQA1 that line the binding pocket of the HLA-DQ molecule, and another residue (9) of HLA-DQB1. **Conclusions**: Imputation and association testing of individual amino acid positions within HLA molecules can help interpret known HLA signals in infectious and autoimmune disease. In two very different diseases, we demonstrate a key role for discrete polymorphisms within the peptide-binding pockets in the presentation of viral or gluten peptides.

2999/F

Estimation of allele frequencies and association mapping using next-generation sequencing data. *SY. Kim[1], A. Albrechtsen[2], Y. Li[3], T. Corneliussen[2], G. Tian[4, 5], N. Grarup[6], T. Jiang[3], T. Sparso[6], G. Andersen[6], D. Witte[7], T. Jorgensen[8], T. Hansen[6], O. Pedersen[6, 9, 10], J. Wang[2, 3], R. Nielsen[2], the LuCamp Consortium.* 1) Department of Integrative Biology/Stats, UC Berkeley, Berkeley CA 94720, USA; 2) Department of Biology, University of Copenhagen, Copenhagen, Denmark; 3) Beijing Genomics Institute, Shenzhen 518000, China; 4) Beijing Institute of Genomics, Chinese Academy of Science, Beijing 101300, China; 5) The Graduate University of Chinese Academy of Sciences, Beijing 100062, China; 6) Hagedorn Research Institute, Copenhagen, Denmark; 7) Steno Diabetes Center, Gentofte, Denmark; 8) Research Centre for Prevention and Health, Glostrup University Hospital, Glostrup, Denmark; 9) Faculty of Health Sciences, University of Aarhus, Aarhus, Denmark; 10) Institute of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark.

Estimation of allele frequencies is of fundamental importance in population genetic analyses and in association mapping. In most studies using next-generation sequencing, a cost effective approach is to use medium or low-coverage data (e.g., <15X). However, SNP calling and allele frequency estimation in such studies is associated with substantial statistical uncertainty because of varying coverage, high error rates, etc. We present a new maximum likelihood method for estimating the allele frequencies in low and medium coverage next-generation sequencing data, based on integrating over uncertainty in the data for each individual rather than calling genotypes. This method can be directly applied to detect associations in case/control studies. We compare our method to methods based on genotype calling using simulations, and show that the likelihood method outperforms the genotype calling methods in terms of: (1) accuracy of allele frequency estimation, (2) distribution of allele frequencies across neutrally evolving sites, and (3) statistical power in association mapping studies. Using real re-sequencing data from 200 individuals obtained using exon-capturing, we show that the patterns observed in the simulations in fact also can be found in real data . In particular, the null distribution of the test statistic computed based on called genotypes shows a significant departure from the chi-square(1) distribution expected using classical asymptotic theory. However, the test statistic calculated using the full likelihood method closely follows the expected distribution. Overall, our results suggest that association mapping and estimation of allele frequencies should not be based on genotype calling in low to medium coverage data. Furthermore, if genotype calling is used, it is better not to filter individuals based on call confidence score.

3000/F

QCALL: SNP detection and genotyping from low coverage sequencing data on multiple diploid samples. *Q.S. Le, R. Durbin.* Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge. CB10 1SA UK.

An efficient approach is to detect SNPs from diploid samples is to sequence many samples at low coverage then to combine data across samples to detect shared variants. Here we present methods to discover and genotype single-nucleotide polymorphism (SNP) sites from low coverage sequencing data, making use of shared haplotype (linkage disequilibrium) information. We first collect SNP candidates based on independent sequence calls at the site with 0.01 posterior probability. We then use MARGARITA with genotype or phased haplotype data from the same samples to collect 20 ancestral recombination graphs (ARGs). We refine the posterior probability of SNP candidates by considering possible mutations at internal branches of the 40 marginal ancestral trees inferred from the 20 ARGs at the left and right flanking genotype sites. Using a population genetic prior on tree branch length and Bayesian inference we determine a posterior probability of the SNP being real, and also the most probable phased genotype call for each individual. We present experiments on both simulation data and real data from the 1000 Genomes Project to prove the applicability of the methods. The current method is limited to samples with available genotypes data. To overcome this limitation, we are developing an approach to place probabilistically new sample with sequencing data in existing ARGs and include the prior probability in Bayesian inference. Consequence, we can analyze any samples as long as we have starting reference ARGs for a related population for examples from HapMap3. Software to implement the methods is available in the QCALL package from www.sanger.ac.uk/software/QCALL/.

3001/F

GWAStoolbox: an R-package which standardizes and accelerates quality control and data handling of multiple GWAS data files. *C. Fuchsberger[1], D. Taliun[2], P.P. Pramstaller[2], C. Pattaro[2] on behalf of the CKDGen consortium.* 1) Department of Biostatistics, University of Michigan, Ann Arbor, MI; 2) Institute of Genetic Medicine, European Academy, Bolzano, Italy.

Large-scale meta-analyses of genome-wide association studies (GWAS) have led to the discovery of numerous novel loci for a variety of complex traits. Despite the methodological challenges in combining a large number of studies, standardization and in depth quality control of all the data sets involved in the meta-analysis is of crucial importance for the reliability of final findings. Results from a single GWAS typically consist of large files (~250Mb) and the number of SNPs that are currently being analyzed is typically >2.5M and will increase rapidly with imputation based on the 1000 Genome Project. Recently published meta-analyses of GWAS were often composed of 10s of studies. Future collaborative efforts, such as the Metabochip project, will include even more. Therefore, problems must be quickly identified to avoid delays of the entire meta-analysis process and to prevent spurious results. We have developed the GWAStoolbox, an R-package which standardizes and accelerates quality control (QC) of individual-study result files undergoing meta-analysis. After setting up a simple configuration file, GWAStoolbox is capable to process any number of GWAS files, and to produce a full QC report for each study, typically in a matter of minutes. Each QC report comprises three parts: (1) formal checking to assess if all files meet given formatting guidelines; (2) quality checking at the individual study level, including the identification of unexpected values for the main statistics and assessment of p-value inflation; (3) global checking to uncover systematically different studies, which might be caused for example by improper phenotype transformation. Moreover, the package contains functions to facilitate data handling and annotation, and graphical tools to support the interpretation of results. We illustrate GWAStoolbox based on a recent meta-analysis of kidney related GWAS as part of the CKDGen consortium. In brief, the computational time for processing 20 studies on a desktop pc was ~36 min (<2min per file) and required in total ~600Mb of memory. Many different kinds of file formatting problems were quickly uncovered. Checks on the distribution of the main parameters and systematic across-study comparisons enabled us to identify studies with systematic biases in the analysis process. Finally, the fast QC process enabled a quick turn-around so that individual-study analysts could fix the problems without causing major delays to the whole consortium.

3002/F

An algorithm to infer haplotypes of copy number variations from noisy high-throughput data. *M. Kato[1], S. Yoon[1], N. Hosono[2], A. Leotta[1], J. Sebat[3], T. Tsunoda[2], M.Q. Zhang[1,4].* 1) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; 2) Center for Genomic Medicine, RIKEN, Yokohama, Japan; 3) Department of Psychiatry, University of California, San Diego, CA; 4) Center for Systems Biology, University of Texas, Dallas, TX.

Accurate and complete information on haplotypes and individuals' diplotypes is required for population-genetic analyses; however, microarrays do not provide data on a phased diplotype (e.g., 1 copy in one haplotype and 2 copies in the other) at a copy number variation (CNV) locus, but only provide data on a diploid number, which is the total number of copies/bases over a diplotype (3 copies in the above example). We previously developed algorithms to infer CNV haplotypes from diploid numbers (Kato et al, Am J Hum Genet, 2008; Kato et al, Bioinformatics, 2008); however, these algorithms cannot correctly handle microarray data in which the signal intensities deriving from different diploid numbers are not clearly separated due to noise.

Here we report an algorithm and computational tool to infer CNV haplotypes and individuals' diplotypes from noisy microarray data, incorporating uncertainty due to noise into the expectation-maximization procedure. The uncertainty is represented by likelihoods that a given observed signal intensity may be derived from different underlying diploid numbers. This tool can handle a combination of integer copy numbers, single nucleotide variations in CNV regions (e.g., AAB, unlike AB of single nucleotide polymorphism), and single nucleotide polymorphisms.

We performed simulation studies based on the known diplotypes of 600 individuals in a European population as well as an error model obtained from real microarray data. The new algorithm outperformed the previous algorithms: the error rate of haplotype frequency estimation was 1-2% in the new one compared with 12-18% in the previous ones. In addition, the new algorithm also corrected diploid numbers wrongly determined due to noise in a customary method: the rate of wrong diploid numbers was 4-5% in the new one compared with 21-34% in the customary one. Encouraged by this success, we applied this algorithm to real microarray data and obtained phased CNV genotypes along the genome for the CEU population. These phased genotypes will be basic information for various analyses in population genetics of CNVs.

3003/F

ParaHaplo 2.0: A Program Package for Haplotype-estimation and Haplotype-based Whole-genome Association Study using Parallel Computing. *K. Misawa[1], N. Kamatani[1,2].* 1) Research Program for Computational Science, Research and Development Group for Next-Generation Integrated Living Matter Simulation, and Fusion of Data and Analysis Research and Development Team; 2) Laboratory for Statistical Analysis, RIKEN Center for Genomic Medicine.

Background: The use of haplotype-based association tests can improve the power of genome-wide association studies. Since the observed genotypes are unordered pairs of alleles, haplotype phase must be inferred. However, estimating haplotype phase is time consuming. When millions of single-nucleotide polymorphisms (SNPs) are analyzed in genome-wide association study, faster methods for haplotype estimation are required.

Methods: We developed a program package for parallel computation of haplotype estimation. Our program package, ParaHaplo 2.0, is intended for use in workstation clusters using the Intel Message Passing Interface (MPI). We compared the performance of our algorithm to that of the regular permutation test on both Japanese in Tokyo, Japan and Han Chinese in Beijing, China of the HapMap dataset.

Results: Parallel version of ParaHaplo 2.0 can estimate haplotypes 100 times faster than a non-parallel version of the ParaHaplo.

Conclusion: ParaHaplo 2.0 is an invaluable tool for conducting haplotype-based genome-wide association studies (GWAS). The need for fast haplotype estimation using parallel computing will become increasingly important as the data sizes of such projects continue to increase. The executable binaries and program sources of ParaHaplo are available at the following address: http://en.sourceforge.jp/projects/parallelgwas/releases/.

3004/F

LocusZoom: Regional visualization of genome-wide association scan results. *R.P. Welch[1,2], R.J. Pruim[3], S. Sanna[4], T.M. Teslovich[2], P.S. Chines[5], T.P. Gliedt[2], M. Boehnke[2], G.R. Abecasis[2], C.J. Willer[2].* 1) Bioinformatics Graduate Program, University of Michigan Medical School, Ann Arbor, MI 48109; 2) Department of Biostatistics and Center for Statistical Genetics, University of Michigan, Ann Arbor, MI 48109; 3) Department of Mathematics and Statistics, Calvin College, Grand Rapids, MI 49546; 4) Istituto di Neurogenetica e Neurofarmacologia (INN), Consiglio Nazionale delle Ricerche, c/o Cittadella Universitaria di Monserrato, Monserrato, Cagliari, Italy 09042; 5) National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland.

Genome-wide association studies (GWAS) are a recently developed strategy whereby a large number of samples are genotyped for millions of single nucleotide polymorphisms (SNPs), which are then tested for association with a particular disease or trait of interest. GWAS have reported hundreds of SNPs associated with disease. Many of these SNPs are in linkage disequilibrium with others nearby, implicating large genomic regions that may contain the causal disease variant(s). Each region contains a number of important features for understanding the association signal, including the association p-values, linkage disequilibrium patterns in various populations, recombination hotspots, the position of genes, and genomic annotations such as regulatory regions, conserved sequences, and transcription factor binding sites. To visualize all of this information simultaneously, we developed LocusZoom (http://csg.sph.umich.edu/locuszoom), a web tool that creates regional plots for genome-wide association results or similar types of data such as candidate gene studies. LocusZoom plots contain all of the features listed above, and can be extended to include user-defined annotations. We support plotting not only a user's association results, but those from other publicly available datasets such as the Kathiresan et al. 2009 GWAS scan on lipid traits. Users can generate single plots interactively, or by using our batch mode that creates plots for each SNP provided in a specification file. We support SNPs in rsid or 1000G naming format, which allows plotting of SNPs from the 1000 Genomes Project or newly discovered SNPs from sequencing experiments. Our software has been used to produce publication-quality plots for multiple complex disease traits, including type 2 diabetes (DIAGRAM), BMI (GIANT), and lipid levels (GLGC). LocusZoom is available via a web interface and as a stand alone application which can run on most Linux/Unix platforms. In both cases, we use the lattice graphics package in R to render plots. Source code is also available for download. Since LocusZoom was released in February 2010, we have created over 1,000 plots per month for external users.

3005/F

Genetic analysis of biological pathway data through genomic randomization. *B. Yaspan[1,2], W. Bush[2,3], E. Torstenson[1,2,3], C. McMunn[2,4], D. Ma[5], M. Pericak-Vance[5], M. Ritchie[1,2,3], J. Sutcliffe[1,2,6,7], J. Haines[2].* 1) Department of Molecular Physiology and Biophysics, Vanderbilt University Medical Center, Nashville, TN; 2) Center for Human Genetics Research, Vanderbilt Univ, Nashville, TN; 3) Department of Biomedical Informatics, Vanderbilt University Medical Center, Nashville, TN; 4) Computational Genomics Core, Vanderbilt University Medical Center, Nashville, TN; 5) The John P. Hussman Institute for Human Genomics, University of Miami, Miami, FL; 6) Vanderbilt Kennedy Center, Vanderbilt University Medical Center, Nashville, TN; 7) Department of Molecular Neuroscience, Vanderbilt University Medical Center, Nashville, TN.

Genome Wide Association Studies (GWAS) have become a standard approach for large-scale common variation characterization and for identification of single genes of even modest effect size predisposing to disease. However, due to issues of moderate sample sizes and particularly multiple testing correction, many variants of smaller effect size are not detected within a single allele analysis framework. Thus, small main effects and potential epistatic effects are likely not consistently detected by GWAS using standard analytical approaches that consider only single SNP alleles. Here we propose unique methodology that aggregates variants of interest (for example, genes in a biological pathway) using GWAS results. Multiple testing and type I error concerns are minimized using genomic randomization, an empirical approach, to estimate significance. Biologic pathways are parsed into independently inherited “features”, linkage disequilibrium (LD) blocks or SNPs in linkage equilibrium, based on the genes within counting the number which are empirically significant. A randomized set of features of similar size (in terms of SNPs tested and LD structure) is generated and significance is compared to the features in the biologic pathway. Randomization corrects for common pathway-based analysis biases such as SNP coverage and density, LD, gene size and pathway size. PARIS (Pathway Analysis by Randomization Incorporating Structure) applies this randomization and directly accounts for linkage disequilibrium effects. Whereas most pathway-based analytical methods require reanalysis of the raw genotype data, PARIS is independent of association analysis method and is thus applicable to GWAS datasets of all study designs. Using the KEGG database we apply PARIS to the publicly available Autism Genetic Resource Exchange (AGRE) GWA dataset, revealing several pathways with significant enrichment (top hits p<0.001: pentose and glucuronate interconversions, ubiquitin mediated proteolysis, synthesis and degradation of ketone bodies, riboflavin metabolism).

3006/F

DiNAMIC: A Method for Assessing the Statistical Significance of DNA Copy Number Aberrations. *V. Walter[1, 3], A.B. Nobel[1, 2, 3], F.A. Wright[1, 3].* 1) Department of Biostatistics, University of North Carolina, Chapel Hill, NC; 2) Department of Statistics, University of North Carolina, Chapel Hill, NC; 3) Lineberger Comprehensive Cancer Center, Chapel Hill, NC.

DNA copy number gains and losses are commonly found in tumor tissue, and some of these aberrations play a role in tumor genesis and development. Although high resolution DNA copy number data can be obtained using array-based techniques, no single method is widely used to distinguish between recurrent and sporadic copy number aberrations. Here we introduce Discovering Copy Number Aberrations Manifested In Cancer (DiNAMIC), a novel method for assessing the statistical significance of recurrent copy number aberrations. DiNAMIC largely preserves the correlation structure found in the underlying DNA copy number data, which may yield additional power to detect recurrent aberrations. Moreover, extensive simulation studies show that DiNAMIC controls false positive discoveries in a variety of realistic scenarios. We use DiNAMIC to analyze two publicly available tumor datasets, and our results show that DiNAMIC detects multiple loci that have biological relevance. DiNAMIC is a useful tool for researchers interested in detecting recurrent DNA copy number aberrations.

3007/F

IntRapid: software for rapid testing of gene-gene interactions in genome-wide association studies. *K. Bhattacharya, R. Magi, A.P. Morris.* Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK.

Genome-wide association (GWA) studies have established themselves as the most successful approach to mapping the genetic determinants of complex traits. However, the primary analyses of GWA studies focus on the marginal effects of single SNPs, and have helped to understand only a small proportion of the genetic component of phenotypic variation. There is increasing evidence from model organisms that complex traits may be influenced by interplay between genes, so that the effect of a causal SNP at one locus is modified by genotypes at other loci. GWA interaction studies may thus help to map additional causal loci, providing a better understanding of the genetic architecture underlying complex traits.

One of the key challenges in performing GWA interaction studies is computational burden. To address this challenge we have developed the IntRapid software to perform rapid testing of gene-gene (GxG) interactions in GWA studies of dichotomous or continuous traits. To achieve this, we undertake a two-stage interaction testing strategy. In the first stage, all pairs of SNPs are tested using a rapid GxG interaction test. For a dichotomous trait, this is equivalent to the PLINK “fast epistasis” analysis. For a continuous trait, the distribution is dichotomised at the median, with half the sample treated as “cases” and half as “controls”. In the second stage, all pairs of SNPs with rapid interaction *p*-values meeting a pre-defined significance threshold are carried forward for testing in a traditional generalised linear modelling framework. Within this framework, we can assess the significance of interaction effects, adjusting for potential non-genetic risk factors, which cannot be fully assessed in the first stage. The open source IntRapid software is freely available for download from www.well.ox.ac.uk/IntRapid, together with documentation and examples, and scripts for converting standard PLINK and SNPTEST format input data files.

We have applied IntRapid to GWA studies of seven common diseases undertaken by the Wellcome Trust Case Control Consortium. Using a rapid interaction threshold of $p<10^{-4}$, analysis of each disease cohort (~2000 cases, ~3000 controls) takes ~20 days on a single processor. Our results highlight interactions between loci that suggest novel mechanisms and pathways underlying disease susceptibility, and warrant follow-up in independent cohorts for confirmation.

3008/F

A library and tools for manipulating and analysing next generation sequence data. *M. Trost, H.M. Kang, P. Anderson, B. Li, W. Chen, C. Fuchsberger, X. Zhan, G.R. Abecasis.* University of Michigan, Ann Arbor, MI.

As the volume of next generation sequencing and genotyping data increases, there is great demand for high quality, high performance data processing and analysis tools. We are actively developing a set of open source tools and libraries to meet this need. Our C++ library and tools facilitate interaction with common data structures used in the analysis of genetic data, including family information stored in pedigree files, raw sequence data stored in FASTA and FASTQ files, and alignment information stored in SAM, Sequence Alignment and Mapping, and BAM, Binary Sequence Alignment and Mapping Format, files. Our library robustly reads and writes each of these file types, providing rapid, validated access to the information they contain. The library is designed to provide a clear, easy to use interface to a variety of file formats and facilitate the development of data analysis tools.

We illustrate a variety of freely available tools that build upon the underlying library to facilitate file format checking, editing of annotation tags, file merging, duplicate marking and removal, base quality recalibration, variant calling and filtering (including family-structure aware variant callers), checking of sample identities, and various quality assessments. The implementation is memory efficient enough that recalibration or duplicate removal of an ~10 GB BAM file with 70 million reads can typically be accomplished within a gigabyte of memory consumption. Our library and software are publicly available from: http://genome.sph.umich.edu/wiki/Software.

3009/F

Copy Number Variation Accuracy in Genome Wide Studies. *P. Lin, S. Hartz, L. Bierut, J. Rice, COGA Collaborators, COGEND Collaborators, GENEVA.* Department of Psychiatry, Washington University , St. Louis, Missouri, USA.

Copy Number Variations (CNVs) are a major source of variation between individuals and are a potential risk factor in many diseases. Numerous diseases have been linked to deletions and duplications of these chromosomal segments. Data from genome-wide association studies (GWAS) and other microarrays may be used to identify CNVs by several different programs, although the reliability of the results has been questioned. However, no study has tested the reliability of CNV identification in a genome-wide scale. In this study, we estimated the sensitivity and positive predicted rate for CNV calling using a mathematical model based on duplicates in our samples. We found that the positive predictive rate increases with number of probes in the CNV and the size of the CNV, with the highest positive predicted rates in CNVs of at least 500kb and at least100 probes. Our analysis also indicates that identifying CNVs reported by multiple programs can greatly improve the positive predicted rate. These tools can be used by investigators to identify CNVs in genome-wide data with greater reliability.

3010/F

Robust Relationship Inference In Genome Wide Association Studies. *W.M. Chen[1,2], A. Manichaikul[1,2], J. Mychaleckyj[1], S.S. Rich[1], K. Daly[3], M. Sale[1,4,5].* 1) Center for Public Health Genomics, Univ Virginia, Charlottesville, VA; 2) Dept of Public Health Sciences Division of Biostatistics and Epidemiology, Univ Virginia, Charlottesville, VA; 3) Dept of Otolaryngology, Univ Minnesota, Minneapolis, MN; 4) Dept of Medicine, Univ Virginia, Charlottesville, VA; 5) Dept of Biochemistry and Molecular Genetics, Univ Virginia, Charlottesville, VA.

Genome-wide association studies (GWAS) have been widely used to map loci contributing to variation in complex traits and risk of diseases in humans. Accurate specification of familial relationships is crucial for family-based GWAS, as well as in population-based GWAS with unknown (or unrecognized) family structure. The family structure in a GWAS should be routinely investigated using the SNP data prior to the analysis of population structure or phenotype. Existing algorithms for relationship inference have a major weakness of estimating allele frequencies at each SNP from the entire sample, under a strong assumption of homogeneous population structure. This assumption is often untenable. Here, we present a rapid algorithm for relationship inference using high-throughput genotype data typical of GWAS that allows the presence of unknown population substructure. The relationship of any pair of individuals can be precisely inferred by robust estimation of their kinship coefficient, independent of sample composition or population structure (sample invariance). We present simulation experiments to demonstrate the algorithm has sufficient power to provide reliable inference on millions of unrelated pairs and thousands of relative pairs (up to the 3rd-degree relationships). Application of our robust algorithm to HapMap and GWAS datasets demonstrates it performs properly even under extreme population stratification, while algorithms assuming a homogeneous population give systematically biased results. Our extremely efficient implementation performs relationship inference on millions of pairs of individuals in a matter of minutes, dozens of times faster than the most efficient existing algorithm known to us (e.g., as implemented in PLINK). Our robust relationship inference algorithm is implemented in a freely available software package, KING.

3011/F

GenAMap: An integrated analytic and visualization software platform for structured GWA and eQTL analysis. *R.E. Curtis[1,2], E.P. Xing[2,3].* 1) Joint Carnegie Mellon-University of Pittsburgh PhD Program in Computational Biology, Carnegie Mellon University, Pittsburgh, PA; 2) Lane Center for Computational Biology, Carnegie Mellon University, Pittsburgh, PA; 3) School of Computer Science, Carnegie Mellon University, Pittsburgh, PA.

GenAMap is a software project that integrates the power of structured GWA with a usable interface and novel visualizations. GenAMap is specifically designed for large-scale association analysis, especially for analyses that take advantage of structure in a genome-wide collection of markers and thousands of traits or gene expression data structured as a network, time-series, or hierarchy. GenAMap provides novel ways of exploring these kinds of data through human-computer interaction. GenAMap currently supports algorithms based both on classical methods and novel structured sparse regression techniques, such as the GFlasso. GenAMap enhances performance of these algorithms with a complex parallel-computing system that runs all algorithms on a remote cluster. Once results for the association analysis have been obtained, GenAMap provides users with natural visualization schemes that enhance discovery by enabling researchers to visualize the structure of the data while exploring the association results. We consider one or two association studies which highlight the usefulness of GenAMap's visualization techniques.

3012/F

Enrichment analysis of gene-sets and gene-centric SNP-sets reveals evidences of potential shared mechanisms underlying hypertension across race groups. *L. de las Fuentes[1], W. Yang[2], A. Morrison[4], M. Fornage[4], J. Chu[2], V.G. Dávila-Román[1], E. Boerwinkle[4], C.C. Gu[2,3].* 1) Department of Medicine, Cardiovascular Division, Washington University, St. Louis, MO; 2) Division of Biostatistics, Washington University, St. Louis, MO; 3) Department of Genetics, Washington University, St. Louis, MO; 4) Human Genetics Center, University of Texas School of Public Health, Houston, TX.

Shared mechanisms likely underlie complex diseases such as hypertension (HT) despite GWAS studies that typically identify SNPs with little overlap between races. We hypothesize that actions of shared mechanisms vary in different populations and/or under different environmental pressure, resulting in different signal strengths for components missed by conventional single-SNP scans. A customized gene-set enrichment analysis method called VSEA (variable set enrichment analysis) was applied to evaluate aggregated effects of GWA for mean arterial blood pressure (MAP) in the Atherosclerosis Risk in Communities (ARIC) Study. The dataset consisted of 3132 African Americans (AA; 832,077 SNPs after QC) and 9315 Caucasians (CA; 828,744 SNPs). VSEA evaluated enrichment of MAP association in 1376 known pathways (from GO, KEGG & BioCarta), 3872 novel networks (self-curated), and 1252 single genes (402 CVD candidate gene, plus top/bottom 2000 MAP-associated SNPs). Analyses were performed within race and agreement between races was examined for empirical evidence of shared mechanisms for HT. Briefly, (1) in both races, significant enrichment (adjusted $p<0.05$) was discovered in multiple known pathways, many previously implicated in HT (e.g., "NF-kB signaling", "Regulation of PGC1α"); (2) 21 gene-sets were common to both races, with the "mitosis" pathway figuring prominently, interesting since this process was recently associated with HT; (3) gene-based analyses found enrichment for 139 and 67 genes in CA and AA, respectively, but only 8 shared to both races (IL21, ACADL and others); 402 CVD candidate genes fared well with a typical inflation factor of ~1.12; (4) significant enrichment was found in 218 and 315 novel networks in CA and AA, respectively, with 16 enriched networks common to both races. The most prominently enriched novel network involves methyltransferase (METTL7A), iron absorption (SLC11A2), and taste receptor (TAS1R2); (5) cross-validation with the results from an independent CA sample (n=592) confirmed 10~30 commonly enriched known pathways. In summary, our analyses provide strong evidence for shared mechanisms of HT across races. More commonality between races was found as the unit of analysis changed from single SNPs to genes to gene-sets. Thus, analyses of aggregated effects are needed to detect relevant components of putative shared mechanisms of HT even if the factors contribute to varying signal strength between populations remain to be identified.

3013/F

KELVIN 2.1: A tool for modeling genetic architecture for complex disorders. *Y. Huang[1], S. Seok[1], W. Valentine-Cooper[1], J. Burian[1], L. Mangin[1], B. Nouanesengsy[1], A. Modi[1], V. Vieland[1,2].* 1) Battelle Ctr Math Med, Res Inst at Nationwide Children's Hospital, Columbus, OH; 2) Dept Pediatrics, Ohio State University, Columbus, OH.

KELVIN (v2.1) is a comprehensive statistical genetics software package developed under the PPL framework. It currently supports more than 20 different types of analyses, including marker-to-marker or trait-to-marker analysis; dichotomous trait, quantitative trait, and QT threshold models; linkage and trait-marker linkage disequilibrium (association) analysis; and gene x gene interaction models. Using an accurate and efficient numerical integration method, values of the unknown trait parameters are integrated out of the likelihood, rather than fixed at arbitrary values. Through Bayesian sequential updating, evidence is accumulated in a mathematically rigorous manner across multiple sets of data, while allowing for heterogeneity both within and between data sets. All statistics under the PPL framework are on the probability scale, they converge to 0 with evidence against and to 1 with evidence in favor of linkage and/or association, and their interpretation is direct and straightforward. Recent upgrades to KELVIN provide unique flexibility, allowing simultaneous analysis of different data structures, from case-control data to extended pedigrees, and allowing different marker maps and/or pedigree peeling algorithms to be used for different pedigrees within a single analysis. Analyses can be done on a single node or can be distributed across a cluster. KELVIN can be run on multiple platforms (Linux, Mac OS X, Windows XP/Vista/7), and a newly implemented version provides a web interface allowing users to upload data, set up and submit analyses, and to download results, through a user-friendly interface. KELVIN also includes tools for graphing results, including a utiliy specifically designed for plotting genome-wide association or linkage scans (not restricted to PPL analyses) with zoom in/out functionality, plot overlays, and flexible annotation features. KELVIN is continuously being updated and polished as a tool for modeling genetic architecture for complex disorders.

3014/F

Evaluation of 1000Genomes data for imputation in genome-wide association studies: analysis of Fasting Glucose in 5,402 individuals from the NFBC66 cohort. *V. Lagou[1,2], R. Mägi[1], M.I. McCarthy[1,2], A.P. Morris[1], I. Prokopenko[1,2].* 1) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK; 2) Oxford Centre for Diabetes, Endocrinology and Metabolism (OCDEM), University of Oxford, Oxford, UK.

In the past few years, genome-wide association studies (GWAS) have significantly contributed to unravelling the genetic architecture of phenotypes of biomedical importance. Genotype imputation is a widely used procedure in the analysis of GWAS and their large-scale meta-analyses. Availability of novel data from the 1000Genomes project and HapMap III, and development of new imputation tools can enable us to improve the quantity and quality of GWAS data. Our study has three key aims. First, we explore properties of the reference panels available for imputation, namely 1000Genomes (112 CEU, August 2009 release, Sanger alignment of haplotypes)/HapMap III CEU (1KG+HM3) in comparison to HapMap II CEU (HM2), respectively. Second, we compare the performance of IMPUTE v1 and IMPUTE v2 software, in genome-wide imputation for the population-based Northern Finland Birth Cohort 1966 (NFBC66), 5,546 subjects genotyped in Illumina HumanCNV-370DUO array. Third, we investigate the quality of imputed data on in the analysis of association for Fasting Glucose (FG) quantitative phenotype. We performed genome-wide imputation using NFBC66 ~328K genotyped SNPs and 5,402 subjects available after genotyping quality control. We were able to impute successfully ~2.2M and ~7.2M additional SNPs from HM2 and 1KG+HM3, respectively, on a Linux environment. It took ~198 hours and ~1410 hours of CPU time for whole-genome imputation using IMPUTE v1 (HM2) and IMPUTE v2 (1KG+HM3), respectively. We have performed GWA analysis of FG using an additive genetic model implemented in SNPTEST. We investigated the accuracy of imputation for both sets of reference panels. We evaluated the performance of IMPUTE v1 and v2 for common and rare variants in comparison with directly genotyped SNPs in a specific chromosomal region. The false positive rates (FPR) and false negative rates (FNR) were lower in IMPUTE v2 (FPR= 1.75%, FNR=1.4%) than IMPUTE v1 (FPR=2.3%, FNR=21.8%). Our results highlight that imputation from multiple reference panels with high density of polymorphic variants (1KG+HM3) increases the accuracy and quantity of imputed data, although it requires 7-fold increase in computing time in comparison to HM2 panel. IMPUTE v2 with larger and denser reference panels performs better than IMPUTE v1 at predicting the genotypes for common and rare SNPs. Accurate imputation of rare SNPs is of great importance in GWAs, as these markers may be involved in complex diseases pathophysiological pathways.

3015/F

Efficient haplotype reconstruction for pedigree data with zero individual genotype mismatches. *Q. Li[1], M. Fallin[2], J. Bailey-Wilson[1].* 1) Inherited Disease Research Branch, National Human Genome Research Institute, National Institutes of Health, Baltimore, MD; 2) Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD.

Haplotype phase imputation is useful for most haplotype based association tests and for missing genotype imputation. However, phase imputation in extended pedigrees is not trivial. For large haplotype blocks, the number of possible diplotype configurations grows exponentially with the number of markers in the block. There are several haplotype phase estimation or reconstruction algorithms, and a few recent methods handle extended pedigree data. However, haplotypes with rare frequencies are often left out in reconstruction. In application, this can result in misclassification of imputed genotypes. These erroneous genotype assignments are considered particular to individuals, and are usually ignored. Based on population haplotype frequencies, we developed an efficient algorithm to impute phases (and therefore genotypes) for haplotype blocks of up to 8 SNPs, in trios as well as in extended pedigrees. To ensure no individual genotype assignment errors, we consider all possible haplotypes, even those with extremely rare frequencies. We evaluated our phase imputation method using simulated data with masked genotypes within pedigrees. We examined the misclassification proportions of imputed genotypes when rare haplotypes are left out, and compared our methods with other methods: PHASE, HAPLORE, PedPhase, and PhyloPed.

3016/F

Haploscope: a graphical tool for the display and investigation of haplotypes in populations. *F.A. San Lucas[1], N.A. Rosenberg[2], P. Scheet[1].* 1) Dept. of Epidemiology, University of Texas M.D. Anderson Cancer Center, Houston, TX; 2) Dept. of Human Genetics, University of Michigan, Ann Arbor, MI.

Haplotype structure is often depicted pictorially by using tools that rely on visualizations of raw data or pairwise linkage disequilibrium patterns. We propose an alternative that provides a novel graphical representation of haplotype frequencies and that enables natural comparisons of haplotype structure across subgroups of a population (e.g. cases and controls, or separate subpopulations). In particular, we provide a tool for visualization of the haplotype cluster frequencies that are produced by a statistical model of haplotype structure across a genome.

Haploscope is a graphics program that generates visual representations of population haplotype variation. It is based on output from the specific statistical model for population haplotype variation used in the software fastPHASE (P. Scheet & M. Stephens, AJHG 78:629-644, 2006), though it can also be applied to other cluster-based models for haplotypes. The program displays summaries of population-specific haplotype frequencies and aids direct comparisons of haplotype composition among subgroups. Haploscope is an extension the program Distruct, which generates graphical images of clustering patterns within populations from cluster data. In a Distruct image, each vertical line corresponds to an individual, and is partitioned into K colored components that represent membership in K distinct clusters. Haploscope generates similar images where the clusters represent haplotype -- rather than population -- structure. Haplotype clusters are represented as colors, and genomic markers are depicted as vertical bars partitioned into colored segments that correspond to membership coefficients in the clusters. An example of Haploscope output can be found in M. Jakobsson et al. (Nature 451:998-1003, 2008). Various options for generating the images enable the user to control the range of marker positions to include in the cluster image, as well as the order of clusters. Cluster colors and other graphical labels and features are also configurable. Thus, the flexibility offered by Haploscope in the graphical representation provides investigators with the ability to report visually appealing and informative plots of haplotype structure.

3017/F

Coordinated New Versions of the Pedigree Simulation Packages SLINK and SUP. *A.A. Schaffer[1], M. Lemire[2], J. Ott[3,4], G.M. Lathrop[5], D.E. Weeks[6].* 1) NCBI/NIH/DHHS, Bethesda, MD, USA; 2) Ontario Institute for Cancer Research, Toronto, Ontario, CANADA; 3) Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing, CHINA; 4) Laboratory of Statistical Genetics, Rockefeller University, New York, NY USA; 5) Centre National de Genotypage, Evry, FRANCE; 6) Departments of Human Genetics and Biostatistics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA.

Simulation of replicates of pedigrees with genotypes filled in at random is an important tool to evaluate the power of a linkage study and to assess the empirical significance of linkage as well as association study results. SLINK (J. Ott PNAS 86(1989), 4175-4178; D. E. Weeks et al. AJHG 47(1990), A204) is one widely-used package for pedigree simulations. Over 15 years ago, SLINK was modified to incorporate faster algorithms from early versions of FASTLINK (Cottingham et al. AJHG 53 (1993), 252-263). One limitation of SLINK, as with most methods based on the Elston-Stewart pedigree likelihood algorithm, is the small number of markers that can be generated. The software package SUP (M. Lemire BMC Genetics 7(2006):40) is an elegant wrapper for SLINK that circumvents the limitation on number of markers by using pseudomarkers generated by SLINK to simulate a much larger number of markers on the same chromosome. Initial versions of SUP were limited in their functionality due to a limit on the number of alleles in SLINK and contorted in their design because SLINK did not output some internal variables needed by SUP. We have released new coordinated versions of SLINK (3.0; available from http://watson.hgen.pitt.edu) and SUP (v090804; available from mlemire.freeshell.org/software or http://watson.hgen.pitt.edu) that integrate the two software packages and remove the previous limitations on SUP. SLINK v3.0 includes improvements from later versions of FASTLINK that make it faster and simplify the set-up procedure. There is no restrictive limit on the number of alleles and the simulated genotypes at the trait locus can be output directly. SUP v090804 has been rewritten to handle pedigrees of arbitrary size and the typical usage is substantially streamlined, as compared to previous versions. Both new releases include new documentation and examples.

3018/F

Genetic Map Interpolator. *N. Mukhopadhyay[1], X. Tang[2], D.E. Weeks[1,2].* 1) Dept Human Genetics, Univ Pittsburgh/Sch Pub Hlth, Pittsburgh, PA; 2) Dept Biostatistics, Univ Pittsburgh/Sch Pub Hlth, Pittsburgh, PA.

Many statistical algorithms for analyzing genetic data require genetic maps of the markers, which specify the recombination rates between adjacent markers. However, while it is relatively easy to extract physical map positions from the on-line databases, it is more difficult to extract genetic map positions. Furthermore, some of the genetic map tools require that one input not only the marker ID, but also the marker's physical position in a specified older build of the genome. The Genetic Map Interpolator (GMI) package is designed to create interpolated genetic maps of single nucleotide polymorphism (SNP) markers. Starting from a list of SNP names, GMI uses the R packages biomaRt and RMySQL to fetch the most up-to-date SNP and microsatellite physical positions from Ensembl, and then combines these with the Rutgers combined genetic and physical map to estimate the corresponding Kosambi genetic positions by linear interpolation for these SNPs. The resulting information is then output in map files formatted to be read in by our data-reformatting program, Mega2. GMI has a number of useful features: (1) It automatically looks up and uses the physical positions for each marker from the most recent Ensembl build. (2) For each SNP that is not initially found in Ensembl, GMI automatically checks to see if that SNP has been assigned a different rs number. (3) GMI automatically figures out which chromosome each marker is on, so knowledge of a marker's chromosome is not required to run GMI. (4) For each of these database queries, the list of SNPs being queried is broken up into smaller chunks so that each individual query can be completed within a few seconds. This has been done to prevent premature timing out of web connections, and in order to obey the limit guidelines required for Entrez queries. GMI is a Unix-based program written in Perl and R, and is portable over most Unix platforms. We have used and tested it extensively on both Intel and PPC-based Macs. Both GMI and Mega2 are available for free from (http://watson.hgen.pitt.edu/register/). This reasearch is supported under NIH grant R01GM076667 (Weeks, Daniel E).

3019/F

A statistical approach for the identification of inter-population variation in recombination rates. *Q. Fan[1], SM. Saw[1,2], YY. Teo[1,3].* 1) Department of Epidemiology and Public Health, National University of Singapore, Singapore; 2) Singapore Eye Research Institute, Singapore; 3) Department of Statistics and Applied Probability, National University of Singapore, Singapore.

Context: Recombination is a driving force for linkage disequilibrium (LD) decay and it tends to cluster in highly localized regions named 'hotspots'. Using population-based recombination rate estimation method, several studies suggested that the recombination hotspots were evolutionarily conserved over broad scales among human populations, but not at the local level. However, the pattern of the fine-scale recombination rate variation due to hotspots across human populations is not well characterized partially because of the hotspot detection method is complicated and not generally consistent. Objective: The goal of the current study is to quantify the fine-scale variation in recombination rates from different well-defined human populations, especially in the locations and intensities of recombination hotspots. We further explore the association of those top-ranked regions with variation between population-specific patterns of LD, local positive selection and potential gene inversion, which has implication on the identification of genetic variants across populations. Methods: We propose a statistical method for the assessment of recombination rate variations between two populations, identifying the top regions in the human genome that exhibit these inter-population differences. Simulations were performed in various scenarios in terms of the hotspot peak strength and locations to test the sensitivity and specificity of this method. We apply this method to analyze the recombination rate data of 1.6 million SNPs from Chinese, Asian Indian and Malays in Singapore, and from Phase 2 of the HapMap. Results: Simulation indicates this method has high power (> 80%) to detect the existence of strong or moderate recombination rate peaks in one population but no hotspot peak or non-overlapping peak in another population; and it is not influenced by SNP density.The false discovery rate is generally below 20%. We observe this approach can be used to identify fine-scale variation in recombination rates between populations, and correlates with genomic regions exhibiting evidence of LD variations. The success of this approach also suggests the potential for prioritizing regions undergoing positive natural selection and gene inversion. Conclusion: The results demonstrate that our proposed method could lead to the location of the extent of the inter-population recombination rates variation.

3020/F

Synthesis-View: visualization and interpretation of SNP association results for multi-cohort, multi-phenotype data and meta-analysis. *S.M. Dudek, S.A. Pendergrass, D.C. Crawford, M.D. Ritchie.* Center for Human Genetics Research, Vanderbilt Univ, Nashville, TN.

Significant GWAS findings are being further investigated for replication and characterization, both in the populations in which the initial GWAS findings were discovered (such as European-Americans) as well as in new cohorts and populations. To increase sample size, meta-analysis is often used to combine results from multiple research sites. In addition, multiple independent and correlated phenotypic measurements may be included in the analyses, such as cardiovascular disease and related biomarkers (lipids, inflammation, etc). Visualization of these data, including p-values or other metrics of significance, allele frequencies, sample sizes, effect size, and direction of effect is integral to being able to interpret as well as share the complex and multi-layered results of these follow-up studies. The software "Synthesis View" has been developed to visually synthesize multiple pieces of information of interest from these studies with the flexibility to perform multiple types of data comparisons. Synthesis View was extended from the previous software "LD-Plus" which also uses a flexible data display format of multiple data "tracks" that can be viewed (Bush, et al., 2010). Through the use of stacked data-tracks information on SNP genomic locations, presence of the SNP in a specific study or analysis, as well as related information such as genetic effect size and summary phenotype information, is plotted according to user preference. With Synthesis view, trends from many different kinds of information can be visualized in a more integrated way than by using tabular data alone. This is important to understanding in greater depth the relationships between SNPs, strata, sample size, and phenotype differences, with the increasing complexity of emerging datasets. Synthesis-View is freely available for non-commercial research institutions, for full details see https://chgr.mc.vanderbilt.edu/synthesisview.

3021/F

Imputation of Untyped Markers for HyperGEN African American Population. *Y.J. Sung[1], C.C. Gu[1], H. Tiwari[2], D.K. Arnett[2], U. Broeckel[3], D.C. Rao[1].* 1) Div Biostatistics, Washington Univ, St Louis, St Louis, MO; 2) School of Public Health, University of Alabama at Birmingham; 3) Department of Medicine, Medical College of Wisconsin, Milwaukee.

Genetic imputation methods impute SNPs that are not genotyped in a study but are present on a reference panel such as the HapMap. They are popular for increasing statistical power and also for comparisons across studies using different platforms. However, the choice of reference panels for an admixed study sample is less straightforward. For African Americans (AA), the commonly used approach is "cosmopolitan" approach that combines HapMap Phase II CEU and YRI haplotypes into a single reference panel. We evaluated 3 strategies (http://www.sph.umich.edu/csg/abecasis) using MACH for imputing in 1,258 HyperGEN AAs who were genotyped with Affymetrix 6.0. The first strategy used the common "cosmopolitan" approach that consists of SNPs polymorphic in both CEU and YRI data. The second strategy used also "cosmopolitan" approach, but consists of SNPs polymorphic in either CEU or YRI data. The third strategy involved imputing twice with CEU and YRI separately and then merging the imputed data sets. These three strategies imputed 2.20 million, 3.01million, and 3.19 million SNPs, respectively. Based on results for chromosme 22 alone, the third strategy, imputation with CEU and YRI separately yielded discrepancy of 19.6%. For these discrepant calls, imputation with YRI was chosen for the merged data set based on HyperGEN AA' higher admixture proportion with YRI. The first and second strategy yielded concordance of 97.8%, whereas the first and third strategy yielded concordance of 94.9%. The accuracy rate was 99.4% for the first and second and 98.8% for the third strategy. Results from genome-wide imputations will be presented. We advocate using the second strategy because it is as accurate as the commonly-used (first) strategy and able to impute an additional 0.8 million SNPs that are ancestry-informative.

3022/F

KSNaP: A New Tool for Integrating SNP, Gene and Biomolecular Pathway Information. *N. Nock[1], R. Shields[2], C. Gray-McGuire[1,3].* 1) Department of Epidemiology & Biostatistics, Case Western Reserve University, Cleveland, Ohio, 44106, USA; 2) Department of Computer Science, Case Western Reserve University, Cleveland, Ohio, 44106, USA; 3) Department of Arthritis and Immunology, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma, 73104, USA.

The widespread use of high-throughput single nucleotide polymorphism (SNP) chip arrays has created an imminent need to efficiently identify common biological pathways from genomewide and large-scale custom SNP data sets. We have developed a software program called "KSNaP", which interfaces with widely utilized pathway (e.g., Kyoto Encyclopedia of Genes and Genomes, KEGG) and SNP (e.g., The Single Nucleotide Polymorphism National Center for Biotechnology Information (NCBI) database, dbSNP) databases to: 1) identify common biological pathways from user-provided SNP (and/or gene) data sets; and, 2) facilitate the integration of publicly available annotation information for SNPs (e.g., type, quality, allele frequency) in a given biological pathway. We will describe the features of KSNaP and illustrate its utility in analyzing SNP data from a systemic lupus erythematous study of 20,000 candidate genes and, in performing a pathway-level analysis of SNPs in the adipocytokine signaling pathway.

3023/F

Visualizing multidimensional support intervals for genetic models. *B. Nouanesengsy[1,2], S. Seok[1], V. Vieland[1].* 1) Nationwide Children's Hosp, Columbus, OH; 2) The Ohio State University, Columbus, OH.

Many genetic analyses involve log likelihoods (LogLs) written as functions of underlying genetic parameters such as gene frequencies, penetrances, etc. While maximum likelihood (ML) can be used to obtain the vector of parameter values "best" supported by the data, it is also frequently of interest to explore the multidimensional support interval (MDSI) itself. For instance, the ML model might support dominant inheritance with a specific penetrance, but dominant models with penetrances across a broad range, or even some recessive models, might also fall within an n-unit support interval of the maximum, particular as functions of variable disease allele frequencies or other parameters. Visualization offers an intuitive approach to exploring such multidimensional features of likelihood surfaces, which are otherwise difficult to capture. We have developed a visualization software program which creates groups of 2D plots (one for each genetic parameter) giving an overview of how each parameter affects the LogL. Each of these 2D plots displays the projection of the LogL space onto a two dimensional subspace, formed by the LogL and one parameter. An additional "overview" 2D plot is created by using the distance of each sampled point to a point of interest. This overview plot provides a high-level visual summary of the multidimensional space. The user can then define a support threshold, which defines the rectangular space MDSI, containing all portions of the parameter space for which the LogL exceeds the threshold. Multiple tools for exploration of the MDSI are provided. Regions of interest can be highlighted in one 2D plot, resulting in automatic highlighting of the corresponding regions in the other 2D plots. Additional 2D plots displaying pair-wise correlations among parameters are also available. 3D distance and correlation plots are currently under development to facilitate simultaneous viewing of 3 parameters at a time (say, three penetrances). Together, these visualization tools can provide information about the shape and size of LogL peaks and the correlations among parameters within regions of maximum support.

3024/F

Using full-information maximum likelihood estimation on sibling data, to determine whether we can combine variables across samples in genome-wide association studies. The example of cognitive data. *A.C. Wood[1], F. Rijsdijk[2], A. Arias-Vasquez[3, 4], N. Rommelse[3], K. Johnson[5,6], P. Andreou[2], B. Albrecht[7], H. Uebel[7], T. Banaschewski[8], M. Gill[5], I. Manor[9], A. Miranda[10], F. Mulas[11], R.D. Oades[12], H. Roeyers[13], A. Rothenberger[7], H.C. Steinhausen[14,15,16], J. Buitelaar[3], J. Sargeant[17], S. Faraone[18,19], P. Asherson[2], J. Kuntsi[2].* 1) Departments of Epidemiology and Section on Statistical genetics, University of Alabama at Birmingham, Birmingham, AL; 2) MRC Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, King's College London, UK; 3) Department of Psychiatry, Donders Institute for Brain, Cognition and Behavior, Centre for Neuroscience, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands; 4) Department of Human Genetics, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands; 5) Department of Psychiatry, Trinity Centre for Health Sciences, St. James's Hospital, Dublin, Ireland; 6) School of Psychology, Queen's University Belfast, Belfast, Northern Ireland; 7) Child and Adolescent Psychiatry, University of Göttingen, Göttingen, Germany; 8) Department of Child and Adolescent Psychiatry and Psychotherapy, Central Institute of Mental Health, University of Heidelberg, Mannheim, Germany; 9) S. Herzog Memorial Hospital, Research Department, Jerusalem, Israel; 10) Department of Developmental and Educational Psychology, University of Valencia, Valencia, Spain; 11) Department of Neuropaediatrics, La Fe University Hospital, Valencia, Spain; 12) Clinic for Child and Adolescent Psychiatry and Psychotherapy, University of Duisburg-Essen, Essen, Germany; 13) Department of Experimental Clinical and Health Psychology, Ghent University, Belgium; 14) Department of Child and Adolescent Psychiatry, University of Zurich, Zurich, Switzerland; 15) Child and Adolescent Clinical Psychology, Institute of Psychology, University of Basel, Basel, Switzerland; 16) Child and Adolescent Psychiatry, Psychiatric Hospital Aalborg, University Hospital Aarhus, Aalborg, Denmark; 17) Department of Clinical Neuropsychology, Vrije Universiteit, Amsterdam, The Netherlands; 18) Department of Neuroscience, SUNY Upstate Medical University, Syracuse, New York, USA; 19) Department of Psychiatry, SUNY Upstate Medical University, Syracuse, New York, USA.

Many Genome Wide Association Studies (GWAS) into psychiatric disorders use alternative phenotypes to the clinical manifestation of the disorder. For example, reaction time (RT) variability, has been strongly associated with attention deficit hyperactivity disorder behaviors (ADHD) at the phenotypic and genetic levels. It is hoped that this is more proximal to the biological etiology of ADHD than the diagnosis and so a truer representation of the underlying genetic liability for ADHD. Despite potential advantages, intermediate phenotypes can be more expensive and time consuming to collect than questionnaire data, and so prohibitive to large sample sizes, highlighting a need for collaborative research strategies. However, genetic heterogeneity across samples may decrease power in GWAS. We aimed to use full information maximum likelihood estimation to examine if there is justification for combining data across two different measures, where no participants had data across both task measures. Data were collected from ADHD probands, their siblings and control sibling pairs on IQ and measures of mean RT, RT variability, and omission and commission errors. RT and error data were collected across three tasks: 'fast task' 'go/no-go task' and 'stop task'. All participants had IQ data; 685 families only had error and RT data from the 'fast task' and 'go/no-go task'. A further 385 families only had error and RT data from the 'stop task'. Prior theory suggested that only the RT variables may be similar across tasks. Multivariate structural equation modeling (SEM) was employed, using all variables, in a familial model using the known correlations of latent familial and individual-specific etiological factors between members of a sibling pair. Mean RT across the two different tasks showed a familial correlation (rF) =.70 (95% confidence interval (CI): -.06 -1.00). The estimates for RT variability were rF= .81 (.81-.100). This indicated, for the first time, that RT variability data could be combined across different tasks and different samples for future GWAS studies. Additionally, bootstrapping methods were calculated in R and used with the SEM for a simulated sample size of 5,000. This gave similar point estimates, but much tighter CIs, with both forms of RT data showing significant rF across samples. This confirms the suitability of aggregating RT variability data and highlights the need to investigate methods for examining the genetic heterogeneity across samples.

3025/F

A small number of candidate gene SNPs reveal geographical ancestry. *N. KODAMAN[1], J.R. SMITH[2], L.B. SIGNORELLO[2,3], K. BRADLEY[2], J. BREYER[2], S. COHEN[2], J. LONG[2], Q. CAI[2], W.J. BLOT[2,3], C. MATTHEWS[2], S.M. WILLIAMS[1].* 1) CENTER FOR HUMAN GENETICS RESEARCH, VANDERBILT UNIVERSITY, NASHVILLE, TN; 2) DEPARTMENT OF MEDICINE, VANDERBILT UNIVERSITY, NASHVILLE TN; 3) INTERNATIONAL EPIDEMIOLOGY INSTITUTE, ROCKVILLE, MD.

Ancestry Informative Markers (AIMs) are genetic variants that differ substantially in frequency among geographical populations. They are frequently used in conjunction with PCA or STRUCTURE to infer ancestry and subsequently to control for stratification in genetic epidemiological studies. The number of AIMs necessary to infer ancestry and provide adequate information to control for stratification is not clear. Original estimates suggested as many as 300 AIMs were necessary, but more recently, Allocco et al. (2007) found that as few as 50 SNPs chosen randomly from the HapMap database predicted ancestral continent of origin with an average accuracy of 95%. This observation raises the question of whether AIMs are necessary to estimate ancestry in candidate gene studies if the study has a sufficient number of markers. Here, using genomic data from an obesity-related candidate gene study on 2547 African-American and Caucasian participants, we assessed the proportion of African/European ancestry in individuals by running STRUCTURE (k=2) with 276 AIMs, and then compared the results to analyses using randomly chosen subsets of 100, 50, and 25 AIMS, as well as 100, 50, and 25 SNPs chosen randomly from 1144 SNPs in 44 obesity candidate genes. Each subset of AIMs and SNPs was chosen randomly 100 times, and each randomized sample was analyzed with STRUCTURE 25 times. We found that all of the subsets of AIMs and SNPs generated reliable estimates of ancestry. For example, the correlation between quantitative ancestry estimates using 276 AIMs and only 50 random SNPs chosen from among the candidate genes was approximately 0.95. Our results confirm Allocco et al's conclusion on the informativeness of random SNPs, while further showing that even SNPs randomly chosen from a small number of hand-picked genes can accurately place individuals to their continent of origin and be used to estimate the degree of admixture in mixed-race individuals. Our findings suggest that future candidate gene studies on African-American and Caucasian populations could be conducted more cost-effectively by forgoing the use of AIMs to control for population stratification and just using SNPs from the candidate genes.

3026/F

More efficient experimental designs for copy number variation (CNV) studies utilizing aCGH technology. *S.K. McDonnell[1], S.M. Riska[1], E.C. Thorland[2], E.W. Klee[1], N.E. Kay[3], S.N. Thibodeau[2], J.E. Eckel-Passow[1].* 1) Department of Health Sciences Research, Mayo Clinic, Rochester, MN; 2) Department of Laboratory Medicine and Pathology, Mayo Clinic College of Medicine, Rochester, MN; 3) Division of Hematology, Mayo Clinic, Rochester, MN.

Introduction: Most CNV detection methods use microarray based technologies that output relative intensity values rather than absolute copy number (CN), necessitating the use of a baseline sample to estimate relative CN. In most studies utilizing aCGH technology, a common reference sample is co-hybridized to every array using the same dye to provide the baseline for CN estimation (reference design). Copy number is then calculated as the ratio of the intensity of the experimental sample relative to the same-array reference sample at each probe. Because the reference sample is often not of biological interest and is only used to estimate CN, we hypothesized that more cost-efficient designs exist that do not require a reference sample be co-hybridized on every array. These cost-efficient designs construct and utilize an average baseline for estimating CN. Here, we evaluate the effectiveness of an average baseline and how many reference samples are necessary to construct the average. Methods: We constructed multiple average baseline samples by averaging 2,...,n reference samples and used the average intensity at each probe as the baseline for estimating CN (average baseline design). We evaluated both the variability and sensitivity of the estimated CN associated with each average baseline sample and compared it with the reference design using empirical data. The median absolute deviation (MAD) of the CN estimates across all probes for a given subject was used as a robust measure of variability. Sensitivity was defined as the probability that the average baseline design detected a segment that was identified using the reference design. Data from a chronic lymphocytic leukemia (CLL) cohort study (n=50) run on the Agilent aCGH 1M array and data from a prostate cancer (PC) case-control study (40 cases, 20 controls) run on a custom Agilent aCGH array targeting chromosome 22 (105k probes) were used to test our hypotheses. Results: The distribution of MAD values stabilizes when the average baseline sample consisted of ≥10 (~ 25% of total study subjects) reference samples and is close to (PC data) or better than (CLL data) than the MAD values obtained from a reference design. With respect to sensitivity, most segments identified for a patient sample using the reference design were also identified for an average baseline consisting of 10 reference samples. Future work will evaluate the effect of competitive binding when co-hybridizing two experimental samples.

3027/F

A statistical approach to the adaptive design of optimal replication studies. *G. Rudolf, E. van den Oord, J. Bukszar.* Center for Biomarker Research and Personalized Medicine, School of Pharmacy, Virginia Commonwealth University, Richmond, VA.

Although replication studies are generally assumed to be critical for following up results from large scale genetic studies, they are often designed intuitively and in an ad hoc fashion. We developed a general statistical approach to design optimal replication studies. Our framework can be used to 1) minimize the costs of replication studies, 2) provide statistically motivated decision rules for declaring significance in replication studies (e.g. as opposed to arbitrary rules such as p-values smaller than 0.05 suggest replication), and 3) design replication studies "adaptively" by using the initial findings. This latter property avoids that replication studies are entirely based on assumptions that may be incorrect potentially leading to goals not being achieved or goals which could have been achieved at much lower costs. Our methods are developed in the context of two-stage GWAS studies, but can be readily adapted to different scenarios. Study design is a multi-objective problem, where we aim to simultaneously achieve a low false discovery rate (FDR), high average power (APW), and low cost. Design decisions include marker selection, sample selection, and critical value selection. We concentrate on the problem of minimizing the cost of the study while maintaining prescribed levels of FDR and APW. However, our optimization framework is sufficiently flexible to allow handling different requirements, such as maximizing the APW of a study with a fixed budget while controlling the FDR. An important additional concern is that in order to obtain estimates for FDR and APW we need information about the fraction of markers with no real effect (p0), and the distribution of the test statistic under both the null and the alternative hypothesis. This leads to the use of parameter estimation methods for p0 and the (average) effect size. A benefit of adaptively designing the followup stage of the study after the first stage results are observed is the opportunity to replace initial assumptions on parameter values with estimators before the follow-up stage is performed. Another advantage is that first-stage test statistics can be used to estimate the fraction of markers with effect in the second stage. We present simulation-based computational results comparing our methods with existing non-adaptive two-stage designs, demonstrating significant advantages including lower costs as well as avoiding underpowered study designs.

3028/F

A Method to Prioritize Quantitative Traits for Sequencing in Family-Based Studies. *K.P. Shah, J.A. Douglas.* Department of Human Genetics, University of Michigan, Ann Arbor, MI.

Genome-wide association studies have been used to examine the role of common variants in complex quantitative traits, however, the impact of rare variants on these traits has been largely unexplored. One strategy to identify rare variants associated with quantitative traits is to sequence unrelated individuals in the tails of the trait distribution using high-throughput sequencing technologies. We present a new method that leverages the relatedness of individuals in the context of family-based studies to prioritize quantitative traits that are most likely to be influenced by rare variation and therefore are good candidates for such sequencing studies. The hypothesis underlying our method is that phenotypically extreme individuals will be more closely related if the trait is influenced by a rare variant of strong effect as opposed to being purely polygenic (null hypothesis). To test this hypothesis, we condition on the observed pedigrees and derive the distribution of mean pairwise kinship coefficients through repeated simulation of a purely polygenic quantitative trait with heritability equal to that of the trait of interest. If the observed mean pair-wise kinship coefficient of individuals in the tails of the quantitative trait distribution is significantly larger than that under our simulated polygenic model, then there is evidence for a rare variant carried by phenotypically extreme individuals. Through simulation, we evaluated the power to detect the presence of an associated rare variant in a sample of 1,481 women from 178 extended Amish families from our genetic study of breast density. Conditional on our observed pedigrees, we have ≥50% power for quantitative traits ranging in heritability from 20-80% when the underlying variant explains ≥10% of the phenotypic variance and has a minor allele frequency (MAF) <5%. In general, the most powerful tail size corresponds to the expected number of carriers of the rare variant given the sample size and MAF. As proof of principle, we apply this test to a quantitative trait for which a previous sequencing study has identified a rare variant of strong effect. Using SNP data, we are also evaluating the strategy of estimating regions of the genome shared identical by descent by phenotypically extreme individuals to localize the associated variant. Application of these methods may be useful in prioritizing and mapping quantitative traits in family-based sequencing studies.

3029/F

A comparison of multiple testing correction methods using 2.5M imputed SNPs. *X. Gao.* Div Statistical Genomics, Washington Univ, St Louis, MO.

Multiple testing corrections are an active research topic in genetic association studies, especially for genome-wide association (GWA) studies, where tests of association with traits are conducted at hundreds of thousands single nucleotide polymorphisms (SNPs). Failure to address multiple comparisons appropriately can introduce excess false positive results and make subsequent studies following up those results inefficient. Permutation tests are considered the gold standard in multiple testing adjustment; however, this procedure is computationally demanding, especially for GWA studies where imputation has been conducted. Notably, the permutation thresholds for this huge amount of SNPs in real data sets have not been reported. Many researchers have recently developed algorithms to rapidly approximate the permutation thresholds with similar accuracy to the permutation test. In this study, we compare recently published multiple testing correction methods and measure their performance using 2.5M SNPs. We also derived permutation thresholds based on 10,000 permutations of GWA studies using 2.5M imputed SNPs and simulated phenotypes. Our results show that simpleM gives the closest approximation to the permutation threshold while requiring the least computation time. The thresholds derived in this study can provide multiple testing guidelines to current GWA studies using imputed SNPs.

3030/F

Effects of ignoring relatedness among study subjects in genotype imputation analyses. *S. Nelson[1], C.C. Laurie[1], D. Crosslin[1], B.L. Browning[2], M.L. Marazita[3].* 1) Department of Biostatistics, University of Washington, Seattle, WA; 2) Department of Statistics, University of Auckland, Auckland, NZ; 3) Department of Human Genetics, University of Pittsburgh, Pittsburg, PA.

Genotype imputation is the process of inferring unobserved genotypes in a study sample based on the haplotypes observed in a more densely genotyped reference sample, allowing for both improved power in individual genome-wide association studies (GWAS) and for meta-analysis of studies assayed on different platforms. We used BEAGLE software (Browning and Browning 2009) and a HapMap3 reference to perform genotype imputation in a dataset of 3,520 individuals of genetically-defined European ancestry, containing an assortment of duos, trios, and other nuclear and extended families. We ignored these family relationships in this imputation analysis, despite the violation of assumptions in the BEAGLE imputation method. To assess the impact of ignoring relatedness, we generated two additional sets of chromosome 1 imputation results in: (1) a maximal subset of 1,960 unrelated subjects run with a default seed and (2) the same subset of unrelated subjects run with a non-default seed. We then compared the estimated allele dosages among the three sets of results at 77,104 imputed SNPs across all 1,960 subjects from the unrelated subset. The mean correlation between allelic dosages from the primary approach and comparison set (1) was 0.998. The mean correlation between comparisons set (1) and (2) was also 0.998, indicating that ignoring relatedness in our primary analysis had minimal impact on imputation results. Furthermore, the distribution of dosage correlations across all imputed SNPs in the comparison of the primary approach and set (1) was very similar to the distribution when comparing sets (1) and (2). We also found high concordance rates in the maximal set of unrelated samples when comparing most likely imputed genotypes from the primary approach and set (1). When imputed SNPs were binned by minor allele frequency (MAF), the mean concordance ranged from 99.93% (MAF<2%) to 97.95% (MAF>20%). These findings indicate that the differences between imputation into the entire sample of related individuals and imputation into the maximal subsample of unrelated individuals are mostly attributable to the sequence of pseudorandom numbers generated during the imputation process. Our results suggest that, for large data sets, ignoring relatedness during imputation yields imputed genotype probabilities that are similar in quality to those obtained from imputation into unrelated individuals. NIH grant DE-018903, DE-014899, NIH contract HHSN268200782096C.

3031/F

A test for cryptic relatedness to map disease genes in as few as two affected individuals. *W. Wu[1], C. Huff[1], J. Xing[1], D. Witherspoon[1], T. Tuohy[3], D. Neklason[3], R. Burt[3], S. Guthery[2], L. Jorde[1].* 1) Department of Human Genetics, University of Utah, Salt Lake City, UT; 2) Department of Pediatrics, University of Utah, Salt Lake City, UT; 3) Huntsman Cancer Institute, University of Utah, Salt Lake City, UT.

Traditional gene-mapping approaches usually require either extended families or large sample sizes. New methods have been recently introduced to identify disease genes with much smaller sample sizes using exome or whole-genome data, although these data are still expensive to obtain and computationally challenging to analyze. Here, we present an inexpensive alternative that relies solely on high-density SNP microarray data and is appropriate for sample sizes as small as two affected individuals. From microarray data, we first use GERMLINE 1.4 to identify chromosomal segments that are identical-by-descent (IBD) between pairs of affected individuals who are not known to be related. The expected length of an IBD segment depends primarily on the number of generations since the common ancestor. When a rare disease-causing mutation is present on the segment, the expected length of the segment is increased due to ascertainment on disease status. Therefore, the size of an IBD segment is a powerful statistic for detecting a disease-causing mutation inherited from a relatively recent common ancestor. Using the empirical distribution of IBD segments from a population with matching genetic background, we can assess the statistical significance of the length of the largest IBD segment shared among cases. For two individuals with European ancestry, when a dominant disease-causing allele was inherited from a common ancestor five generations ago, the power to identify the disease-causing locus is approximately 0.88 at the 0.05 level; at 10 generations ago the power is about 0.66. The power improves substantially with either an increase in sample size or with a recessive mode of inheritance. We tested this method with 13 individuals with attenuated familial adenomatous polyposis resulting from an identical disease-causing mutation. This sample allows us to evaluate our method with pairs of individuals in a real world example where the number of generations to the common ancestor is unknown. Among the 62 pairs of individuals that were not known to be related, the test for cryptic relatedness was significant at the known disease-causing locus (APC gene) in 27 pairs. Therefore, the disease region could have been successfully mapped 44% of the time with just two individuals from this sample. In summary, this approach provides a powerful, inexpensive alternative for mapping rare genetic diseases without any family information, and with as few as two affected individuals.

3032/F

Comparison of affection status classification using less affected eye versus severe affected eye in Age Related Macular Degeneration. *L.M. Olson[1], K.L. Spencer[1], J.D. Hoffman[1], N.C. Schnetz-Boutaud[1], A. Agarwal[2], J.L. Kovach[4], S.G. Schwartz[4], P. Gallins[3], G. Wang[3], W.K. Scott[3], M.A. Pericak-Vance[3], J.L. Haines[1].* 1) Ctr of Human Genetics Research, Vanderbilt Univ Med Ctr, Nashville, TN; 2) Department of Ophthalmolgy, Vanderbilt Univ Med Ctr, Nashville, TN; 3) Hussman Insitute of Human Genetics, University of Miami - Miller School of Medicine, Miami, FL; 4) Bascom Palmer Eye Institute, University of Miami - Miller School of Medicine, Miami, FL.

Age-Related Macular Degeneration (AMD) is one of the leading causes of central vision loss among the aging Caucasian population in the United States. Previously published work has shown that genetics play a significant role in risk of AMD disease development. In these studies affection status classification is often reported based on the more severely affected eye. We propose that looking at the less severely affected eye does not change the risk for developing AMD. We created a case/control population with the following characteristics: Age of Exam (AOE) reported, genotyping for known risk factors: rs1061170 (CFH Y402H), rs10490924 (ARMS2 A69S), rs2230199 (C3 R102G) and mtDNA 4917 as well as protective factor rs641153 (CFB R32Q), smoking status (ever/never smoked/unknown) and OD (right eye) & OS (left eye) grades for both eyes. Our final dataset had a total of 988 individuals. First, affection status classification was determined based on the more severely affected eye (Affected=676, Unaffected=312), then univariate and multivariate analysis was completed to give us a baseline for comparisons as this method of affection status classifications is the current standard operating procedure. After reclassifying individuals based on their less affected eye (Affected=590, Unaffected=398), we ran univariate and multivariate analysis and compared the results. No significant deviations were found between the two classification with respect to the risk and protective factors when comparing both the univariate and multivariate odds-ratios.

3033/F

Selective sequencing for efficient fine-mapping of disease loci. *E. Marchani[1], E. Wijsman[1,2,3].* 1) Div Med Gen, University of Washington, Seattle, WA; 2) Dept Biostat, University of Washington, Seattle, WA; 3) Dept Genome Sci, University of Washington, Seattle, WA.

Sequence data is a vital, but expensive, tool for identifying the genetic causes of disease segregating within families. Linkage signals and genome-scan data in families can be used to target the most informative individuals and genomic regions for sequence data collection. These sequences are then used to identify candidate variants to be genotyped in the rest of the family with a less expensive protocol, or a subset of candidate genes for follow-up functional analyses.

We assume genome-scan marker data within a family and a region of interest, defined by candidate genes, associated alleles, or linkage signals. Under the hypothesis that a disease-risk allele is segregating within the family, affected individuals are expected to have inherited the genetic region surrounding that allele from the same ancestor (IBD) with high probability. The exact value of that probability depends on the number and penetrance of segregating risk alleles.

We estimate expected and observed kinship coefficients between pairs of affected relatives, excluding subjects unavailable for sequencing. Expected kinship may be estimated using pedigree structure without marker data or using the complete set of genome-scan markers, while observed kinship is estimated using pedigree data and multipoint marker information within the region of interest (*e.g.*, using MCMC methods). The ratio of observed-to-expected kinship is then used to rank pairs of affected individuals for sequencing, prioritizing pairs of distant relatives who have high IBD probability within the region of interest. This prioritization shortens the chromosomal segments shared IBD, reducing the number of shared variants and the amount time and money investigating these candidates.

Our approach excels when applied to extended pedigrees or genetically heterogeneous traits. Extended pedigrees offer more distant relationships, and therefore the greatest narrowing of IBD regions between prioritized subjects. In the case of genetic heterogeneity within a family, our approach allows investigators to target only pairs of affected relatives who share IBD in the region of interest and to avoid pairs that do not.

3034/F

Selection of rare variants by enrichment in familial samples and functional annotation: a data-oriented approach. *W. Yang[1], S. Wang[1], S. Hunt[3], A. Chakravarti[4], D.C. Rao[1,2], E. Boerwinkle[5], D. Arnett[6], U. Broeckel[7], C. Jaquish C[8], H. Tiwari[9], G. Stormo[2], C.C. Gu[1,2].* 1) Division of Biostatistics, Washington Univ in St Louis, St Louis, MO; 2) Department of Genetics, Washington Univ in St Louis, St Louis, MO; 3) Department of Internal Medicine, University of Utah, Salk Lake City, UT; 4) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 5) Human Genetics Center, University of Texas School of Public Health, Houston, TX; 6) Department of Epidemiology, University of Alabama at Birmingham, Birmingham, AL; 7) Department of Pediatrics, Medical College of Wisconsin, Milwaukee, WI; 8) Division of Cardiovascular Sciences, NHLBI, Bethesda, MD; 9) Department of Biostatistics, University of Alabama at Birmingham, Birmingham, AL.

Backgrounds: Genome-wide studies of rare variants have revealed that “small effect” variants can strongly affect those few who possess them. Methods were developed to “collapse” clusters of rare variants to produce detectable aggregate effects. A key to success is to include only “relevant” mutations in the clusters. We hypothesize that disease-associated variants are enriched in ascertained familial samples particularly for rare variants, and this information can be used to select promising rare SNPs for downstream analysis. Methods: Allele frequencies of 369,623 autosomal SNPs (shared on 2 platforms) in 1270 Caucasians from the family-based HyperGEN (Hypertension Genetic Epidemiology Network) study are compared to those in 9345 Caucasians from the population-based ARIC (Atherosclerosis Risk in Communities) Study. Enrichment of a SNP is measured by significantly elevated minor allele frequency (MAF). These are divided into 4 groups according to MAF: 0.001~0.01 (SNP Set 1), 0.01~0.05 (Set 2), 0.05~0.1 (Set 3), and ≥0.1 (Set 4). In each group, we calculated proportions of SNPs with functional annotation in public databases and the mean imputed functional score (IFS, estimated from cross-species conservation and transcription factor binding capacity of flanking sequences) using an in-house database; and compared the results with those derived from randomly selected SNP sets of equal sizes. Results: A total of 40,329 SNPs had significantly elevated MAF in HyperGEN: (1) SNPs in Sets 1-3 were slightly more likely to be proximal to a known gene, but not for those in Set4. (2) Compared with random SNP sets, SNPs in Set 1 (MAF<0.01) are more likely to contain exonic splicing enhancers (ESE, 0.90% vs 0), exonic splicing silencers (ESS, 0.01% vs 0), and non-synonymous mutations (NS, 0.42% vs 0). The trend holds true for Sets 2&3, but not for the more common SNPs in Set 4. (3) SNPs in Sets 2&3 have the highest probability to be ESE (0.13, 0.12, respectively), ESS (0.009, 0.018) and NS (0.043, 0.04). (4) The mean IFS scores in all 4 sets are substantially higher than those in the random SNP sets (0.39-0.44 vs 0.33-0.34). Moreover, the mean IFS values decrease as SNP MAF increases (0.44, 0.43, 0.42, 0.39 for Sets 1-4, respectively). Conclusion: The analyses provide strong empirical evidence for enrichment of rare variants in ascertained familial samples. It is a simple but potentially very useful way to enhance power of aggregate effects of rare variants.

3035/F

Improving genotype imputation in African populations. *L. Huang[1], M. Jakobsson[2], T.J. Pemberton[3], J.K. Pritchard[4,5], S.A. Tishkoff[6], N.A. Rosenberg[1,3].* 1) Center for Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI; 2) Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden; 3) Department of Human Genetics, University of Michigan, Ann Arbor, MI; 4) Department of Human Genetics, University of Chicago, Chicago, IL; 5) Howard Hughes Medical Institute; 6) Departments of Biology and Genetics, University of Pennsylvania, Philadelphia, PA.

In human populations, Sub-Saharan Africa has consistently been identified as the part of the world with the greatest genetic diversity. This high level of diversity causes difficulties for genome-wide association studies in African populations - for example, by substantially reducing the accuracy of genotype imputation in African populations compared to non-African populations. Here, we perform a detailed investigation of imputation in Africa, using genotypes at 1,272 SNPs in 253 unrelated individuals from 15 Sub-Saharan African populations. We identify the populations that provide the greatest potential for serving as reference panels for imputing genotypes in the remaining groups. Considering reference panels comprised of samples of recent African descent in Phase III of the HapMap Project, we identify mixtures of HapMap reference groups that produce the maximal imputation accuracy in each of the sampled populations. In many of the populations, the optimal mixture of reference groups for imputation can be predicted by using a simple model that relates imputation accuracy to the extent of haplotype sharing between study samples and potential reference samples. Our results suggest strategies that can be used to increase the accuracy of imputation in populations from Sub-Saharan Africa, thereby assisting in the extension of GWA techniques for use in these populations.

3036/F

Non-replication of genome-wide association studies: Winner's Curse or Survival Bias? *L.B. Chibnik[1], P. Kraft[2].* 1) Program in Translational NeuroPsychiatric Genomics, Department of Neurology, Brigham & Women's Hospital, Boston, MA; 2) Department of Epidemiology, Harvard, School of Public Health.

Purpose. Some risk loci discovered using Genome Wide Association (GWA) studies have not been replicated in other studies. One potential reason for non-replication is differential survival bias. We quantify this bias and its effect on the power to replicate an association found in a prospective cohort study (which is not affected by survival bias) within a case-control study (which is). Methods. The odds ratio (OR) expected from a case-control study can be estimated from the OR from a cohort study multiplied by an inflation factor, ψ, where ψ is the relative probability of ascertainment into a case control study for those with the risk allele versus those without. This probability depends on the relative hazard of mortality for the risk allele (HRg), the genotypic frequency, and the time from disease diagnosis to case ascertainment. We calculate the power to replicate an association observed in a cohort study across a range of these parameters in two scenarios, motivated by pancreatic cancer (5-year survival rate of 5%) and ovarian cancer (5-year survival rate of 45%) Results. The inflation factor ψ depends on median survival for the population and time from diagnosis to ascertainment into a case control study. It is inversely related to HRg such that for large HRg the OR in a case control study will be inverted relative to the OR from the cohort study. Starting with a cohort OR of 1.3 and time to ascertainment of 1 year post-diagnosis, for pancreatic cancer we observe a U-shaped relationship between power and HRg. The power to replicate the association decreases before an HRg of 1.5 and increases with larger HRg, reflecting increased power to detect the inverted association. For ovarian cancer, with a higher 5-year survival rate, power slowly decreases as mortality hazard increases. Conclusion. Survival bias has the potential to affect the power to replicate significant findings from a cohort study in a case control study. However, only very specific values of average survival, the relative mortality hazard, time to enrollment, and the association in the cohort study may significantly bias results.

3037/F

Exact Tests of Hardy-Weinberg Equilibrium - A Markov Base Approach. *M. Rao, S. Venkatesan.* Environmental Hlth, Univ Cincinnati, Cincinnati, OH.

The focus of the presentation is on exact tests of Hardy-Weinberg Equilibrium in multi-allelic problems. The exact test that is commonly used is the one developed by Guo and Thompson (1992). The key idea of the test is to fix the allele frequencies and enumerate all possible data sets with the same allele frequencies. Even for moderate number of alleles, the enumeration is a gargatuan task. Borrowing ideas from 'algebraic statistics,' we develop an exact test built upon Markov Bases and Metropolis-Hastings algorithm. This test is an alternative to Guo and Thompson's. A comparison of the proposed new test and Guo and Thompson's test is made.

3038/F

Comparative analyses of different algorithms for copy number variation identified by Affymetrix SNP 6.0 chips. *H. Wang[1, 2], X. Zhang[1], X. Gong[1], S. Li[1,2], F. Zhang[1], Y. Sun[1], Y. Shen[4], S. Xu[3], B. Wu[2,4], L. Jin[1,2,3].* 1) School of Life Sciences, Fudan Univ, Shanghai, China; 2) Institute of Biomedical Research, Fudan U, Shanghai, China; 3) Inst of Computational Biology, Chinese Academy of Sciences, Shanghai, China; 4) Boston Children's Hospital, Harvard Medical School, Boston, USA.

Copy number variation (CNV) is a new logo of disease susceptibility polymorphism in genomic DNA. Although there are many mature and widely used softwares for CNV analysis based on raw data calling, a gold standard is still absent. In order to validate the effectiveness of various software for CNV calling and analysis, we did the following comparison. First, we collected the SNP6.0 chip data from the total 325 samples which contain three pairs of duplicate samples to test for the different software's reproducibility on data analysis. Then Penncnv, Cnag, Birdsuite and Dchip were run to analyze the sample sets and generate CNV data. In terms of the identified CNV number, Dchip generated the most (36568), while CNAG called the least (6481). Birdsuite's and Penncnv's call were close to 22494 and 17594 respectively. Penncnv's Gain-Loss ratios were always under the isoline; CNAG close to the isoline; Dchip and Birdsuite were above the isoline. Analysis of CNV lengths shows that Dchip's CNV call mainly were below 100K, CNAG above 100K, and Birdsuite's and Penncnv's distributions were similar and uniform. The verification of these four algorisms' consistency reveal that nearly 1/3 of the CNV call by Penncnv's and Birdsuite were similar to that called by the other software, while CNAG is about 2/5. Penncnv verified 87%, CNAG 74%, Dchip 49%, and Birdsuite 53% through the three pairs of duplicate samples reproducibility test. Finally, the CNVs with the Q-PCR verification results were also sent to a DGV-like self-developed database. By comparison analysis, Cnag is the most conservative in CNV calling with the lowest false positive rate and the highest false negative rate. Cnag was the most effective algorithm of CNV detection for the known loci. Dchip's algorithm is the least conservative, which generated the most results. Both Birdsuite and Penncnv have very low false positive and false negative rates. The overall performance of Penncnv is the best calling, but the software has no visual interface because of running under Dos. In summary, the most optimized method is to use Penncnv for Raw data analysis, Birdsuite for visualized analysis, CNAG for exporting SNP signal for the association analysis and then Dchip for LOH analysis. With this combination, we imported all reliable CNV and LOH call into the DGV and our own database for the subsequent data mining.

3039/F

CNVineta: A data mining tool for large case-control copy number variation data sets. *M. Wittig[1], I. Helbig[2], S. Schreiber[1], A. Franke[1].* 1) Christian Albrechts University, Institute of Clinical Molecualr Biology, Kiel, Germany; 2) Department of Neuropediatrics, University Clinic Schleswig-Holstein, Campus Kiel, Arnold-Heller-Strasse 3, Building 9, 24105 Kiel, Germany.

Copy number variation (CNV), a major contributor to human genetic variation, comprises 1 kb or longer genomic deletions and insertions. Yet, the identification of CNVs from microarray data is still hampered by high false negative and positive prediction rates due to the noisy nature of the raw data. Here, we present CNVineta, an R package for rapid data mining and visualization of CNVs in large case-control data sets genotyped with single-nucleotide polymorphism oligonucleotide arrays. CNVineta is compatible with various established CNV prediction algorithms, can be used for genome-wide association analysis of rare and common CNVs and enables rapid and serial display of log2 of raw data ratios (LRR) as well as B-allele frequencies (BAF) for visual quality inspection. In summary, CNVineta aides in the interpretation of large-scale CNV data sets and prioritization of target regions for follow-up experiments. CNVineta is available as an R package and can be downloaded from http://www.ikmb.uni-kiel.de/CNVineta/; the package contains a tutorial outlining a typical workflow. The CNVineta compatible HapMap (International HapMap Consortium 2003) data set can also be downloaded from the link above.

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Identification of Copy Number Variations associated with Hepatitis B Virus Infection in Hepatocellular Carcinoma Families and Chronic Liver Diseases. *K.Y. Chang[1], S.W. Chang[1], D.I. Tai[2], C.L. Hsu[1], C.S.J. Fann[1].* 1) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 2) Liver Research Unit, Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Taipei, Taiwan.

Hepatitis B is a major health problem worldwide and a potentially life-endangering liver infection. Previous studies have shown that male was a significant factor for hepatitis severity. In this study, we conducted a genome-wide survey of copy number variations (CNVs) with 625 male Taiwanese. To our knowledge, this is the first CNV study on HBV infection: 321 HBsAg positive cases and 304 HBsAg negative controls were included and genotyped by Illumina HumanHap beadchips. A total of 456,262 SNPs was used in the analysis after quality control filters with an average call rate of 99.95%. To detect CNVs with intensity data of 456,262 SNPs, PennCNV was applied. 52 CNV gains and 390 losses were identified that were different between the case and control groups (Fisher's exact test P < 10-6). The case and control groups showed significantly different patterns of CNV gains on chromosomes 6, 9, 11, 14, 15, 16, and 19 and different patterns of CNV losses on chromosomes 6, 8, 11, 14, 15, and 19. We found that one deleted region (P < 10-21) and one amplified region (P < 10-8) covered HLA-B locus on chromosome 6 that might be associated with persistent HBV infection.

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Relationship between obesity, diabetes and admixture in African American and Hispanic American Women. *R. Nassir[1], L. Qi[2], R. Kosoy[1], L. Garcia[2], J.A. Robbins[3], M.F. Seldin[1].* 1) Rowe Program Gen, Univ California, Davis, Davis, CA; 2) Public Health Sciences, Univ California, Davis, Davis, CA; 3) UCD Health System, General Medicine, UCDMC, Sacramento, CA.

Different ethnic groups have different propensities for obesity and type 2 diabetes. To further assess the relationship between ethnicity and these phenotypes we examined whether differences in continental admixture in a cross-sectional analysis of both African American and Hispanic American adult women in the Women's Health Initiative were associated with these traits. The proportion of European, sub-Saharan African and Amerindian admixture was estimated for each individual participant using a Bayesian clustering method (STRUCTURE) and 92 SNP ancestry informative markers (AIMs). In self-identified African American women (n= 11821) there was a significant positive association between BMI and African admixture (p < 10-4). This relationship had a substantial effect size (estimate = 0.42 +/- 0.08 expressed as a fraction of the trait standard deviation) when BMI was considered as a continuous variable, and age, education, exercise, parity, income and smoking were included as covariates. A dichotomous model (upper and lower BMI quartiles) showed that a unit increase in African admixture was associated with a high odds ratio [OR = 3.25, 2.07 - 5.12, 95% confidence interval (CI)]. In contrast, for the self-identified Hispanic group (n = 5133) there was no association between BMI and admixture. When waste to hip ratio (WHR) was used as an alternate measurement of obesity, very different results were obtained. There was no significant association in the self-identified African American group but there was a strong association in the Hispanic group (estimate = 0.65 +/- 0.08, p<10-4; dichotomous model OR Amerindian admixture = 5.81, CI = 3.60 - 9.37). Finally, diabetes prevalence showed a positive association with both African [Hazard Ratio (HR) = 1.98, p < 10-4] and Amerindian admixture (HR = 2.00, p <10-4) in African American and Hispanic American groups, respectively. In the African American group the HR decreased significantly when BMI was used as a covariate. For Hispanic Americans, WHR but not BMI significantly decreased the HR of the diabetes association with Amerindian admixture. These studies show that 1) African admixture is associated with both obesity (BMI) and diabetes in African American women; 2) Amerindian ancestry is associated with both obesity (WHR) and diabetes in Hispanic American women; and 3) it may be critically important to consider different indices of obesity in different ethnic population groups.

3042/F

Gene mapping study of constitutive skin color in a genetically isolated population. *H-J. Kim[1,4], SH. Paik[2], H-Y. Son[3], SB. Lee[1,4], YS. Ju[3,4], JH. Yeon[2], SJ. Jo[2], J-S. Seo[1,3,4], OS. Kwon[2,6], J-I. Kim[3,4,5].* 1) Department of Biomedical Sciences, Seoul National University Graduate School; 2) Departments of Dermatology, Seoul National University College of Medicine; 3) Department of Biochemistry and Molecular Biology, Seoul National University College of Medicine; 4) Genomic Medicine Institute (GMI), Medical Research Center, Seoul National University; 5) Psoma Therapeutics, Inc., Seoul; 6) Laboratory of Cutaneous Aging and Hair Research, Institute of Dermatological Science, Seoul National University.

To elucidate the responsible genes governing constitutive skin color, we measured the extent of skin pigmentation in buttock, the sun-unexposed area for the life time, and conducted a gene mapping study on skin color in genetically isolated population composed of 344 individuals from 59 families who live in Dashbalbar, Dornod Province, Mongolia. Through the linkage analysis of 1039 short tandem repeat (STR) microsatellite markers, we found novel genomic region regulating constitutive skin color on 11q24.2 with an LOD score of 3.39. Moreover, we found other five candidate regions controlling intrinsic skin color. To test further association in regions of linkage, we selected chromosome 11, 17, and 6 (maximum LOD score > 2.5), and in each chromosome, high linkage peak regions (LOD score >1.5) were analyzed. On linkage region of chromosome 11, we identified 19 significant SNPs (p < 9.29 × 10-6). Further we found two (p =9.74 × 10-7) and one (p=2.00×10-6) significant SNPs on linkage regions of chromosome 17 and chromosome 6, respectively. In that the strongest locus of linkage on 11q24.2 harbors and significant SNP is located adjacent to ST3GAL4, we suggest ST3GAL4 as the novel candidate gene responsible for controlling constitutive skin color. Taken together with our linkage analysis and association study, other candidate genes are DRD2, MPZL3, BRIP1 and ZBTB17.

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Genome-wide detection and characterization of mating asymmetry in human populations. *M. Bourgey[1], J. Healy[1], P. Saint-Onge[1], H. Massé[1], D. Sinnett[1, 2], M-H. Roy-gagnon[1, 3].* 1) Hemotologie-Oncologie, CHU Sainte-Justine, Montreal, PQ, Canada1Sainte-Justine Hospital Research Center, University of Montreal, 3175 chemin de la Côte-Sainte-Catherine, Montreal, Quebec, H3T 1C5, Canada; 2) Department of Pediatrics, Faculty of Medicine, University of Montreal, 3175 chemin de la Côte-Sainte-Catherine, Room 7955, Montreal, Quebec, H3T 1C5, Canada; 3) Department of Social and Preventive Medicine, Faculty of Medicine, University of Montreal, PO Box 6128, Station Centre-Ville, Montreal, Quebec, H3C 3J7, Canada.

The mother can play an important role in shaping disease susceptibility in her offspring through the effects of her genes acting directly on the intrauterine milieu or indirectly through feto-maternal gene-gene interactions. An important source of bias in feto-maternal genetic association studies however is the possibility of confounding due to mating asymmetry (MA). Departures from mating symmetry between parental mating pairs can lead to distortions in the distribution of alleles among case-mothers and create spurious maternal associations. The evaluation of the effects of asymmetry on feto-maternal association testing has been limited by our meager understanding of the dynamics of MA in human populations. We propose a new quantification estimator for mating asymmetry (ChA) along with its related test to determine the significance of asymmetry at a given locus. To achieve a fuller understanding of the dynamics of mating asymmetry in human populations we carried out a genome-wide evaluation of MA using mate-pairs of European and African ancestry from the International HapMap Project. We measured MA for ~1.2 million SNPs from all autosomes of the CEU and YRI population samples and investigated MA variation both within and across populations. Our results show that specific genomic regions are subject to MA among human populations and we show how the levels of MA observed in human population can incur important bias in feto-maternal association testing and significantly inflate false-positive rates.

3044/F

Genome-Wide Association Scan of Osteoporotic Fracture Rate among African American Women in the Women's Health Initiative. *K. Taylor[1], K. North[1], N. Franceschini[1], R. Jackson[2], A. LaCroix[3], J. Robbins[4], B. Lewis[5], M. Stefanick[6], J. Cauley[7].* 1) Epidemiology Dept, University of North Carolina at Chapel Hill, Chapel Hill, NC; 2) Ohio State University, Columbus, OH; 3) Fred Hutchinson Cancer Research Center, Seattle, WA; 4) University of California at Davis Medical Center, Sacramento, CA; 5) University of Alabama, Tuscaloosa, AL; 6) Stanford Prevention Research Center, Stanford University, Stanford, CA; 7) University of Pittsburgh, Pittsburgh, PA.

Background. Osteoporosis is a major public health problem that over a lifetime results in fractures in 40% of aging women, resulting in disability and death. Osteoporosis has a strong genetic component, with 25-85% of the variation in osteoporosis-related traits being attributable to genetic factors. However, most of these data are from Caucasian populations. We used GWAS data in the Women's Health Initiative (WHI) as part of the SNP Health Association Resource (SHARe) program to study genetic determinants of fracture among African American (AA) women. Methods. Data on incident fractures, except fingers, toes, face, skull or sternum, were analyzed for 8155 unrelated AA clinical trial (CT) and observational study (OS) participants in the WHI. Hip fractures were centrally adjudicated in all arms of the WHI. Other fractures were only adjudicated in the CTs, and in select OS centers. The Affymetrix 6.0 GWAS panel was used for genotyping, and YRI and CEU individuals from HapMap were pooled and used as the reference sample for imputation. Individual ancestry estimates, divided into Caucasian, East Asian, Native American, and African ancestry proportions, were calculated using FRAPPE and were used to adjust for population stratification. We used Cox proportional hazard models to evaluate the effect of ~2.5 million SNPs on incident fracture rate, adjusting for ancestry, age, and region (4 geographical regions in the U.S.). Results. No SNPs reached genome-wide significance (P £ 5.0 E-8). For self-reported fractures (874 events, 304 of which were adjudicated), one intronic SNP in MOGAT2, involved in dietary fat absorption, approached significance (rs12284314, P=7.9 E-8; minor allele frequency, 0.21) (hazard ratio: 1.37; 95% CI: 1.22, 1.53). The same SNP was the most significant locus when restricting the analysis to adjudicated fractures (P=2.4 E-7). This novel finding was not reported in any previous osteoporosis-related GWA studies. Given that dietary fat is important for bone health, replication of our findings in other African American populations is warranted.

3045/F

Test of independence in contingency tables of large dimension with ordered categories and its application in population genetics. *J. Ge[1,2], R. Chakraborty[1,2], B. Budowle[1,2].* 1) Inst of Investigative Genetics, Univ North Texas, Health Science Center, Fort Worth, TX; 2) Department of Forensic and Investigative Genetics, University of North Texas Health Science Center, Fort Worth, Texas.

Classic tests of independence of two categorical variables generally use chi-square or likelihood ratio statistics by summarizing data in a two-way contingency table. However, at times the sample size is not large enough to populate the cells of the contingency table, such as when analyzing Y chromosome short tandem repeat (STR) haplotypes that often are observed only once in a sample population database. In this situation, the classic tests yield degenerate distributions (or nearly so). In this study, a new permutation test was developed based on the number and distribution of mismatches between profiles from unrelated individuals. This test is statistically powerful even when the sample size is smaller than the number of categories of either variable. Independence among autosomal STRs, Y chromosome STR and mitochondria DNA (mtDNA) were tested with empirical data, and independence among them was confirmed. The power of the mismatch-based test was calculated. The dependence between autosomal and Y chromosome markers due to population substructure can be detected with greater than 90% accuracy if the subpopulations have similar proportions and at least 800 samples are typed.

3046/F

Reference samples in imputation and its implications in association analysis results. *M. de Andrade[1], M.E. Matsumoto[1], S. Maharjan[1], E.J. Atkinson[1], S.L.R. Kardia[2].* 1) Div Biomed Statistics Informat, Mayo Clinic, Rochester, MN; 2) Dep Epidemiology, Un Michigan, Ann Arbor, MI.

Imputation of untyped genetic markers has been shown to increase genome coverage in genome wide association studies with minimal added cost. However, there are still some questions about which reference samples to use when imputing and how much the reference influences association analysis results. Our evaluation is two-fold. First, we used four reference panels and evaluated the overall quality and accuracy of the imputed markers. Second, we compared association analysis results using known markers and imputed versions of the same markers. To compare the four reference panels, we used two populations from the GENOA cohort: 1385 European Ancestry (EA) sibships and 1535 African Ancestry (AA) sibships. We used MACH (version 1.0) software for the imputation and four reference panels all pulled from the HapMap Phase II data: 1) 60 CEU samples; 2) 60 YRI samples; 3) combination of the 1st and 2nd panels; 4) combination of panel 3 with 90 JPT/CHB samples. For each study population we applied a two-step procedure. Step 1 was run using a set of unrelated subjects (510 for AA and 379 for EA) and one of the 4 panels to infer haplotype phase and recombination rates. In step 2, 2.5 million markers were imputed for subjects using the first stage estimates. To evaluate imputation quality and accuracy we examined the minor allele frequency of the imputed dosage, the average recombination rates, the quality measures and the accuracy of masked genotypes. To compare the association analysis results, we first evaluated the familial relationship implied by the genotype data since our samples consist of related individuals and the imputation was performed assuming unrelated samples. This was done using identity-by-descent calculations based on the known genotypes and on the 4 versions of imputed genotypes. Next, we performed association analysis using the known genotypes and using the imputed genotypes of selected known markers. Our preliminary results show that panel 1 is a good reference for the GENOA EA sibships, panel 3 is a good reference for the GENOA AA sibships, and the association analysis results are not affected if a good reference panel was used.

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Genome-wide association study of Ankylosing Spondylitis identifies four new loci, a tag SNP for HLA-B27, and an interaction between HLA-B27 and variants in ERAP1. *DM. Evans, WTCCC2 and TASC.* Social Medicine, Univeristy Bristol, Bristol, United Kingdom.

Ankylosing Spondylitis (AS) is a common inflammatory arthritis, which can lead to fusion of the spine and other affected joints. In order to identify genetic variants predisposing to risk of AS, we performed a genome-wide association study of 1782 British cases fulfilling modified New York Criteria and 5167 historical controls from the Wellcome Trust Case Control Consortium 2 (WTCCC2). After imputing to Hapmap, the study was combined with existing results from the TASC Consortium using inverse variance meta-analysis and subsequently replicated in 2109 cases and 4410 controls from the UK and Canada. As well as confirming known associations at HLA, IL23R, ERAP1, 2p15 and 21q22 we identified and subsequently replicated risk predisposing variants in RUNX3 (combined p = 3.3 x 10-12), KIF21B (p = 1.1 x 10-11), IL12B (p = 1.8 x 10-8), and LTBR (p = 4.5 x 10-10), and found suggestive association at ANTXR2 (p = 3.8 x 10-7), PTGER4 (p = 7.8 x 10-8), CARD9 (p = 1.2 x 10-6), TRADD (p = 4.1 x 10-6) and TBKBP1-TBX21 (p = 5.9 x 10-8). We identified a single SNP, rs4349859 near the gene MICA, which tagged HLA-B27 with near perfect sensitivity (98%) and specificity (99%) in 531 cases and 729 controls of UK origin. These findings were confirmed in an independent set of 251 Sardinians and also showed that whilst rs4349859 tagged the non-AS associated HLA-B2709 subtype, it did not tag the AS associated HLA-B2707 subtype, indicating that rs4349859 is not AS causative. Finally, we identified an interaction between HLA-B27 and variants within ERAP1 in the WTCCC2 (p = 0.008), TASC (p = 0.004) and replication datasets (p = 0.004). Specifically, risk variants in ERAP1 increased odds of disease in individuals who were positive for HLA-B27, but not those who were B27-ve (combined interaction p = 1.4 x 10-6). This result implies that B27+ve and B27-ve forms of disease have substantially different aetiologies and that ERAP1 contributes to disease risk through its action in trimming peptides prior to loading into nascent HLA class I molecules, rather than by cleaving pro-inflammatory cytokine receptors on the cell membrane. In summary, we have identified four new loci at genome-wide significant levels that affect risk of AS, a single SNP variant that tags HLA-B27 which may be used in the future as a cheap alternative to expensive B27 typing, and have discovered one of the first convincingly replicated examples of a genetic interaction affecting risk of a complex disease.

3048/F

Genes influencing the high sensitivity C-reactive Protein (hs-CRP) levels by the obesity status: The Healthy Twin Study, Korea. *D.E. Kim[1], J. Sung[1], Y.M. Song[2], K. Lee[3], D.H. Lee[1], M.K. Lee[1].* 1) Department of Epidemiology, Seoul National University of Public Health; 2) Department of Family Medicine, Samsung Medical Center, SungKyunKwan University School of Medicine; 3) Department of Family Medicine, Busan Paik Hospital, School of Medicine, Inje University.

Recently, growing body of evidence indicates the role of inflammatory process in the etiology of coronary heart disease(CHD) and carcinogenesis. It is also well known that obesity increase the inflammation status, and increased inflammation may bridge obesity and its sequels. C-Reactive Protein(CRP) is a general marker released during inflammation process. CRP level showed genetic contributions and recent genome-wide association studies revealed several genetic variants regulating CRP levels. The specific goal of the study is to identify the genes accounting for the serum CRP concentration, and comparing the CRP-associated genes with genes showing association after considering the obesity level. overall genetic contribution of CRP level was analyzed by variance component methods (SOLAR) and specific genes were identified by the Family Based Association Test(FBAT). 3445 individuals of Korean twins and their families, including 1239 twins in the Healthy Twin study in Korea were analyzed. Affymetrix Genome-wide Human SNP Array 6.0 Genechip was used for genotyping the subjects. The average CRP concentration of objects is 0.154mg/L(1487 for men, 1958 for women). With solar, estimated heritability is 0.350(±0.031) and after adjusting age, sex, and BMI, heritability estimated about 0.325((± 0.032). In genome-wide association test, SNP A-1993164 and flanking SNPs (in chromosome 8) showed genome-wide significance SNP (p-value : 1.70E-0.05) adjusting age, sex or smoking. The analyses were repeated after adjusting BMI levels. It is assumed that chromosome 8 has a significant role on hsCRP level. As a conclusion, Genetic factors associated with many other environmental factors to determine CRP level.

3049/F

A powerful truncated tail strength method for testing an overall hypothesis in a dataset. *B. Jiang[1], X. Zhang[1], Y. Zuo[2], G. Kang[1,3].* 1) The University of Alabama at Birmingham, Birmingham, AL; 2) Michigan State University, East Lansing, MI 48824; 3) School of Medicine, University of Pennsylvania, Pennsylvania, PA 19104.

In microarray analysis, medical imaging analysis and functional magnetic resonance imaging, we often need to test a large number of single hypotheses (usually larger than 1,000) in one data set. To measure an overall significance for a set of a large number of m (≥ 1,000) independent single hypothesis tests under the overall null hypothesis that all m single hypotheses are true, a tail strength statistic (Taylor and Tibshirani, 2006) and Fisher's probability method are useful and can be applied. Here, we propose a new method that improves the tail strength statistic by only considering the values whose corresponding p-values are less than some pre-specified cutoff. We call it truncated tail strength statistic. Then, we propose a Monte Carlo method to estimate the empirical p-value of the truncated tail strength statistic. Simulation studies show that the truncated tail strength statistic not only controls type one error rate quite well and but also it has significantly higher power than the tail strength method and Fisher's method with a cutoff value of 0.005 in most cases. We applied the truncated tail strength method to two genome-wide data sets by chromosome. Result showed that our truncated tail strength method with a cutoff of 0.005 identified more significant chromosomes than the tail strength method.

3050/F

APOBEC3B gene polymorphism among patients with chronic Hepatitis B Virus infection. *S. SUBODH[1], S. Datta[2], K. MADAN[3], S. PARIHAR[1], K. NARAYANASAMY[1].* 1) THE CENTRE FOR GENOMIC APPLICATION, AN IGIB-IMM CO, NEW DELHI; 2) INDIAN STATISTICAL INSTITUTE, KOLKATA; 3) ALL INDIA INSTITUTE OF MEDICAL SCIENCES, NEW DELHI.

A large number of chronic liver diseases and hepatocellular carcinoma cases in India are attributed to Hepatitis B virus infections. The disease progression may be due to an inter play of viral and host factors. It is imperative to understand these factors to direct our understanding of disease outcomes and therapeutic targets. One such host factor is the APOBEC3B gene (A3B) which is believed to play an important role in innate immunity by posing barrier to viruses such as HBV and HIV. A deletion polymorphism of this gene can seriously alter the functioning of A3B and would be preferentially selected if this variant offered the individual with greater resistance to specific pathogens. To test this hypothesis we analyzed the A3B deletion frequency in 152 patients chronically infected with HBV and those who were carriers. These individuals did not clear the virus and were assumed to have a high A3B deletion allele frequency. Chi-square analysis revealed that the frequency of insertion homozygous individuals (II) was much higher among HBV patients than among controls. On the other hand the deletion homozygous individuals (DD) comprised only 2% of the HBV patient population as compared to 6% in the control population. Additionally, the frequency of the insertion allele (I) was significantly higher among HBV patients. It is possible that A3B be acting in conjunction with other proteins by alternate mechanisms and may not be the major determinant of viral clearance.

3051/F

Statistical Prediction of Classical HLA Typing Using Unphased SNP Data. *X. Zheng[1], J. Shen[2], M. Ehm[2], M. Nelson[2], B. Weir[1], J. Wakefield[1,3].* 1) Departments of Biostatistics, University of Washington, Box 357232, Seattle, Washington 98195-7232, USA; 2) GlaxoSmithKline, Research Triangle Park, NC 27709, USA; 3) Departments of Statistics, University of Washington, Box 357232, Seattle, Washington 98195-7232, USA.

Genetic variation in the Human Leukocyte Antigen (HLA) region, located on chromosome 6p21.3, is known to be highly polymorphic, and typing at classical HLA loci has been an essential tool for basic as well as clinical immunology research. High-resolution HLA typing is limited by the cost of genotyping technologies. A hidden Markov model (HMM) has been developed to predict HLA alleles based on haplotype inference from genotypic data in a recent study (Leslie et al, 2008). Their approach does not take into account the different probabilities of the estimated individual haplotypes from the phasing procedure, and does not allow missing values in the SNP predictors. Here, we introduce a novel statistical method for predicting HLA alleles, which computes the posterior probability from all possible haplotypes in the training data under the model assumptions of Markov property. Our method allows missing SNP genotypes in the training and validation data, and is computationally efficient because no haplotypes are estimated for each individual. Leave-one-out cross-validation is used to demonstrate the performance of our method on prediction accuracy, compared to the previous approach (HMM), for the four HapMap populations. Two additional datasets, 1958 birth cohort data of Welcome Trust Case Control Consortium (n = ~1800), and data for 1564 subjects from several racioethnic groups of GlaxoSmithKline collections, are used to validate the accuracy of our approach. Our results indicate that a panel of 50 ~ 100 SNPs typed at specific loci is sufficient to predict classical HLA alleles with ~90% accuracy in European samples. Finally, our SNP-based method provides an efficient and low-cost way to predict HLA alleles using genome-wide association data, which is useful in many experimental and clinical settings. Our study is supported in parts by NIH grant GM 75091.

3052/F

The story of chromosome 16 rewritten in relation to susceptibility to Crohn's Disease. *D.M. Swallow, W. Lau, N. Maniatis.* Research Department of Genetics, Evolution & Environment, University College London, London, United Kingdom.

Crohn's Disease (CD) is one of two main kinds of Inflammatory Bowel Disease (IBD) with an estimated heritability of approximately 50-60%. Although a Meta-analysis on three genome-wide association studies has detected 32 loci, these account for only 20% of the genetic risk of the disease. In this study we employ a novel association method based on linkage disequilibrium mapping and utilising a multilocus approach to identify new regions associated with CD. We focused on chromosome 16, which has been reported to show significant linkage over a very extensive region, and which cannot fully be accounted for by the one chromosome 16 gene (NOD2) that has been identified. The chromosome was divided into non-overlapping windows measured in LD units, for which the significance of association with disease status was calculated. We detected significant associations between CD and several novel regions implicating multiple genes, some previously reported for ulcerative colitis (CADH1, CADH3), or multiple sclerosis (IRF8), which also has features of an immune dysregulation condition. Introduction of this approach will accelerate the identification of genes and pathways for CD, and will be useful for other complex traits.

3053/F

Association of Polymorphisms in Novel Innate Immune Genes with Gram Negative Sepsis. *I.V. Yang, L.A. Warg, S.D. Alper, D.A. Schwartz.* Center for Genes, Environment, and Health, Departments of Medicine and Immunology, National Jewish Health, Denver, CO 80206.

The role of host susceptibility in the initiation and severity of infections caused by Gram negative (GN) bacteria is incompletely understood. Our project aims to further understand why some individuals develop infection, and of those with infection, why only some go on to have adverse outcomes. We have previously shown that polymorphisms in TLR4 predispose humans to GN sepsis. However, our previous findings also demonstrate that sequence variants of TLR4 account for only a portion of the LPS phenotype in either mice or humans and that other genes are involved in regulating the response to LPS. In this study, we used a combination of genetic and genomic techniques following a challenge with lypopolysaccharide (LPS), an outer membrane component of GN bacteria, in model systems (mice, *C. elegans*, and cell culture) to identify genes other than TLR4 that may regulate the pathophysiologic response to innate immune stimuli in humans. To translate these findings to humans, we performed an association study in patients with GN bacteremia. Tagging SNPs were selected for 35 high priority candidate genes and three 96-plex Illumina Veracode GoldenGate genotyping assays were designed. Genotyping was performed in 300 individuals with GN bacteremia (family Enterobacteriaceae) and 300 control subjects with no signs of infection. Association analysis was performed using standard logistic or linear regression with adjustment for gender, sex, and race. Tests of association were independently performed for several variables, including GN bacteremia, Acute Physiology and Chronic Health Evaluation II (APACHE II) score that is indicative of disease severity, acute lung injury/acute respiratory distress syndrome (ALI/ARDS), disseminated intravascular coagulation (DIC), septic shock, and outcome. Significant associations will be presented.

3054/F

Heritability of serological measures of common infections in participants of the San Antonio Family Heart Study. *R. Rubicz[1], C.T. Leach[2], E. Kraig[3], N. Dhurandhar[4], J. Blangero[1], R. Yolken[5], H.H.H. Göring[1].* 1) Dept. of Genetics, SFBR, San Antonio, TX; 2) Dept. of Pediatrics, University of Texas Health Science Center at San Antonio, San Antonio, TX; 3) Dept. of Cellular and Structural Biology, University of Texas Health Science Center at San Antonio, San Antonio, TX; 4) Pennington Biomedical Research Center, Baton Rouge, LA; 5) Dept. of Pediatrics, Johns Hopkins School of Medicine, Baltimore, MD.

Variation in susceptibility to infectious disease has been documented among individuals and between populations and is attributable to different environmental and genetic factors. Here we test the hypothesis that variation in antibody titer, as well as the overall number of infections, is partly due to genetics. Participants were >1300 Mexican Americans in the San Antonio Family Heart Study. Antibody titers and seroprevalence were determined by ELISA (except Ad-36, which was quantified using a published serum neutralization test) for 13 common infections: *Chlamydophila pneumoniae*; *Helicobacter pylori*; *Toxoplasma gondii*; Adenovirus 36 (Ad-36); hepatitis A; influenza A; influenza B; cytomegalovirus; Epstein-Barr virus; herpes simplex I virus (HSV-1); herpes simplex II virus (HSV-2); human herpesvirus 6; and varicella zoster virus (VZV). Both quantitative antibody titers and discrete serostatus phenotypes (seropositivity/seronegativity) were analyzed. Additive genetic heritability (h^2) was calculated using variance component (VC) pedigree analysis with the computer program SOLAR. To assess the influence of shared environmental factors, a household component was included in the VC model. All but two pathogens (HSV-2, which is sexually transmitted, and VZV) were found to have a significant heritable component at p£0.05, with h^2 ranging from 9.0% (Ad-36) to 45.7% (hepatitis A) for quantitative titers, and 18.4% (Ad-36) to 70.4% (*C. pneumoniae*) for seropositive/seronegative status. However, fewer pathogens were significantly heritable when simultaneously accounting for household. Household was significant for approximately half the pathogens, the largest contribution being 32.4% ($p=7.2x10^{-03}$) for influenza A, possibly due to the infection being passed among relatives. Most participants (80%) had between 6 and 9 infections, and pathogen load was significantly heritable for all pathogens (h^2=37.6% and 27.2%, for the quantitative and discrete traits, respectively), as well as for the herpesviruses (h^2=32.0% and 18.1%). These results demonstrate that individual genetic differences do contribute to serological phenotypes, and that identifying the underlying genetic factors may help illuminate how the immune system functions and our understanding of the variation in disease susceptibility, potentially leading to novel approaches for the treatment of patients suffering from infectious disease.

3055/F

Estimation of the contribution of rare causal variants to complex diseases. *W. Guan[1], M. Boehnke[2], L.J. Scott[2].* 1) Division of Biostatistics, Univ of Minnesota, Minneapolis, MN; 2) Department of Biostatistics and Center for Statistical Genetics, Univ of Michigan, Ann Arbor, MI.

Rapid advances in next-generation sequencing technologies are providing ever more complete surveys of genomic regions of interest and even whole genomes, and these advances are facilitating genetic association studies of an increasingly wide array of common and rare variants. As we plan re-sequencing studies, it is interesting to consider constraints on the range of plausible genetic models based on results of previous genetic studies of complex traits. In particular, results of genome-wide association (GWA) studies and previous linkage studies provide information on the range of causal variant risk allele frequencies (RAF) and effect sizes (genotype relative risk, or GRR) that might underlie common GWA signals and might, more broadly, exist in a given genomic region. In this study, we use power calculations to evaluate the range of models that would result in little evidence for linkage in a linkage scan (a typical finding) and different degrees of evidence for association at genotyped markers in an association study, assuming a multiplicative model of disease risk. We find that prior negative linkage or association results can help to restrict the plausible range of GRR, and little evidence for linkage and significant evidence for association can reduce the plausible ranges of both GRR and RAF in the corresponding region. We apply our approach to large-scale linkage and GWA meta-analyses for type 2 diabetes. Our results suggest that several association signals can only be explained by relatively common causal variants (RAF > .01), while other signals are consistent with a wide range of RAF and GRR.

3056/F

Allelic Heterogeneity in a Genome-wide Association Study of Body Mass Index in 123,000 Individuals. *R. Weyant[1], C. Willer[1], H. Lango Allan[2], M. Weedon[2], T. Ferreira[3], C. Lindgren[3,4], E. Speliotes[5,6], S. Berndt[7], K. Monda[8], G. Abecasis[1], G. Thorleifsson[9], M. Boehnke[1], K. Stefansson[9,10], K. North[8,11], M. McCarthy[3,4,12], J. Hirschhorn[5,14,13], E. Ingelsson[15], R. Loos[16] on behalf of the GIANT Consortium.* 1) Biostatistics, University of Michigan, Ann Arbor, MI; 2) Genetics of Complex Traits, Peninsula College of Medicine and Dentistry, University of Exeter, Exeter, EX1 2LU, UK; 3) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, OX3 7BN, UK; 4) Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford, OX3 7LJ, UK; 5) Metabolism Initiative and Program in Medical and Population Genetics, Broad Institute, Cambridge, Massachusetts 02142, USA; 6) Division of Gastroenterology, Massachusetts General Hospital, Boston, Massachusetts 02114, USA; 7) Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland 20892, USA; 8) Department of Epidemiology, School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514, USA; 9) deCODE Genetics, 101 Reykjavik, Iceland; 10) Klinikum Grosshadern, 81377 Munich, Germany; 11) Carolina Center for Genome Sciences, School of Public Health, University of North Carolina Chapel Hill, Chapel Hill, North Carolina 27514, USA; 12) NIHR Oxford Biomedical Research Centre, Churchill Hospital, Oxford, OX3 7LJ, UK; 13) Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115, USA; 14) Divisions of Genetics and Endocrinology and Program in Genomics, Children's Hospital, Boston, Massachusetts 02115, USA; 15) Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, 171 77 Stockholm, Sweden; 16) MRC Epidemiology Unit, Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge, CB2 0QQ, UK.

Genome-wide association studies have identified variants that are associated with many complex traits. Typically, initial analyses focus on a single variant in each identified locus. This variant is then examined in additional samples and discussed in the context of nearby genes and eQTL signals. Although it is known that many GWAS signals overlap with previously (or subsequently) identified Mendelian loci, the extent to which identified loci harbor multiple associated variants has not been fully explored. The GIANT consortium identified 32 loci associated with BMI in an initial analysis of ~123,000 individuals and subsequent follow-up in ~125,000 individuals. At least three of the 32 loci (near POMC, SH2B1 and MC4R) are known to also harbor variants associated with morbid obesity. To explore allelic heterogeneity for common variants in the 32 novel loci, we repeated genome-wide analyses after conditioning on lead variants for each of the 32 loci. The analysis identified a non-synonymous SNP (rs2229616, minor allele frequency (MAF) = 0.017, effect size -0.33 kg/m2 per minor allele) in MC4R that shows association with BMI (p = 6 x 10-6) and is also independent of the previously reported variant at that locus (rs571312, MAF = 0.283, effect size 0.23 kg/m2 per minor allele). The minor allele at this SNP, V103I (rs2229616) was previously reported to be associated with reduced risk of obesity. There is no evidence of linkage disequilibrium between these two SNPs (r2=0.004) which are ~200kb apart from each other, potentially representing different mechanisms of altering gene function. While our results do not provide evidence for widespread allelic heterogeneity at BMI associated loci, they do illustrate that common variants at the same locus can point to different functional subunits.

3057/F

Genetic and Non-Genetic Risk Estimation in the Coriell Personalized Medicine Collaborative. *C.B. Stack, N. Gharani, E.S. Gordon, T.J. Schmidlen, M.F. Christman, M.A. Keller.* Coriell Institute for Medical Research, Camden, NJ.

Recent genome wide-association studies have identified hundreds of single nucleotide polymorphisms associated with common complex diseases. With the momentum of these discoveries comes a need to communicate this information to individuals. The Coriell Personalized Medicine Collaborative(CPMC®) is an observational research study designed to evaluate the utility of genetics knowledge in medicine. Participants provide saliva samples for genotyping and complete extensive on-line medical history, family history, and lifestyle questionnaires. Only results for selected genetic variants associated with diseases deemed potentially actionable by an independent advisory board are reported. Results for non-genetic risk factors are also provided to convey the multi-factorial nature of reported diseases. We present our methodology for developing risk reports for CPMC® participants. Risk estimates are given as relative risk, derived or reported from valid and representative peer-reviewed publications. Studies used for risk reporting are selected based upon the strength of the design and study quality. Use of relative risk allows for consistent reporting across genetic and non-genetic factors, and across multiple diseases. Also, relative risks are applicable to most individuals, as measures of relative effect are generally stable across populations. Importantly, this approach to risk reporting does not require estimates of population lifetime disease risks, which have been shown to vary substantially across populations. Because communicating risk in relative terms to a lay audience is more challenging, we include detailed descriptions for the risk estimates within our reports. Risk reports are accompanied by information on disease prevalence, heritability and ethnic-specific genotype frequency. The delivery of risk reports is supported by web-based educational material and access to genetic counseling and educational seminars. In developing our reporting methods, we acknowledge some trade of conceptual simplicity for accuracy and transparency. CPMC® risk reports demonstrate an approach to communicating risk of complex disease via the web that encompasses risks due to genetic variants along with risks due to family history and lifestyle factors. This approach can be applied to multigenic risk models, as they are developed and validated. The report format is amenable to integration into an electronic health record (eHR) or personal health record (pHR).

3058/W

Modulation of alternative splicing using morpholino oligonucleotides. *S. Tantzer[1], K. Sperle[1], J. Taube[2], G.M. Hobson[1,2].* 1) Nemours Biomedical Research, Alfred I. duPont Hospital for Children, Wilmington, DE; 2) Department of Biological Sciences, University of Delaware, Newark, DE.

Pelizaeus-Merzbacher Disease (PMD) is a neurological disease often caused by duplication of the proteolipid protein 1 gene (*PLP1*) on the X-chromosome. However, PMD is also caused by small deletions, insertions and single base mutations that affect the coding sequence of the protein or the splicing of the gene. *PLP1* has two major splice isoforms due to alternative 5' splice donor sites for intron 3 leading to either PLP1 or *DM20* mRNA transcripts. Tight regulation of the PLP1/DM20 alternative splice is critically important for central nervous system function; a developmental switch in the isoform ratio from *DM20* toward the *PLP1* isoform occurs during myelination in the central nervous system. We showed that patient mutations within exon 3 or intron 3 that reduce the ratio of *PLP1* to *DM20*, without resulting in amino acid changes, cause PMD. Although there are currently no therapeutic interventions or cures for PMD or for the many other diseases that can be caused by disruption of alternative splicing, molecules that modulate aberrantly spliced products toward a more normal ratio are currently being tested as a therapeutic intervention. We have designed morpholino oligomers (MO) to target the *DM20* 5' splice site to increase the *PLP1* to *DM20* transcript isoform ratio. An oligodendrocyte cell line, Oli-neu was treated with MO and the resulting endogenous *PLP1* to *DM20* ratio was found to be significantly increased. *PLP1* mini-gene splicing constructs containing different patient mutations that affect *PLP1* splicing also showed a significant increase in the *PLP1* to *DM20* ratio after transfection into Oli-neu cells and treatment with MO. Our studies indicate that MOs can be used to alter *PLP1* splice site selection both endogenously and in mutant transfected constructs, suggesting that MOs may be a therapeutic option for PMD caused by aberrant alternative splicing. The results of these studies have implications for other diseases caused by misregulation of alternative splicing.

3059/W

Viral vector tropism for supporting cells in the developing murine cochlea. *A.M. Sheffield[1], S.P. Gubbels[2], M.S. Hildebrand[1], J.A. Chiorini[3], G. Di Pasquale[3], R.J.H. Smith[1].* 1) Department of Otolaryngology—Head and Neck Surgery, University of Iowa, Iowa City, IA; 2) Division of Otolaryngology—Head and Neck Surgery, University of Wisconsin, Madison, WI; 3) National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD.

Gene therapies are currently being developed to provide novel treatments for genetic hearing loss. Many genetic forms of deafness are congenital and gene transfer-based therapies for these forms of deafness would require initiating treatment prior to the onset of significant hearing loss. Recently, Bedrosian and colleagues developed a safe and effective method for in utero transfer of therapeutic agents to progenitor cells in the embryonic murine otocyst. In this study, we recapitulated this approach to investigate viral tropism in the developing inner ear. The cellular heterogeneity that exists within the cochlea makes viral tropism an important consideration for effective inner ear gene therapy. For the first time we have characterized viral tropism for cochlear supporting cells following in utero delivery to their progenitor cells. Supporting cells are the primary site of connexin 26 gap junction proteins that are mutated in the most common form of congenital genetic deafness (DFNB1). We examined the inner ear tropism of three previously untested vectors: AV5-CMV-GFP (first-generation adenovirus), Adf.11d (late-generation adenovirus) and BAAV-CMV-GFP (bovine adeno-associated virus). Adult mice underwent timed matings to generate embryos for transuterine microinjection. Injection of GFP-expressing viral vectors into the left otocyst of mouse embryos was performed via transuterine microinjection at embryonic day 12.5 (E12.5). Auditory brainstem response (ABR) testing was performed at 5 weeks of age on injected mice and littermates to assess for potential hearing loss associated with virus administration. Subsequently, cochleas were analyzed for GFP expression using fluorescence microscopy to assess cellular tropism of each viral vector. Both first- and late-generation adenovectors (AV5-CMV-GFP and Adf.11d) showed robust tropism for organ of Corti supporting cells throughout the cochlea. However, both adenovectors resulted in markedly increased ABR thresholds in the injected cochleas indicating toxicity to normal hearing. Bovine adeno-associated virus also showed tropism for organ of Corti supporting cells, with preferential transduction toward the cochlear apex. BAAV-injected cochleas showed normal hearing at 5 weeks of age when compared to non-injected cochleas. These results suggest BAAV as a promising vector for safe targeting of supporting cell progenitors in the developing murine cochlea.

3060/W

Transduction of choroid plexus epithelia is crucial for gene therapy rescue of a murine model of Menkes disease. *A. Donsante[1], L. Brinster[2], P. Zerfas[2], D. Goldstein[3], J. Prohaska[4], J. Centeno[5], S. Kaler[1].* 1) Unit on Human Copper Metab., NIH,NICHD, Bethesda, MD; 2) Division of Veterinary Resources, NIH, Bethesda, MD; 3) Clinical Neurocardiology Section, NINDS, NIH, Bethesda, MD; 4) Dept of Biochemistry and Molecular Biology, U. of Minnesota, Duluth, MN; 5) Armed Forces Institute of Pathology, Washington, D.C.

The *mottled-brindled* (*mo-br*) mouse manifests a lethal abnormality in copper transport to the brain caused by a mutation in *atp7a*, a P-type ATPase, and is a model for Menkes disease. We treated neonatal *mo-br* mice with either intracerebroventricular recombinant adeno-associated virus serotype 5 (AAV) harboring an atp7a homolog, intracerebroventricular copper, or both treatments. As we previously noted, only AAV+Cu treatment rescued *mo-br* mice. Transduction by AAV occurred primarily in the choroid plexus epithelia at the viral titers ($5x10^8$ vector genomes) employed. Survival was associated with higher brain copper levels and improved activity of dopamine-β-hydroxylase, a copper enzyme metallated in the *trans*-Golgi compartment. In contrast, the activities of cytosolic and mitochondrial copper enzymes did not differ notably among treatment groups. Serial neurobehavioral testing in long-surviving *mo-br* mice using the constant speed rotarod (balance and coordination) and wire hang (neuromuscular strength) was performed weekly beginning at 25d of age and up to 300d of age (3 trials per time point, 60s maximum). AAV+Cu *mo-br* mice were similar to wild type controls on the rotarod at 25d of age [*mo-br* (N=10) 56s, wild type (N=9) 60s]. However, they exhibited significant impairment in later weeks, averaging 15-20s on the rod. Surprisingly, three AAV+Cu *mo-br* mice regained the ability to remain on the rotarod for the full 60s beginning at ages 95d, 144d, and 173d. On the wire hang test, AAV+Cu mice were abnormal at 25d of age (30s vs. wild type 60s; $p<10^{-4}$) but showed improvement with age. At 116d, *mo-br* mice (N=6) stayed on the wire for a mean duration of 54s versus 60s in wild type (N=10). 67% of AAV+Cu *mo-br* mice completed the full 60s at this time point as compared to 10% at 25d of age. At 300 days of age, electron micrographs showed no overt ultrastructural abnormalities and myelin staining appeared similar between treated *mo-br* brain and normal controls.

Our findings suggest that 1) rescue of the *mo-br* mouse is dependent upon improved copper retention in the brain due to transduction of choroid plexus epithelia, 2) copper enzymes metallated in the *trans*-Golgi network are responsible for improved life span in AAV+Cu *mo-br* mice, and 3) recovery of motor function in the rotarod and wire hang test long after administration of therapy may be explained by slower than normal rates of myelination and axonal growth/development.

3061/W

Elucidation of Mechanism and Therapy for Loeys-Dietz Syndrome Using an Allelic Series of Mutant Mice. *D. Loch[1], E. Gallo[1,2], H. Dietz[1,2].* 1) Johns Hopkins University School of Medicine, Baltimore, MD; 2) Howard Hughes Medical Institute.

Loeys-Dietz syndrome (LDS) is a systemic connective tissue disorder with significant phenotypic overlap with Marfan syndrome (MFS). Unlike MFS, LDS carries a strong risk of aneurysm and dissection throughout the arterial tree that is often difficult or impossible to manage surgically, highlighting the need to elucidate pathogenesis and develop medical therapies. LDS is caused by heterozygous missense mutations in the kinase domain of the type I or type II subunits of the transforming growth factor-beta receptor (TβRI or TβRII, respectively). Expression of mutant receptor subunits in cells naïve for the corresponding receptor fails to support TGFβ signaling, yet analysis of LDS patient aortic tissue has shown paradoxically enhanced TGFβ signaling, like MFS. The angiotensin II type 1 receptor blocker losartan has shown the ability to attenuate both TGFβ signaling and aneurysm progression in mouse models of MFS and is often prescribed for LDS patients on a theoretic rather than empiric basis. To address the issue of mechanism, we have created three mutant mouse models of LDS; two knock-in strains with missense mutations in TβRI (M318R) or TβRII (G357W) and a transgenic strain that ubiquitously over-expresses TβRII mutant G357W. Strains haploinsufficient for the TβRI or TβRII gene have also been analyzed. Both knock-in strains fully recapitulate the LDS vascular phenotype, with widespread arterial disease including arterial tortuosity, aneurysm and dissection. G357W transgenic mice also develop vascular disease, with increased severity seen in homozygosity, despite full TGFβ signaling capacity in cultured cells, arguing against a dominant-negative mechanism. In contrast, both haploinsufficient mouse strains show normal longevity and no evidence of vascular disease. LDS knock-in mice show increased canonical TGFβ signaling in aortic tissue (phosphorylated Smad2 and PAI1 expression) and activation of noncanonical TGFβ signaling cascades (ERK1/2), suggesting a gain-of-function mechanism. A blinded and placebo-controlled trial of losartan in LDS mice demonstrated prevention of aortic root dilatation with reduced canonical and noncanonical TGFβ signaling, in accordance with previous findings in MFS. This study highlights the utility of studying pathogenesis in models that recapitulate the physiologic complexity of the human system and suggests that therapeutic strategies developed for MFS may find broader application in disorders of vessel wall homeostasis.

3062/W

The Link between innate immunity and Gaucher disease: Implications for therapy. *A. Farwah[1], O. Alpan[4], P. Mistry[3], D. Unutmaz[2], O. Goker-Alpan[1].* 1) LSD Research and Treatment Unit, Center for Clinical Trials, Springfield, VA; 2) NYU School of Medicine, Departments of Microbiology and Pathology, New York, NY; 3) Yale University School of Medicine, New Haven CT; 4) Laboratory of Immunopathogenesis, O & O Alpan, LLC, Springfield VA.

Lipid engorged macrophages are the hallmark for Gaucher disease (GD), which are not metabolically inert, but strongly express markers of macrophage activation. Similarly, the plasma of GD patients shows more than 1000 fold increase of chitotriosidase, a chitinase secreted from activated macrophages. While these markers respond to therapy, they are not specific for different organ involvement. In GD, the mechanisms of macrophage activation remain unclear, but non-specific inflammatory response, poor wound healing and insulin resistance may indicate that there are multiple pathways involved. Activation of macrophages could impact other innate immune system components such as NK cells, and adaptive immunity through modifying T cell and B cell function. Therefore, understanding the mechanisms of macrophage activation could provide mechanistic insights in GD. We hypothesized that in GD macrophage activation could occur both through an alternative pathway, such as CCL18-mediated activation and by more direct effects of lipid metabolites. We then assessed the effects of macrophage activation on NK function modulation, macrophage function and response to therapy. NK cell numbers and activity were evaluated in peripheral blood mononuclear cells. NK cells have the ability to induce cell death in tumor cells. K562, human erythroleukemia cell line was loaded chromium and lysis of the K562 targets by NK was measured by the amount of chromium in the supernatant. NK and T cell subsets were evaluated by flow cytometry. Chitotrisidase levels were followed as a marker of alternative macrophage activation. Although the number of circulating NK cells was normal, there was a significant decrease in target killing in vitro. Despite normalization of chitotriosidase and other GD biochemical markers with enzyme replacement therapy, inflammatory markers in peripheral blood remained elevated. In GD, pathways leading to macrophage activation are heterogeneous. NK cell dysfunction contributes to secondary macrophage activation, which may alter the expected outcomes and response to therapy in some patients with GD. Therapeutic interventions that suppress macrophage activation may control the inflammatory component of GD, which in turn could alleviate the development or progression of bone, pulmonary and neurological complications.

3063/W

Protocol for early initiation of growth hormone therapy in Prader-Willi Syndrome yields good clinical results. *J. Atkin[1], A. Dye[2], D. Hardin[3], D. Repaske[2], K. Anglin[2].* 1) Molecular/Human Genetics, Nationwide Children's Hospital/Ohio State University, Columbus, OH; 2) Endocrinology, Nationwide Children's Hospital/Ohio State University, Columbus, OH; 3) Ohio State University, Columbus, OH.

Growth Hormone (GH) therapy in children with Prader-Willi Syndrome (PWS) has been shown to be beneficial in decreasing body fat percentage, improving lean body mass, and increasing linear growth and resting energy expenditure. However, earlier initiation of growth hormone therapy could have direct benefits on psychomotor and cognitive development. Thus, although GH therapy seems beneficial for children with PWS, it is unclear at what age to safely initiate this therapy. We therefore report a protocol for early initiation of growth hormone therapy in infants with PWS. Our multidisciplinary PWS team involves a collaboration of genetics, pediatric endocrinology, and nutrition specialists who see patients every three months. Our PWS clinic has been successfully using a protocol for early initiation (ages 3-4 months) of growth hormone therapy with good clinical results. Developmental milestones are monitored at each visit and patients with early initiation of GH therapy demonstrate earlier acquisition of developmental milestones. All patients undergo polysomnography assessments to evaluate for central apnea and sleep disordered breathing prior to the initiation of growth hormone. Low-dose growth hormone is started and gradually increased with careful attention to subsequent sleep studies and side effect profiles with close follow-up. Conclusion: Introduction of growth hormone therapy as early as 3-4 months of age in the Prader-Willi population can be safe and effective when closely monitoring for obstructive/central apnea with repeated polysomnography assessments and side effect evaluations. Growth hormone is beneficial for improving body composition and motor development which may impact cognitive development and should be considered in early infancy.

3064/W

Everolimus for subependymal giant-cell astrocytomas (SEGAs) in patients with tuberous sclerosis (TS). *D.A. Krueger[1], M.M. Care[1], K. Holland-Bouley[1], K. Agricola[1], C. Tudor[1], P. Mangeshkar[1], A. Weber Byars[1], T. Sahmoud[2], D.N. Franz[1].* 1) Division of Neurology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 2) Novartis Pharmaceuticals Corporation, Florham Park, NJ.

Objective: TS is a potentially devastating disorder caused by mutations in *TSC1* or *TSC2* and characterized by hamartoma formation in multiple organ systems, including the brain. SEGAs develop in 5-15% of TS patients and represent a significant medical risk, including the potential for sudden death secondary to acute hydrocephalus. Neurosurgical resection, the standard treatment of SEGAs, carries a significant risk of peri- and postoperative complications, and incomplete resection, which invariably occurs, necessitates repeat procedures. However, no effective alternative currently is available. This open-label, phase-II study evaluated the effect of treatment with the mTOR inhibitor everolimus on SEGA volume in patients with TS. **Methods:** Patients aged ≥3 years with a definitive TS diagnosis and serial SEGA growth based on evidence from magnetic resonance imaging (MRI) scans received oral everolimus (starting dose, 3 mg/m^2 daily) titrated to trough concentrations of 5-15 ng/mL. The primary endpoint was change from baseline in SEGA volume after 6 months of treatment as assessed by central radiological review of MRI scans. Seizure frequency was assessed by 24-hour video electroencephalograms (EEGs). **Results:** Median treatment duration for 28 patients enrolled between 1/07 and 12/08 was 21.5 months (range 4.7-34.4). Everolimus was associated with clinically meaningful reductions in SEGA volume ($p<0.001$). Twenty-one patients (75.0%) experienced reductions in SEGA volume ≥30% during the first 6 months. Mean reduction in left and right ventricular volume was 3.22 and 3.15 cm^3, respectively, from baseline to month 6. No new lesions, worsening hydrocephalus, or symptoms attributable to increased intracranial pressure developed. No surgical resection or other SEGA treatment was required. Grade 3 adverse drug reactions were single cases of sinusitis, pneumonia, viral bronchitis, tooth infection, stomatitis, and leukopenia; no grade 4 events occurred. Clinically relevant reductions in overall clinical and subclinical seizure frequency ($p=0.022$) also were observed. Of 16 patients with evaluable EEG data, 9 experienced a decrease from baseline in seizure frequency, 6 reported no change (5 of whom were event-free at baseline), and 1 patient experienced an increase (median change 1.0, $p=0.012$). **Conclusions:** Everolimus offers a viable alternative to surgical resection and appears to be well tolerated.

3065/W

Novel Enzyme Replacement Therapy for Gaucher Disease: Clinical program with Plant Cell Expressed Recombinant Glucocerebrosidase (prGCD) - taliglucerase alfa. *E. Almon-Brill[1], R. Chertkoff[1], M. Maas[2], M. Petakov[3], E. Terreros Muñoz[4], S.E. Solorio-Meza[5], D. Amato[6], G.S. Duran[7], F. Giona[8], R. Heitner[9], H. Rosenbaum[10], P. Giraldo[11], A. Mehta[12], A. Zimran[13].* 1) Protalix Biotherapeutics, Carmiel, Israel; 2) Academic Medical Centre Department of Diagnostic Radiology, Amsterdam, The Netherlands; 3) Belgrade University Medical School Institute of Endocrinology, Diabetes and Diseases of Metabolism, Belgrade, Serbia; 4) Centro Médico Nacional Siglo XXI Departamento Clínico de Hematología México, D.F. México; 5) Hospital de Especialidades No. 1 del Centro Médico Nacional del Bajío Unidad de Investigación en Epidemiología, León, Gto Mexico; 6) Mount Sinai Hospital Department of Medicine Mount Sinai Hospital, Toronto, Ontario, Canada; 7) Pontificia Universidad Católica de Chile , Departamento de Pediatría, Santiago, Chile; 8) Ematologia, Dipartimento di Biotecnologie Cellulari ed Ematologia, Università La Sapienza, Roma, Italy; 9) Department of Pediatrics, Johannesburg Hospital and University of Witwatersrand, Johannesburg, South Africa; 10) Hematologic department, Rambam Medical Center, Haifa , Israel; 11) Haematology Department, Miguel Servet University Hospital, Zaragoza; 12) Department of Hematology, Royal Free Hospital and University College London , London, UK; 13) Gaucher Clinic, Shaare Zedek Medical Center, Jerusalem, Israel.

Taliglucerase alfa is a carrot-cell-expressed recombinant human β-glucocerebrosidase for treating Gaucher disease developed and produced by Protalix Biotherapeutics. A Phase III double-blind, randomized, parallel dose groups (60units/kg /infusion and 30units/kg /infusion) clinical trial was completed. The 9-month,Pivotal trial treated-naïve adult symptomatic patients. Safety endpoints were drug-related adverse events and antibody formation. Primary efficacy endpoint was reduction in spleen volume. Secondary endpoints were: change from baseline of: hemoglobin levels, liver volume, and platelet count. Exploratory parameters included biomarkers and Quantitative Chemical Shift Imaging Patients from 11 centers worldwide were enrolled and equally randomized to each dose group. No serious adverse events were reported; drug-related adverse events were mild/moderate and transient. Two patients (6%) developed IgG, none of which presenting neutralizing activity, two other patients developed hypersensitivity reactions. There was a statistically significant difference from baseline for the primary efficacy measures, i.e splenic reduction ($p<0.0001$) in both 60 U/kg dose and 30 U/kg dose treatment groups. The primary endpoint was already achieved after six months of treatment in both treatment groups. Statistically significant improvements compared with baselines were observed in the secondary endpoints, including increase in hemoglobin level, decrease in liver size and increase in platelet count at the 60 U/kg dose. Statistically significant improvements compared with baselines were observed in hemoglobin level and liver size and significant nominal elevation in platelet count in the lower dose of 30 U/kg. Taliglucerase alfa 30 and 60 units/kg administered by intravenous infusion for 9 months in 31 patients with moderate to severe Gaucher disease was found to be safe and efficacious in a clinically relevant and statistically robust manner. Additional clinical trials including an extension study, switch over study from imiglucerase to taliglucerase, early access program and pediatric study are currently on-going and will be addressed.

3066/W

Preliminary Analysis of Cervical Cord Involvement in Mucopolysaccharidosis VI Patients Aged <6-Years Old Before and After Enzyme Replacement Therapy with Naglazyme® (galsulfase). *C. Hendriksz[1], P. Harmatz[2], R. Giugliani[3], H. Nicely[4], A. Waite[4], MPS VI Clinical Surveillance Program Investigators.* 1) Clinical Department, Birmingham Children's, Birmingham, B4 6NH, United Kingdom; 2) Children's Hospital and Research Center Oakland, Oakland, CA 94609; 3) Medical Genetics Service/HCPA, Department of Genetics/UFRGS and INAGEMP, Porto Alegre, RS, Brazil; 4) BioMarin Pharmaceutical Inc., Novato, CA 94949.

OBJECTIVES: Deficiency of N-acetylgalactosamine 4-sulfatase leads to accumulation of undigested glycosaminoglycans (GAG) dermatan sulfate/chondroitin 4-sulfate in untreated patients. GAG storage leads to skeletal dysplasia and dural thickening, accentuated in rapidly progressing patients. This retrospective analysis assesses incidence of cervical cord involvement in patients with mucopolysaccharidosis VI (MPS VI) <6 years of age, relative to enzyme replacement therapy (ERT). METHODS: Baseline and routine data were examined from 141 patients (54 USA; 87 EU) collected to date in the BioMarin Pharmaceutical MPS VI Clinical Surveillance Program (CSP). Analyses selected <6-yr old patients on ERT and included magnetic resonance imaging (MRI) describing cervical cord compression (CCC) as "present" or "absent" and rating MRI as "stable", "worse" or "improved" since last examination. PRELIMINARY RESULTS: Twenty-seven patients (27/141; 19%) <6 yr of age were identified, all of whom had received at least one ERT infusion. Of these patients, 14/27 (52%) had MRI at any time; 10/27 (37%) had "baseline" MRI anytime prior to ERT and up to 6 months post-ERT treatment. Of these patients, 2 (20%) had compression at baseline; 8 patients (80%) did not. Of the 8 patients without compression at baseline, 2 patients (25%) remained unchanged, while 6 patients (75%) developed compression at different lengths of follow-up. CONCLUSION: Thirty-seven percent (10/27) of <6 yr old patients in the CSP had cervical compression documented by MRI regardless of treatment time, suggesting comorbidity in this patient population. In those with sufficient follow-up, no observable trend was determined between ERT and CCC, although sample size was limited. MRI assessments at diagnosis, baseline and follow-up are necessary to understand the prevalence and natural history of cervical cord compression as well as the impact of surgery and ERT on this condition. Caution should be used in generalizing these data to the entire MPS VI patient population.

3067/W

Clinical and immunological response in patients with type 1 Gaucher disease transitioning from imiglucerase to velaglucerase alfa: ongoing extension of study TKT034. *R. Mardach-Verdon[1], G. Pastores[2], A. Zimran[3], A. Tylki-Szymanska[4], A. Mehta[5], M. Heisel-Kurth[6], C. Eng[7], L. Smith[8], P. Harmatz[9], J. Charrow[10], D. Elstein[3], D. Zahrieh[11], E. Crombez[11], G. Grabowski[12].* 1) Dept Genetics, S CA Kaiser Permanente, Los Angeles, CA; 2) NYU School of Medicine, New York, NY; 3) Shaare Zedek Medical Center, Jerusalem, Israel; 4) Children's Memorial Health Institute, Warszawa, Poland; 5) Royal Free Hospital, London, UK,; 6) Children's Hospitals of Minnesota, Minneapolis, MN; 7) Baylor College of Medicine, Houston, TX; 8) Children's Mercy Hospital, Kansas City, MO; 9) Children's Hospital & Research Center Oakland, Oakland, CA; 10) Children's Memorial Hospital, Chicago, IL; 11) Shire Human Genetic Therapies, Cambridge, MA; 12) Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

Purpose: To assess long-term enzyme replacement therapy with velaglucerase alfa in patients with type 1 Gaucher disease who were clinically stable on imiglucerase therapy. **Methods:** Study TKT034 was a 1-year, open-label, multicenter trial in which patients with type 1 Gaucher disease aged ≥2 years with stable hemoglobin concentrations and platelet counts while receiving imiglucerase (for ≥30 consecutive months, at a dose unchanged for ≥6 months) transitioned to the same number of units of velaglucerase alfa (15-60 U/kg every other week by 1-hour intravenous infusion). After 1 year, patients were offered enrollment in an ongoing extension study. The primary endpoint was safety; secondary endpoints evaluated efficacy. Adverse events, hematology, and anti-velaglucerase alfa antibodies were measured at baseline and throughout the study (anti-imiglucerase antibodies were assayed at baseline). Liver and spleen MRI were done at baseline, 6 months, and 1 year in TKT034, and annually during the extension. **Results:** In study TKT034, 40 patients were enrolled and received ≥1 dose of velaglucerase alfa (median age, 37 [range 9-71 years], median prior imiglucerase use, 67 months [range 22-192 months]). At baseline, median hemoglobin concentration was 13.8 g/dL (range 10.4-16.5 g/dL) and median platelet count 162 x10^9/L (range 29-399 x10^9/L). Three patients were anti-imiglucerase positive at baseline. However, none of these patients developed antibodies to velaglucerase alfa during the trial, nor did patients who were antibody-negative at baseline. Velaglucerase alfa was generally well tolerated with most reported AEs of mild or moderate severity; no patient experienced a life-threatening AE; one patient experienced an SAE (hypersensitivity reaction) that led to discontinuation. Clinical parameters remained stable over the first year of treatment, and 38 patients continued into the extension. To date (18 months), mean changes for hemoglobin concentration and platelet counts are negligible; liver and spleen volumes will be recorded at Month 24.

Conclusion: In study TKT034, patients with type 1 Gaucher disease successfully transitioned from imiglucerase to velaglucerase alfa, maintaining clinical parameters over the long-term. Velaglucerase alfa was generally well tolerated, and appeared to show a favorable immunogenicity profile.

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A multicenter, randomized, double-blind, head-to-head, phase III study of velaglucerase alfa enzyme replacement therapy compared with imiglucerase in patients with type 1 Gaucher disease. *A. Mehta[10], M-F. Ben Dridi[1], DE. Gonzalez[2], A. Zimran[3], M. Kabra[4], EA. Lukina[5], P. Giraldo[6], I. Kisinovsky[7], A. Bavdekar[8], H. Ben Turkia[1], N. Wang[9], E. Crombez[9], K. Bhirangi[9].* 1) La Rabta Hospital, Tunis, Tunisia; 2) Sanatorio Español, Asunción, Paraguay; 3) Shaare Zedek Medical Center, Jerusalem, Israel; 4) All India Institute of Medical Sciences, New Delhi, India; 5) National Research Center for Haematology, Moscow, Russia; 6) Hospital Universitario Miguel Servet, Zaragoza, Spain; 7) Your Health S. A., Buenos Aires, Argentina;; 8) KEM Hospital Research Centre, Pune, India;; 9) Shire Human Genetic Therapies, Cambridge, MA, USA; 10) Dept Hematology, Royal Free Hosp, London, United Kingdom.

Purpose: Enzyme replacement therapy (ERT) with recombinant glucocerebrosidase (imiglucerase) has been the standard of care for Gaucher disease since the early 1990s. This study compared efficacy and safety of velaglucerase alfa (recently approved in the US) with imiglucerase in patients with type 1 Gaucher disease. Methods: Study 039 was a 9-month, global, randomized, double-blind, non-inferiority study comparing velaglucerase alfa with imiglucerase (60 U/kg every other week) in treatment-naïve patients aged ≥2 years, with anemia and either thrombocytopenia or organomegaly. The primary endpoint was the difference between the groups in mean change from baseline to 9 months in hemoglobin concentration (performed in intent-to-treat [ITT] and per-protocol [PP] populations). Results: 35 patients in 9 countries were randomized; 34 received study drug (ITT population: 17 velaglucerase alfa; 17 imiglucerase). Baseline characteristics were similar in the 2 groups; overall, 18/34 (53%) were female, the age range was 3-73 years (9/34 [27%] were <18 years), and 20/34 (59%) were splenectomized. The PP population included 15 patients in each arm. After 9 months, the estimated mean treatment difference for hemoglobin concentration from baseline between velaglucerase alfa and imiglucerase was 0.14 and 0.16 g/dL in the ITT and PP populations, respectively, with a lower bound of the 97.5% 1-sided confidence interval of -0.60 g/dL in both populations, greater than the pre-defined non-inferiority margin of -1.0 g/dL. These results indicate that the primary endpoint was met. There were no statistically significant differences in the secondary endpoints, including platelet counts, or spleen and liver volumes. The majority of AEs were mild or moderate in severity; no patient discontinued due to an AE. One treatment-emergent SAE was considered probably related to study drug (allergic skin dermatitis in a patient in the velaglucerase alfa group). No patient treated with velaglucerase alfa developed antibodies to velaglucerase alfa; 4 patients (23.5%) treated with imiglucerase developed antibodies to imiglucerase. Conclusion: This head-to-head study demonstrates the efficacy of velaglucerase alfa compared with imiglucerase in adult and pediatric patients with type 1 Gaucher disease, with velaglucerase alfa meeting the primary and all secondary efficacy endpoints. In addition, the results suggest possible differences in antigenicity between velaglucerase alfa and imiglucerase.

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SBC-102, a recombinant enzyme replacement therapy, corrects key abnormalities due to lysosomal acid lipase deficiency. *A.G. Quinn[1], A. Harvey[1], M. Chen[1], L. Christmann[1], M. Leavitt[1], W. Hu[1], T. Nagy[2], A. Lents[1], R. Richards[1].* 1) Synageva BioPharma Corp., Lexington, MA; 2) Dept of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA.

Lysosomal acid lipase (LAL) deficiency is a rare disease associated with significant morbidity and mortality, which affects individuals from infancy through adulthood. In infants LAL deficiency, sometimes called Wolman's disease, is a rapidly progressive and fatal condition characterized by malabsorption, growth failure and significant weight loss. In other patients the disease manifests with marked hepatomegaly, liver fibrosis, type II hyperlipidemia and accelerated atherosclerosis. The profound clinical effects of LAL deficiency are due to a failure to break-down cholesteryl esters and triglycerides in lysosomes, which leads to massive accumulation of lipid in many organs and a marked disturbance in cholesterol and lipid homeostatic mechanisms. We have developed methodologies for the production of recombinant therapeutic proteins in egg white (EW), including lysosomal enzymes, which have substantial advantages over cell culture based systems. We have expressed recombinant human LAL protein using this approach and demonstrated that the purified protein, SBC-102, is highly effective in correcting LAL deficiency in vitro and in an in vivo model. SBC-102 has N-glycans with terminal GlcNAc and mannose structures as well as mannose-6-phosphate moieties. In vitro studies with fluorescently labeled SBC-102 show cellular uptake into macrophages and fibroblasts via mannose and mannose-6-phosphate receptors respectively. Lysosomal localization was confirmed using confocal microscopy and LysoTracker. Incubation of fibroblasts from a patient with LAL deficiency demonstrated normalization of LAL enzymatic activity. Studies in a preclinical animal model of LAL deficiency have shown that IV injections of SBC-102 correct abnormalities associated with LAL deficiency including: 1) a reduction in liver, spleen and gut size relative to body weight; 2) normalization of gross pathology and histo-pathological abnormalities; and 3) decreases in tissue lipid content. **Conclusions:** 1. SBC-102, a rhLAL produced in EW has properties which allow effective lysosomal targeting to key target cells. 2. Enzyme replacement with rhLAL in an in vivo preclinical model demonstrates that this protein reduces lipid substrate levels in diseased tissues and corrects abnormalities associated with enzyme deficiency. These studies indicate that SBC-102 warrants further investigation as a therapy for LAL deficiency in patients.

3070/W

Hypophosphatasia in Children: Enzyme Replacement Therapy Using Bone-Targeted, Tissue-Nonspecific Alkaline Phosphatase. *M.P. Whyte[1], C.R. Greenberg[2], D. Wenkert[1], W.H. McAlister[3], K.L. Madson[1], A.L. Reeves[1], K.E. Mack[1], L. Bourrier[4], J.E. Mayhew[5], A.M. Skrinar[5], H. Landy[5].* 1) Center for Metabolic Bone Disease & Molecular Research, Shriners Hospital for Children, St Louis, MO; 2) University of Manitoba, Winnipeg, Canada; 3) Mallinckrodt Institute of Radiology, St. Louis, MO; 4) Clinical Research Unit, Manitoba Institute of Child Health, Winnipeg, Canada; 5) Enobia Pharma, Montreal, Canada.

Hypophosphatasia (HPP) features low serum alkaline phosphatase (ALP) activity caused by deactivating mutation(s) within the gene that encodes the tissue-nonspecific isoenzyme of ALP (TNSALP). The natural substrates for TNSALP that accumulate extracellularly include inorganic pyrophosphate (PPi), an inhibitor of mineralization, and pyridoxal 5'-phosphate (PLP), the principal form of vitamin B6. Rickets occurs because PPi blocks hydroxyapatite crystal growth within the skeletal matrix. HPP severity spans stillbirth from profound skeletal hypomineralization to osteomalacia late in adult life. There is no established medical treatment. ENB-0040 is a bone-targeted, human recombinant, TNSALP fusion protein that preserved skeletal mineralization and survival in a TNSALP knockout mouse model of severe HPP (J Bone Miner Res 23:777, 2008). Patient trials began in 2008. In a 6-mo, open-label study initially of 6 pts (£ 3 yrs) with life-threatening HPP, substantial skeletal remineralization, weaning from respiratory support, and improved motor development occurred with ENB-0040 (2 mg/kg IV followed by 1-3 mg/kg SC 3x/wk). Now, we report a phase II, open-label assessment of ENB-0040 in 13 children with HPP, ages 5-12 yr (2 girls, 11 boys), randomized to receive either 2 or 3 mg/kg SC 3x/wk for 6 mo. One pt awaiting scoliosis surgery was withdrawn, and 12 finished Wk 12. The complete (24-week) study will be reported. Transient injection site erythema was common, but there were no drug-related SAEs. Serum PPi and PLP levels, significantly elevated in all patients at baseline, declined in all and were normal in 8 and 10, respectively, at Wk 12. Increases in circulating parathyroid hormone from enhanced skeletal mineralization occurred in most pts, but without symptomatic hypocalcemia from "hungry bones". Skeletal radiographic improvement was present in nearly all pts by Wk 12; in some as early as Wk 6. All pts reported increased strength, endurance, and mobility within several weeks of ENB-0040 treatment. There was a mean increase in walking distance of 84 meters on a 6-minute walk test at Wk 12 (baseline of 325 m was subnormal), and mean strength and agility composite scores increased from the 3rd to the 10th percentile on the BOT-2. This change accompanied the appearance of running, jumping, and hopping skills. Pain troubled 7 pts at baseline, and only 3 at Wk 12. ENB-0040 is a promising enzyme replacement therapy for children with hypophosphatasia.

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Arbaclofen Treatment Is Associated with Global Behavioral Improvement in Fragile X Syndrome (FXS): Results of a Randomized, Controlled Phase 2 Trial. *W.T. Brown[1], R. Hagerman[2], B. Rathmell[3], P. Wang[3], R. Carpenter[3], M. Bear[4], E. Berry-Kravis[5].* 1) Dept Human Gen, NYS Inst Basic Res, Staten Island, NY; 2) UC Davis, Sacramento, CA; 3) Seaside Therapeutics, Cambridge, MA; 4) HHMI & MIT, Cambridge, MA; 5) Rush Univ Med Ctr, Chicago, IL.

Background: Arbaclofen is a GABA-B agonist, and is one of the first targeted treatments for a genetic disorder resulting in neurodevelopmental impairment. The rationale for testing arbaclofen in FXS includes data from mouse and drosophila models of FXS, and from the anecdotal use of racemic balcofen in patients with FXS. Arbaclofen's mechanism is relevant to the mGluR theory of FXS, as pre-synaptic GABA-B stimulation inhibits glutamate release. This study was designed to explore the safety and efficacy of arbaclofen for the treatment of behavioral symptoms in FXS. Methods: A double-blind, placebo-controlled, crossover trial was performed at 12 sites in the United States. 63 subjects (55 male, 8 female) with a full mutation of FMR1, and who met severity criteria on the Aberrant Behavior Checklist - Irritability (ABC-I) subscale, were enrolled. Up to 3 concomitant psychoactive medications were allowed. In each treatment period, study drug was flexibly titrated, then continued at the optimal titrated dose for 4 weeks total. Multiple behavioral and cognitive assessment were performed at baseline, 2 weeks, and 4 weeks in each treatment period. Results: 49 subjects completed the study and had no major protocol deviations. In this group, clinicians ($p= 0.05$) and parents ($p<0.10$) both reported a blinded preference for arbaclofen vs. placebo. These results were more robust among subjects who met criteria for autism ($p<0.01$) or who had higher baseline severity (ABC-I score ≥ 18, $p<0.01$). Similarly, significantly more subjects were "responders" on the Clinician Global Impression - Improvement scale when receiving arbaclofen vs. placebo (35% vs 18% overall, 50% vs. 6% in the autism group, 44% vs. 11% in the high severity group). The ABC-I scale was not sensitive to these effects. However, a post-hoc analysis showed that subjects with higher ABC-Social Withdrawal baseline scores had significant improvement on that scale, consistent with parent reports of improved socialization and communication. Arbaclofen was very well-tolerated and showed no notable weight gain or metabolic side effects. A majority of subjects enrolled in an open-label extension study, and some have withdrawn from their concomitant medications, including from antipsychotics. Conclusions: Arbaclofen shows significant potential for the treatment of behavioral symptoms in FXS, and possibly for the treatment of core social symptoms in autism.

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Testing the metabotropic glutamate receptor theory in fragile X syndrome: a randomized, placebo-controlled, proof-of-concept study of AFQ056, a novel, sub-type selective mGluR5 inhibitor. *S. Jacquemont[1,8], A. Curie[2,8], Y. He[3], C. Paulding[3], M.G. Torrioli[4], F. Chen[1,5], N. Hadjikhani[5], D. Martinet[1], J. Meyer[3], J.S. Beckmann[1], G. Neri[6], F. Gasparini[7], T. Hilse[7], A. Floesser[7], J. Branson[7], D. Johns[7], V. des Portes[2], B. Gomez-Mancilla[7].* 1) Service de Génétique Médicale, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland; 2) Service de Neurologie Pediatrique, HFME, Hospices Civils de Lyon - Université Lyon 1, Lyon, France; 3) Biomarker Development, Novartis Institutes for Biomedical Research; 4) Università Cattolica del Sacro Cuore, Cattedra di Neuropsichiatria Infantile, Rome, Italy; 5) Brain Mind Institute, EPFL, Lausanne, Switzerland; 6) Università Cattolica del Sacro Cuore, Istituto di Genetica Medica, Rome, Italy; 7) Neuroscience Discovery, Novartis Pharma AG, Basel, Switzerland; 8) Both authors contributed equally to the study.

Fragile X syndrome (FXS) is the most common cause of inherited mental retardation and is associated with behavioral problems including hyperactivity, attention deficit disorder and autism. It is caused by the expansion of a CGG repeat in the FMR1 gene, leading to hypermethylation, transcriptional silencing of FMR1 and absence or reduced levels of the translational repressor FMR1 protein (FMRP). The metabotropic glutamate receptor (mGluR) theory hypothesizes that without FMRP, uncontrolled protein synthesis occurs in response to activation of synaptic Group I mGluRs and may be the underlying cause of some of the clinical symptoms of FXS. Research in animal models of FXS suggests that mGluR5 may be a valid target for a therapeutic intervention in FXS treatments. The aim of this study was to assess the efficacy of AFQ056, a novel, sub-type selective inhibitor of mGluR5, to improve the behavioral symptoms in people with FXS, and test the validity of the mGluR hypothesis. This was a multicenter, randomized, double-blind, placebo-controlled, two-treatment, two-period cross-over study of AFQ056 in 30 men aged between 18 and 35 years with FXS (NCT00718341). Patients were randomly assigned to receive either AFQ056 then placebo, or placebo then AFQ056. Each treatment period lasted 28 days and the two periods were separated by a wash-out period of at least one week. Patients received 50 mg AFQ056 bid on Days 1-4, 100 mg bid on Days 5-8, 150 mg bid on Days 9-20, 100 mg bid on Days 21-24 and 50 mg bid on Days 25-28. The primary efficacy assessment of behavior was the Aberrant Behavior Checklist - Community (ABC-C) score at Day 19/20. Secondary efficacy assessments included the Clinical Global Impression (CGI) scale, Vineland Adaptive Behavior Scale (VABS), Repetitive Behavior Scale - Revised (RBS-R), Social Responsiveness Scale - Adult Research Version (SRS), Visual Analog Scale of behavior (VAS), KITAP test battery and the Peabody Picture Vocabulary Test - Revised (PPVT-R) scores. This proof-of-concept study was designed to test the effect of inhibiting mGluR5 with AFQ056 on the behavioral symptoms of FXS, and assess the safety of 50-150 mg bid AFQ056 in a population with FXS. A biomarker was shown to distinguish between responders and non-responders to AFQ056 treatment on the behavioral symptoms of FXS. We would like to acknowledge the patients who participated in this study and the Clinical Investigation Center, Hospices Civils de Lyon, Lyon, France.

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In utero gene-environment interactions influence asthma susceptibility phenotype in rat models of atopy and airway hyperresponsiveness. *N. Carpe[1,2], I. Mandeville[1], F. Kaplan[1,2].* 1) Montreal Children's Hospital Research Institute, Montreal, Quebec, Canada; 2) Department of Human Genetics, McGill University, Montreal, Canada.

Rationale. Asthma, affecting 6.8 million children in the US, is a leading cause of childhood morbidity. Existing evidence suggests that increased prevalence of asthma derives from early exposures acting on pre-existing asthma-susceptible genotypes. Abnormalities of the lung already exist in infants who subsequently develop asthma. We showed previously that diverse transcriptome signatures are associated with distinct developmental respiratory phenotypes in rat strains that model the traits of airway hyperresponsiveness (AHR, Fisher)and atopy (Brown Norway, BN). Hypothesis. We hypothesized that, in these models, in utero allergen exposure would amplify individual variation in asthma risk and perturb innate patterns of lung gene expression. Methods. Fisher, BN and Lewis (control) rats were sensitized to ovalbumin(OVA) or saline 14 days prior to mating. Pregnant dams were challenged with aerosolized OVA or saline and pups were collected at postnatal days (PN)1, 7 or 14. Outcome assessments included lung function (PN14), bronchoalveolar lavage (BAL) cell differentials (PN1-14), and serum IgE levels(PN14). Expression of genes previously implicated in asthma and/or with strain specific expression patterns were assessed by Real-Time PCR. Results. Fisher pups of saline exposed dams displayed elevated airway resistance following methacholine challenge compared to Lewis and BN. All pups exposed to OVA in utero showed increased lung resistance. This increase was most pronounced in Fisher pups. Fisher pups of saline exposed dams consistently exhibited elevated BAL neutrophils compared to Lewis and BN (PN1-14). In utero OVA-exposed Fisher pups also showed a significant increase in BAL neutrophils when compared to saline-exposed Fisher pups at PN7. Maternal OVA exposure led to a significant increase in BAL eosinophil levels in BN pups compared to saline-exposed BN pups and to OVA-exposed Lewis and Fisher pups. There was also a trend towards increased serum IgE levels in in utero OVA-exposed BN pups. In utero OVA exposure amplified differences in lung gene expression of Serpina1 and Ficolin B(Fisher) and Ly-6B and Egfr(BN)compared to Lewis pups. Conclusion. In utero exposures that interact with pre-existing respiratory genotypes in the developing lung influence asthma susceptibility traits. The delineation of specific molecular and environmental determinants of respiratory susceptibility has implications for both pediatric and adult pulmonary health.

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Development of Neocentromere-based minichromosome gene delivery system. *Y. Inaba[1, 2], L.H. Wong[1], K.H.A. Choo[1].* 1) Chromosome and Chromatin Research Laboratory, Murdoch Childrens Research Institute, Parkville, Victoria, Australia; 2) Department of Paediatrics, The University of Melbourne, Parkville, Victoria, Australia.

Human engineered chromosomes (HECs) have been generated to overcome many of the hurdles presented by other gene delivery systems such as viral vector system. They are fully functional chromosomes containing the minimal genomic content compatible with correct mitotic function, and have the capacity to carry large transgene or gene clusters (> 300 kb). The neocentromere of marker chromosome Mardel 10, derived from chromosome 10 provides a highly desirable source for HEC construction. Mardel 10 neocentromere-based minichromosomes (NC-MiCs) were generated as a therapeutic gene vector, by truncation of the chromosome arms and successful incorporation of a loxP site specific sequence. The availability of purified NC-MiC is essential to achieving successful HEC delivery. Currently Flow cytometry is the most efficient method for sorting single chromosomes at a high level of purity. NC-MiC1 (3.5Mb in size) was successfully isolated with high purity confirmed by reverse chromosome painting. The same approach was applied to sort NC-MiC6 (1.4Mb) and the use of multiple gates lead to 20-40 times enrichment in the sorted samples. Such levels of purification of minichromosomes less than 2Mb in size has not been previously reported and opens the way for the use minichromosome preparations carrying less background genetic material. While comparing the NC-MiC6 quantity in each sorted samples, we developed a new DNA quantification method. It is relatively simple and quick, and importantly more sensitive (with a confirmed threshold up to 0.12pg) than currently available DNA quantification methods. The next phase of our approach will focus on the integration of therapeutic genes into NC-MiC and its delivery into various cell lines.

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Long-term phenotypic correction of a lethal mouse model of methylmalonic acidemia using rAAV9-mediated gene therapy and metabolic improvement after re-boosting at 1 year. *J.S. Sénac, R.J. Chandler, C.P. Venditti.* Genetics and Molecular Biology Branch, NHGRI, NIH, Bethesda, MD.

Methylmalonic acidemia (MMA) is an inherited metabolic disorder most commonly caused by the deficient activity of methylmalonyl-CoA mutase (MUT). The disorder carries a poor prognosis for long-term survival. We have recently demonstrated that gene therapy with an adeno-associated virus serotype 8 (rAAV8) vector can both rescue $Mut^{-/-}$ mice from neonatal lethality and provide long-term phenotypic correction. Over time, hepatic Mut RNA levels and Mut protein significantly decreased in the rAAV8 treated $Mut^{-/-}$ mice, suggesting that translation to human subjects may require multiple rounds of gene delivery throughout life. Our goal was to develop a new gene therapy vector using an alternative AAV serotype. We engineered an rAAV9 vector to express the murine Mut cDNA under the control of an enhanced chicken beta actin promoter (rAAV9-CBA-mMut). rAAV9-CBA-mMut was delivered directly into the liver of newborn $Mut^{-/-}$ mice at a dose of $1x10^{10}$ GC (N=9). A small group (N=5) of older treated $Mut^{-/-}$ mice were re-injected via the intraorbital route after 1 year with $2x10^{9}$ GC per gram. Effects on survival, growth, metabolites, in vivo propionate oxidative capacity were used to determine the efficacy of rAAV9 gene therapy. Untreated $Mut^{-/-}$ mice (N=58) uniformly perish, with most dying during the newborn period. However, rAAV9 treated $Mut^{-/-}$ mice survived for more than 1 year. On an unrestricted mouse diet, the treated $Mut^{-/-}$ mice displayed elevated methylmalonic acid levels in the plasma yet were vigorous and have maintained 80% of the weight of age, sex and diet matched controls. Expression of the Mut transgene, as early as 24 hours post injection, appears to correlate with early phenotypic correction. rAAV9 treated mice that received a second dose at one year showed metabolic improvements as early as 72 hours post injection and experienced a full correction of propionate oxidative capacity 2 weeks post injection. These experiments demonstrate that rAAV9 is an efficient gene therapy vector for the treatment of MMA in a mouse model that faithfully replicates a severe form of the disorder, even at lower dose than that used in previous studies with rAAV8. Re-treating older $Mut^{-/-}$ mice with a dose of rAAV9 similar to that used in human clinical trials produced a measurable effect, rapidly. Our data support the use of rAAV9 as a vector with potential human translation in the treatment of MMA.

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CELL AND GENE THERAPY FOR ANTIGEN-SPECIFIC TOLERANCE INDUCTION AGAINST THERAPEUTIC PROTEINS. *G. Sule[1], M. Suzuki[1], R. Cela[1], J. Rodgers[3], B. Lee[1,2].* 1) Baylor College of Medicine , Department of Molecular and Human Genetics, Houston, TX; 2) Howard Hughes Medical Institute; 3) Baylor College of Medicine, Department of Pathology & Immunology Houston, TX.

Immune reaction towards a particular therapeutic protein leads to alleviation of the beneficial effects of the protein. This is particularly seen in Hemophilia A patients, where about a third of them develop inhibitory antibodies to recombinant protein as a consequence of the repeated protein infusions. Induction of tolerance towards therapeutic protein would prevent such an antibody response and hence prolong the efficacy of the therapeutic protein. Tolerogenic dendritic cells (DCs) have the unique ability to initiate and direct the host immune response towards peripheral tolerance. Cytokines such as IL-10 and TGF-β1 have been identified as key mediators of tolerogenic response. Thus, we hypothesize that the genetic modification of DCs to produce immune modulatory cytokines such as TGF-β1 and IL-10 will enable us to generate antigen specific immune tolerance. Our in vitro studies show, transducing DCs by helper-dependent adenovirus to express hTGF-β1 and vIL-10 renders them semi-mature. These DCs demonstrate tolerogenic characteristics by, preventing T cell proliferation, and increasing the frequency of regulatory T cells. Adoptive transfer of antigen-loaded, transduced DC to mice prior to protein treatment induced suppression of the immune response towards the protein. These mice also show a decrease in protein specific antibody formation. This report demonstrates that autologous cell therapy for antigen-targeted immune suppression may be developed to facilitate long-term therapy. Additionally, this study would have broader implications in solid organ transplantation, immune response to protein replacement therapies, and auto-immune diseases like diabetes and inflammatory bowel disease where proinflammatory responses are undesirable.

3077/W

Characterization of host sensing mechanism of helper-dependent adenovirus for gene therapy of Hemophilia A. *M. Suzuki[1], V. Cerullo[1,4], T. Bertin[1], C. Racel[1], C. Clarke[1], R. John[2], B. Lee[1,3].* 1) Molec & Human Gen, Baylor College Med, Houston, TX; 2) Immunology, Baylor College Med, Houston, TX; 3) Howard Hughes Medical Institute; 4) University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland.

Hemophilia A is a common inherited coagulopathy caused by deficiency of factor VIII (FVIII) activity. Previously, we focused on gene therapy of hemophilia A using helper dependent adenovirus (HDAd) encoding FVIII in both dog and mouse models. However, host antibody response to hFVIII has been a limitation of these therapies. We hypothesized that the innate immune response to HDAd may correlate with the induction of adaptive immune response to transgene product delivered with these vectors. Recently we demonstrated that TLR/MyD88 signaling is required for maximal innate and acquired (Th1) immune responses following systemic administration of helper-dependent adenoviral vectors (HDAds). However, MyD88-deficient mice injected with HDAdLacZ exhibited only partial reduction of innate immune cytokine expression compared with that of wild-type (WT) mice, suggesting MyD88-independent pathways also respond to HDAds. Our results indicate that NOD2, a NOD-like receptor known to detect muramyl dipeptides in bacterial peptidoglycans, also contributes to innate responses to HDAds and empty adenoviral particles lacking vector DNA, but does not mediate humoral or Th1 immune responses. Indeed, NOD2-deficient mice exhibited significantly higher transgene expression than did WT mice. Macrophages, especially Kupffer cells, play an important limit for adenovirus gene therapy in vivo. Bone marrow-derived macrophages from NOD2-deficient mice also exhibit lower innate response and higher transgene expression in vitro compared to WT. These results indicate that the intracellular sensor NOD2 is required for innate responses to adenovirus protein and limiting transgene expression. Our results suggest that the use of HDAd still faces hurdles, especially induction of acute toxicity through pathogen-recognition receptors. We need to understand better the molecular mechanisms of how host cells recognize HDAd and induce innate responses to improve the therapeutic index of these vectors.

3078/W

Identification of triplet repeat disease-causing alleles by a novel pull-down method and disease-causing allele specific silencing by RNAi. *M. Takahashi[1], S. Watanabe[1], M. Murata[1,2], H. Furuya[3], I. Kanazawa[1,2], K. Wada[1], H. Hohjoh[1].* 1) National Institute of Neuroscience, NCNP, Kodaira, Tokyo, Japan; 2) National Center Hospital, NCNP, Kodaira, Tokyo, Japan; 3) Neuro-Muscular Center, National Omuta Hospital, Fukuoka, Japan.

Nucleotide variations including single nucleotide polymorphisms (SNPs) in the coding region of disease genes are essential as targets for a potential tailor-made RNA interference (RNAi) treatment, which is promising for intractable diseases such as triplet repeat diseases, and for which it is vital to find such nucleotide variations linked to diseases. Here we show rapid determination of coding SNPs (cSNPs) haplotypes in triplet repeat disease-causing alleles by a novel and easy-to-use pull-down method, followed by SNP typing. The method is based on the hybridization and fractionation techniques using biotinylated CAG ribonucleotide repeat probe (biotin CAG RNA probe). The cDNAs synthesized from total RNAs prepared from Huntington's disease (HD) patients' specimens were treated by the pull-down method, and the resultant cDNAs were examined by conventional PCR analysis for expansion of CAG trinucleotide repeats. As a result, abundant PCR amplicons derived from aberrantly expanded CAG repeats were markedly detected, suggesting that biased collection of the mutant *HTT* cDNAs was performed by the method. The subsequent SNP typing using the cDNAs further revealed the SNP haplotypes of the mutant *HTT* alleles; and then, based on the haplotypes, we demonstrated disease-causing *HTT* allele specific silencing by RNA interference targeting the determined allelic nucleotides at the SNP sites. The current procedure has also achieved a great deal of reduction of processing time to determine the disease-causing alleles: it takes about 6 hours for the determination, whereas conventional procedures usually take at least one week. We further indicate that the procedure may be available for other triplet repeat disease genes, for example, *ATXN3*, *CACNA1A*, and *ATN1*, to identify their disease-causing alleles. Taken together, our current procedures may contribute to achievement of a tailor-made RNAi treatment for intractable triplet repeat diseases.

3079/W

Adeno-Associated Virus Serotype 8 (AAV8) Gene Transfer Rescues a Neonatal Lethal Murine Model of Propionic Acidemia. *R.J. Chandler[1, 2], S. Chandrasekaran[1], T.M. Cowan[3], C.P. Venditti[1].* 1) National Human Genome Research Institute, National Institutes of Health, Bethesda, MD; 2) Institute for Biomedical Sciences, The George Washington University, Washington, DC; 3) Department of Pathology, Stanford School of Medicine, Stanford, CA.

Propionic Acidemia (PA) is an autosomal recessive inborn error of metabolism caused by a deficiency of the mitochondrial enzyme propionyl-CoA carboxylase, encoded by the *PCCA* and *PCCB* genes. Defects in either the *PCCA* or *PCCB* gene can cause PA. Despite dietary and cofactor therapy, PA patients still suffer from life-threatening metabolic instability as well as numerous chronic disease related complications. A murine model of PA (*Pcca*$^{-/-}$) was created that replicates the severe neonatal manifestations of PA and perishes within the first 48 hours of life. A recent study describes adenoviral gene delivery of the human *PCCA* cDNA to *Pcca*$^{-/-}$ mice and reports a modest increase in life expectancy (maximum increase of less than 40 hours) without demonstrating PCCA expression or metabolic correction. Therefore, whether gene transfer is a potential treatment for PA remains uncertain. To determine the efficacy of AAV gene transfer as a therapy for PA, we engineered an AAV8 vector to express the human *PCCA* cDNA under the control of a combined CMV-enhancer-chicken beta actin promoter and injected 1x10^10 genome-copies of the virus into the liver. Survival effects, phenotypic correction, transgene expression and biochemical parameters were studied in the treated mice to determine the efficacy of gene transfer. We rescued a total of 9 *Pcca*$^{-/-}$ mice from certain death with AAV8 mediated gene transfer. Two treated *Pcca*$^{-/-}$ mice were sacrificed at day 16 of life to confirm PCCA expression. Immunoblots of liver extracts from the treated *Pcca*$^{-/-}$ mice revealed levels of hepatic PCCA protein greater than wild-type mice. No hepatic Pcca protein was detected in the untreated *Pcca*$^{-/-}$ mice. The treated *Pcca*$^{-/-}$ mice also exhibited markedly reduced plasma levels of propionylcarnitine and 2-methylcitrate compared to the untreated *Pcca*$^{-/-}$ mice, which indicates significant levels of propionyl-CoA carboxylase enzymatic activity were achieved after gene transfer. At the time of this report, the oldest treated *Pcca*$^{-/-}$ mice were over one month of age and not discernable from their wild-type littermates. In summary, AAV gene delivery of PCCA effectively rescues *Pcca*$^{-/-}$ mice from neonatal lethality, restores growth and substantially ameliorates metabolic markers of the disease state. These experiments are the first to demonstrate that gene transfer might be used as a treatment for PA, a devastating and often lethal disorder desperately in need of new therapeutic options.

3080/W

Early intrathecal and intravenous enzyme replacement therapy improves gene expression alterations in cortical neurons in canine mucopolysaccharidosis I. *P.I. Dickson[1], B.L. Tippin[1], N.M. Ellinwood[2], S.Q. Le[1], S. Kan[1], S. Karsten[1].* 1) LA BioMed Harbor-UCLA Med Ctr, Torrance, CA; 2) Department of Animal Science, Iowa State University, Ames, IA.

Introduction: Glycosaminoglycan (GAG) storage in brain normalizes after intrathecal enzyme replacement therapy (IT ERT) for canine mucopolysaccharidosis I (MPS I), but downstream effects on neuronal function are not known. Methods: We studied normal dogs (n=4, age 14-22 m), untreated MPS I dogs (n=4, 17-28 m), and MPS I dogs treated with IT and intravenous (IV) ERT (n=4, 15-18 m). Treated dogs received 0.05 mg/kg IT ERT diluted in artificial spinal fluid delivered into the cistern magna every three months starting from 12-112 d old and weekly 0.58 mg/kg IV ERT starting from 3-23 d old. Treatment continued for 56-80 weeks. Animals were sacrificed 48 hours after the final IT ERT dose and brains removed, quick frozen and stored at -80°C until analysis. Laser capture microdissection was performed by NeuroInDx (Signal Hill, CA) of frontal cortical neurons stained with Cresyl violet. Total RNA extracted from ≥200 neurons per dog underwent a two-round T7-based amplification labeling process and was hybridized onto canine whole genome microarrays (Agilent). Data analysis was performed with the TM4 software suite (http://www.tm4.org/) and DAVID, PANTHER, OMIM and KEGG databases. Results: There were 320, 1380, and 280 differentially regulated genes, respectively in Untreated vs. Normal, Treated vs. Normal, and Untreated vs. Treated dogs. The expression pattern of ~75% of genes with altered expression showed normalization of 15% or more with IT+IV ERT. Thirteen processes showed ≥2-fold enrichment in Untreated MPS I vs. Normal. These included synaptic transmission, transmission of nerve impulse, actin cytoskeleton organization and biogenesis, cellular homeostasis, visual perception, sensory perception of light stimulus, cellular chemical homeostasis, cellular ion homeostasis, proteolysis, actin filament-based process, RNA processing, ion homeostasis, and cell maturation. The majority of genes in these processes showed a normalized expression pattern after IT+IV ERT. Conclusion: Gene expression is altered in frontal cortical neurons of MPS I dogs, affecting critical pathways. Early IT+IV ERT may improve cortical neuronal gene expression in the MPS I dog.

3081/W

Discovery and Evaluation of a Non-Iminosugar Glucocerebrosidase Inhibitory Series with Chaperone Activity. *O. Motabar[1,2], W. Zheng[2], J. Marugan[2], E. Goldin[1], W. Westbroek[1], N. Southall[2], E. Sidransky[1].* 1) Medical Genetics Branch, NHGRI, NIH, Bethesda, MD; 2) NIH Chemical Genomics Center, NHGRI, NIH, Rockville, MD.

Gaucher disease is a lysosomal storage disorder (LSD) caused by a deficiency in the enzyme glucocerebrosidase (GC). Small molecule chaperones have been proposed as a promising therapeutic approach for the treatment of LSDs. Most of the small molecule chaperones described in the literature have iminosugar scaffolds. Here, we present the discovery and evaluation of a new series of GC inhibitors with quinazoline cores. This series was discovered through the quantitative high throughput screening (qHTS) of 326,770 compounds, in which several quinazoline analogues were identified. Structure-activity relationship (SAR) optimization yielded compounds with an IC50 close to 300 nM. These compounds were able to inhibit the hydrolysis of the natural substrate, and were selective for GC when tested against other lysosomal enzymes. Thermal denaturation experiments demonstrated the capacity of this series to stabilize GC. Immunocytochemistry on control and GC deficient fibroblasts with anti-GC and anti-LAMP2 antibodies was performed followed by laser scanning confocal microscopy. Upregulation and colocalization of GC to the lysosomes was seen with some of the compounds from this series. Systematic synthetic modifications enhancing the usefulness of these compounds are presented as part of the SAR. This new class of GC inhibitors may open new avenues for the development of alternative therapies for patients with Gaucher disease.

3082/W

Global Differential Gene Profiling of Patients with VCP-Associated Myopathy and Paget Disease of Bone. *A. Nalbandian[1], E. Dec[1], S. Ghimbovschi[2], S. Aizik[3], J. Vesa[1], M. Badadani[1], G. Watts[4], C. Smith[5], B. Martin[5], E. Hoffman[2], V. Kimonis[1].* 1) Department of Pediatrics, Division of Genetics and Metabolism, University of California, Irvine, Irvine, CA; 2) Children's National Medical Center, Research Center Genetics Medicine, Washington, DC; 3) Department of Pediatrics, General Clinical Research Center, University of California, Irvine, CA; 4) School of Medicine, Health Policy and Practice, University of East Anglia, Norwich, Norfolk, UK; 5) Department of Neurology, University of Kentucky Medical School, Lexington, KY.

Inclusion body myopathy associated with Paget's disease of the bone and frontotemporal dementia (IBMPFD) is caused by mutations commonly in the ubiquitin binding domain of the Valosin Containing Protein (VCP) gene on 9p12-13. IBMPFD is characterized by progressive, proximal muscle weakness, ubiquitin and TDP-43 inclusions and LC3+ and p62+ vacuoles in muscle fibers, malfunction in the bone remodeling associated with increased osteoclastic activity, and premature frontotemporal dementia. VCP is a ubiquitously expressed member of the type II AAA+ ATPase family and is involved in several cellular processes including post-mitotic nuclear envelope reformation, Golgi reassembly, cell cycle progression, endoplasmic reticulum degradation of defective proteins and DNA damage repair. To elucidate the affected signaling transduction axes in IBMPFD quadriceps samples from 16 patients and unaffected relatives, we determined expression profiles by Affymetrix GeneChip Human Genome Array. Differentially dysregulated genes analyzed by DAVID included the actin cytoskeleton, ubiquitin-mediated proteolysis, lysosomal and MAPK signaling transduction pathways. Statistical analyses of the obtained expression data revealed that 279 genes were differentially expressed in patients' muscle ($p<0.01$). Most of the dysregulated genes were shared with other muscle dystrophies except for the striking downregulation of Platelet-Derived Growth Factor Receptor Alpha (PDGFR-α), Nitric Oxide Synthase 3 (NOS3), and Fibroblast Growth Factor Receptor 2 and upregulation of Cytochrome P450, and family 19, subfamily a, polypeptide 1 (CYP19A), validated by qPCR. Defective PDGFR-α expression is involved in the progression of muscular dystrophies in the promotion of muscle fibrosis as well as in the active stage of tissue destruction. Nitric oxide (NO) mediates fundamental physiological actions on skeletal muscle and NOS3 levels are altered in muscular dystrophy. Additionally, studies have revealed that FGFs/FGFRs levels are tightly regulated and play essential functions in skeletal development and adult homeostasis. Future studies will clarify how PDGFR-α, NOS3 and FGF signaling may result in impaired autophagic degradation, increased apoptosis, defective myotube formation, and associated muscle weakness in IBMPFD patients. Our study is the first to compare gene expression profiling in IBMPFD patients, providing insights into the pathways involved in VCP disease and potential future therapies.

3083/W

Long-Term Follow-up of Patients with Leber's Optic Neuropathy Treated with Co-enzyme Q or Idebenone. *S.C. Benes[1], D.W. Hauswirth[2], K. Vesper[3], O. Bartlett[4].* 1) Eye Ctr Columbus, Columbus Children's Hosp, Columbus, OH; 2) Columbus Children's Hospital, Ohio State University; 3) Ohio State College of Veterinary Medicine; 4) Salida Hospital, Colorado.

This observational study of 24 affected patients with Leber's optic neuropathy describes the reversibility of central vision loss in patients treated with either co-enzyme Q or idebenone and followed for 18-25 years. Of the 8 with the 11778 mutation, 2 had recovery of vision (in 1 eye and 2 eyes); of the 6 with the 3460 mutation, 1 recovered vision in both eyes; of the 5 with 15257 mutation, 3 recovered vision in both eyes; the 1 patient with both the 13708 and 15257 mutations recovered vision in both eyes. Our patients with other mutations had no improvement with either co-enzyme Q or idebenone (2 with 4917, 1 with only 13708, 1 with 14484). References: Mashima Y, Hiida Y, Oguchi Y. Remission of Leber's hereditary optic neuropathy with idebenone. Lancet. 1992;340:368-369 Mashima Y, Kigasawa K, Wakakura M, Oguchi Y. Do idebenone and vitamin therapy shorten the time to achieve visual recovery in Leber hereditary optic neuropathy? J Neuro-ophthalmol. 2000;20:166-170 Huang cc, Kuo HC, Chu CC, Kao LY. Rapid visual recovery after coenzyme Q10 treatment of Leber optic neuropathy. J Neuro-ophthalmol. 2002;22:66 Cortelli P, Montagna P, Pierangeli G, et al. Clinical and brain bioenergetics improvement with idebenone in a patient with Leber hereditary optic neuropathy: A clinical and P-MRS study. J Neurol Sci. 1997;148:25-31 Johns DR, Smith KH, Miller NR. Leber's hereditary optic neuropathy: clinical manifestations of the 3460 mutation. Arch Ophthalmol. 1992;110:1577-1581.

3084/W

Copper and bezafibrate cooperate to rescue cytochrome c oxidase deficiency in cells of patients with SCO2 mutations. *L. Salviati[1], A. Casarin[1], G. Giorgi[1], E. Trevisson[1], M. Doimo[1], V. Pertegato[1], R. Rizzuto[2], S. DiMauro[3], M.M. Davidson[3], E.A. Schon[3].* 1) Pediatrics, University of Padova, Padova, PD, Italy; 2) Biomedical Sciences, University of Padova, Italy; 3) Neurology, Columbia University, New York, NY.

Cytochrome c oxidase (COX) deficiency is a clinically and genetically heterogenous mitochondrial disoreder usually causes by defect in ancillary factors required for the assembly of the COX holoenzyme, encoded by COX-assembly genes. Mutations in SCO2 present with COX deficiency associated to neonatal-onset, rapidly fatal, cardiomyopathy and encephalomyopathy. SCO2 encodes a protein involved in copper delivery to COX, and it has been shown that supplementation with copper salts can rescue the COX deficiency in cells of patients with mutations in this gene. Recently, it has been also shown that treatment with bezafibrate (BZF), a drug that induces mitochondrial biogenesis by stimulating the PGC1-alpha/PPAR-gamma pathway, may ameliorate the COX deficiency in cells and in mice with mutations in COX10, another COX-assembly gene. We have investigated the effect of BZF on cells with mutations in SCO2. We found that respiratory chain complex displayed different responses to BZF, and COX activity increased of about 40% in all cell lines we tested (both WT and COX-deficient). SCO2 cells reached about 80% COX activity compared to untreated controls. The increase in COX activity was associated with an increase in cellular ATP. The effect was dose dependent with a maximum with BZF concentrations around 400 uM. We did not observe a plateau with higher doses of the drug, but rather there was a relative reduction of COX activity. Combined treatment with 100 uM CuCl2 and 200 uM BZF (which are only marginally effective when administered alone) could achieve complete rescue of COX activity in SCO2 cells. We show that CuCl2 treatment increases the steady-state levels of the mutant Sco2p, suggesting that the mechanism of action involves a stabilization of the mutant protein, which in turn allows a more efficient assembly of COX stimulated by BZF. These data are crucial to design future therapeutic trials with these compounds for this otherwise fatal disorder. In fact, the additive effect of these two compounds will allow to employ lower doses of each drug, avoiding potential toxic effects.

3085/W

Environmental enrichment modulates phenotypic manifestations of a mouse model of Rett Syndrome trough a non-standard transcriptional program. *P.A. Silva[1], B. Kerr[1], K. Walz[2], J.I. Young[1, 2].* 1) Centro de Estudios Cientificos, Valdivia, Chile; 2) John P. Hussman Institute for Human Genomics. Miller School of Medicine, University of Miami, 1501 NW 10th Ave, Miami, Fl 33136.

Rett syndrome (RTT) is an X-linked disorder characterized by psychomotor retardation, purposeless hand movements, autistic-like behavior, abnormal gait and one of the leading causes of mental retardation in females. RTT is caused by mutations in the gene encoding methyl-CpG binding protein 2 (MeCP2). We here report the effects of environment enrichment (EE) on the phenotypic manifestations of a RTT mouse model that lacks MeCP2 (Mecp2-/y). We observed that EE delayed and attenuated some neurological alterations presented by Mecp2-/y mice and prevented the development of motor discoordination and anxiety-related abnormalities. To define the molecular correlate of these effects of EE on the RTT-like phenotypes, we analyzed the expression of several synaptic marker genes whose expression is increased by EE in several mouse models. We found that EE induced downregulation of several synaptic markers, suggesting that neurons lacking MeCP2 are unable to respond to EE through canonical pathways and that the partial prevention of RTT-associated phenotypes is achieved through a non-conventional transcriptional program. The EE-induced decrease in mRNA levels for synaptic proteins in Mecp2-/y mice suggests a reduction in synapse number. Since our EE program started when mice were of 3 weeks of age, these results could reflect an interference in the process that culminates with synapse number compensation or a loss of synapses during EE. Our data strengthen the hypothesis that alterations in synaptic function underlie the pathophysiology of Rett syndrome.

3086/W

The effect of different levels of dystrophin on muscle function and pathology in mouse models. *M. van Putten, V.D. Nadarajah, E. van Huizen, M.A. Hulsker, T. Messemaker, P.A.C. 't Hoen, G.J.B. van Ommen, J.T. den Dunnen, A.M. Aartsma-Rus.* Human Genetics, Leiden University Medical Center, Leiden, Netherlands.

In patients with Duchenne Muscular Dystrophy (DMD) frame shifting or non sense mutations in the *DMD* gene lead to the synthesis of truncated, non functional dystrophin proteins. Fibers lacking dystrophin are more injury-susceptible, which exhausts the regenerative capacity leading to severe muscle wasting, functional impairment and death in the mid twenties. Several therapeutic approaches currently under investigation aim to restore the synthesis of functional dystrophin to improve the phenotype. It is largely unknown which levels of dystrophin are minimally needed for restoration of muscle function, integrity and protection against exercise induced damage. Similarly, a correlation between dystrophin levels and these parameters has never been studied in detail. To address these issues we have generated a mouse model with varying, low levels of dystrophin. These mice were generated by crossing female mice carrying a mutation in the Xist promoter (coordinating X-inactivation) with dystrophin negative *mdx* males. During embryogenesis, due to the preferential inactivation of the maternal X chromosome the mutant *DMD* gene will be active in 60-90% of the cells resulting in low dystrophin levels. In female offspring (*mdx-xist*) dystrophin levels varied from 5-40%. Dystrophin positive fibers were spread randomly across the muscles. *Mdx-xist*, *mdx* and wildtype females were divided in groups that participated in a 12 week functional test regime, aiming to either assess muscle function (grip strength, rotarod, 2 and 4 limb hanging wire tests), muscle integrity (2x per week horizontal treadmill running) or muscle function after chronic exercise (3x per week horizontal treadmill running directly followed by a functional test). Overall, *mdx-xist* mice outperformed *mdx* mice in the functional tests, had improved histopathology and normalized pro-inflammatory biomarker expression in a dystrophin level dependent manner. Furthermore, body weight and creatine kinase levels were lower in the *mdx-xist* mice. Based on these findings the *mdx-xist* mouse offers great possibilities to study the effect of different levels of dystrophin on different outcome measures.

3087/W

Stabilization of Transthyretin by Tafamidis in Transthyretin Amyloidosis Patients Across Different Amyloidogenic Variants. *J. Packman[1], R. Labaudinière[1], J. Fleming[1], E. Souther[2], D. Grogan[1].* 1) FoldRx Pharmaceuticals, Inc., Cambridge, MA; 2) Genzyme Genetics Analytical Services, Los Angeles, CA.

Background: Homotetrameric transthyretin (TTR) is one of 20 proteins that form fibrillar deposits in human amyloidoses. There are >100 mutations associated with TTR amyloidosis (ATTR). The two most common are V30M and V122I (associated with polyneuropathy and cardiomyopathy, respectively). Wild-type (wt) and mutated variants are involved in amyloid disease, but a mutation in TTR accelerates fibrillogenesis. The rate determining step is tetramer dissociation leading to monomers that misfold and misassemble into amyloid fibrils. Two trans-suppressor TTR variants (T119M and R104H) confer protective clinical effects by increasing resistance of TTR tetramer to dissociation. Tafamidis, by binding to and stabilizing tetrameric TTR, is a potent inhibitor of TTR dissociation, mimicking the overall stabilization effect shown by the trans-suppressors. The ability of tafamidis to stabilize TTR in ATTR patients with different mutations was evaluated. Methods: Plasma samples were obtained from patients administered 20 mg tafamidis in 3 clinical trials: an 18-month, randomized, double-blind, placebo-controlled trial in V30M ATTR patients (N=125) and two 12-month, open-label trials—one in nonV30M ATTR patients (N=21) and one in V122I or wt ATTR patients (N=35). Using an immunoturbidimetric assay, samples were incubated under urea denaturing conditions and the ratio of tetramer after and before denaturation was determined. The mean and 95% confidence interval (CI) for the percent stabilization were previously determined in healthy volunteers to determine a stabilization cutoff. Values obtained from ATTR patients above this cutoff were deemed to be "stabilized". Results: Nine nonV30M mutations (V122I, Leu58His, Phe64Leu, Thr60Ala, Gly47Ala, Ile70Val, Ser77Tyr, Asp38Ala, Ser77Phe), and wt and V30M were evaluated. At the first assessed time point, stabilization was observed for all TTR mutations in tafamidis-treated patients: V30M (n=63) 98%, nonV30M (not V122I, n=19) 95%, V122I (n=4) 100%, as well as wt (n=31) 97%. Only 7% of V30M placebo-treated patients (n=60) were stabilized. Results were similar at the end of treatment. Conclusions: TTR stabilization during chronic dosing with tafamidis was observed for V30M, nonV30M, and wt genotypes in ATTR patients that should translate clinically to reduced disease progression.

3088/W

SMA therapy with valproate (VPA): Why do we have Positive- and Non-Responders? *B. Wirth[1], L. Heesen[1], I. Hölker[1], T. Bauer[2], C. Müller[2], J. Dimos[3], R. Heller[1], L. Garbes[1].* 1) Institute of Human Genetics, Institute of Genetics, and Center for Molecular Medicine Cologne, Cologne, Germany; 2) Institute of Pharmacology, Cologne, Germany; 3) iPierian, San Francisco, USA.

Spinal muscular atrophy (SMA) is the most frequent genetic cause of childhood lethality. SMA is caused by functional loss of *SMN1* but all patients retain an almost identical copy gene, *SMN2*. However, *SMN2* is unable to compensate for the loss of *SMN1* since it produces only low amounts of full-length transcripts, while the majority is alternatively spliced. Within the last years a plethora of compounds has been identified that activate transcription and/or restore the correct splicing of *SMN2* or stabilize the SMN2 protein *in vitro* or in SMA-like mice. We showed that valproic acid (VPA), a short-chain fatty acid histon deacetylase (HDAC) inhibitor, significantly increases SMN levels *in vitro*, *ex vivo* and in VPA-treated SMA patients. However, only about 1/3 of SMA patients are such responders, whereas the remaining are non- or even negative responders. To answer the question why only a subset of patients positively responds to VPA, we obtained skin biopsies from all SMA patients included in our previous study and treated them with VPA. We found a strong correlation between both cell systems - fibroblasts and white blood cells. Next, we used fibroblast mRNA from positive- and negative-responders, either mock or VPA-treated to identify transcriptome-wide expression differences. qRT-PCR helped us to confirm four candidate genes - two of them significantly up-regulated and two transcripts down-regulated in positive-responders compared to non-responders. Interestingly, one of the latter genes is involved in fatty acid metabolism suggesting a disturbed VPA turnover in non-responders. To clarify this issue we are currently analyzing VPA metabolism in fibroblasts derived from both groups. In addition, overexpression and knockdown studies showed that the four genes belong to a common pathway and are influencing each other. Lastly, we already succeeded in generating induced pluripotent stem cells (iPS) from fibroblast lines of positive- and non-responders. Differentiating these cells into motoneurons will allow us to verify our fibroblast data in the actual SMA target cells. Understanding the molecular mechanisms and pathways influencing VPA response will have a major impact on therapy of SMA and of other diseases like epilepsy and mood disorders, where VPA is frequently used. Furthermore, pre-therapeutic analysis of candidate gene expression will avoid ineffective drug administration and advance SMA patients' perspective.

3089/W

Division of Pediatric Genetics, Joe DiMaggio Children's Hospital, Hollywood FL. *P. Benke.* Pediatric Gen, Joe Dimaggio Children's Hosp, Hollywood, FL.

Last year we described preliminary observations of treatment of a boy with Ehlers Danlos Syndrome IV (EDIV) with Losartan. This boy had small size, easy bruisability, hyperextensible joints, thin skin, thin hair, and poor subcutaneous tissue with highly visible veins. A c.3554 G>A mutation in Exon 49 of the Col3A1 gene was shown (Univ. Washington). Losartan (50-75 mg/day) appeared to increase abdominal wall skin thickness, and decrease venous prominence of the abdomen, but did not improve easy bruisability, growth or weight gain. A second child with EDIV has since been treated for 8 months, and again, abdominal skin thickness and abdominal vein appearance showed some improvement, but there was no effect on easy bruisability, growth or weight gain. The first patient suffered a traumatic blow to the lower abdomen, resulting in a large hematoma as the child began year 2 of therapy. The finger tips and skin of the hands became thin and tight. Since the latter may have been a complication of therapy, and the therapy was not having an effect on bruising or growth, it was suggested to the family that the medication be discontinued. They agreed and therapy was withdrawn. The tight, thin finger tip skin did not improve after Losartan therapy was discontinued. For the second child, a dilated subclavian vein was discovered during the course of therapy of Losartan 50 mg/day. It was recommended that Losartan be discontinued in this child as well. Easily measurable findings such as dilated aortic arch are not part of the presentation of EDIV. For the important matters that are, success with Losartan treatment has not been observed.

3090/W

DEVELOPING A CELLULAR ROADMAP FOR CORRECTORS OF CFTR MISFOLDING. *L.C. Pyle[1], W.E. Balch[2], G. Lukacs[3], I. Braakman[4], W.B. Guggino[5], P.J. Thomas[6], C. Penland[7], H.B. Pollard[8], J.L. Brodsky[9], R.A. Frizzell[10], E.J. Sorscher[1], W.R. Skach[11].* 1) Gregory Fleming James Cystic Fibrosis Research Center, University of Alabama at Birmingham, Birmingham, Alabama; 2) Department of Cell Biology, The Scripps Research Institute, La Jolla, California; 3) Department of Physiology, McGill University, Montreal, Quebec; 4) Department of Chemistry, Utrecht University, Utrecht, Netherlands; 5) Johns Hopkins Cystic Fibrosis Center, Johns Hopkins University, Baltimore, Maryland; 6) Department of Physiology, University of Texas Southwestern Medical Center, Dallas, Texas; 7) Cystic Fibrosis Foundation, Bethesda, Maryland; 8) Department of Anatomy, Physiology and Genetics, Uniformed Services University, Bethesda, Maryland; 9) Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania; 10) Department of Cell Biology and Physiology, University of Pittsburgh, Pittsburgh, Pennsylvania; 11) Department of Biochemistry & Molecular Biology, Oregon Health & Science University, Portland, Oregon.

High throughput screening (HTS) has emerged as a powerful tool to probe large regions of chemical space and identify therapeutic agents with new biological properties. Because HTS is generally based on a rapid, simple, and binary cellular or biochemical phenotype, it does not require a priori knowledge of a precise cellular target. The strategy has proven particularly well suited for diseases with complex cellular physiology such as Cystic Fibrosis in which inherited mutations disrupt intracellular trafficking and target CFTR for rapid degradation. A potential weakness of HTS, however, is that active compounds often lack a known mechanism of action. This limits the ability to predict potential long-term toxicities and more importantly, frustrates attempts to identify and rationally develop alternate molecular scaffolds that act via different cellular pathways. Here we report on the results of an international consortium established to augment HTS efforts aimed at correcting intracellular trafficking of the F508del variant of cystic fibrosis transmembrane conductance regulator (CFTR). Our strategy was to identify intracellular pathway(s) affected by compounds or conditions that correct CFTR trafficking using a diverse array of cell-based and in vitro assays that query distinct aspects of CFTR biology. The assays employed are hierarchically overlaid on a modified scheme of the secretory pathway, which we refer to as the CFTR roadmap. Central nodes of the scheme were designed to direct consortium efforts and define global effects on CFTR function, structure, surface expression/stability, ER associated degradation, intracellular trafficking, and biophysical properties of CFTR folding. Within each major node, specific assays were also developed to more precisely interrogate the corresponding cellular machinery involved. This empirical, data-driven analysis yields a complex cellular 'fingerprint' or 'signature' that provides valuable insight into mechanism and can be used to compare different correction strategies. The approach and assay panel are intended for collaborative access by members of the CF research community and provide a complementary and novel platform to HTS efforts for genetic and acquired disease states. Supported and funded by CFFT.

3091/W

The Pharmacological Chaperone AT1001 Increases rhα-Gal A-mediated Cellular Uptake and Substrate Reduction in vitro and in a Mouse Model of Fabry Disease. *A.E. Schilling, R. Khanna, B.L. Young, J.J. Flanagan, Y. Lun, L. Pellegrino, M. Frascella, J. Feng, R. Soska, B. Ranes, H. Do, D.J. Lockhart, K.J. Valenzano, E.R. Benjamin.* Amicus Therapeutics, Cranbury, NJ.

Fabry disease is an X-linked lysosomal storage disorder caused by mutations in the gene that encodes α-galactosidase A (α-Gal A), and is characterized by pathological accumulation of globotriaosylceramide (GL-3). Regular infusion of recombinant human α-Gal A (rhα-Gal A; agalsidase beta, Fabrazyme®, Genzyme Corp. or agalsidase alpha, Replagal™, Shire Human Genetic Therapies, Inc.), termed enzyme replacement therapy (ERT), is the primary treatment for Fabry disease. However, rhα-Gal A has low physical stability (especially at neutral pH and body temperature), a short circulating half-life, and variable access and uptake across different tissues. The iminosugar AT1001 (migalastat hydrochloride) is a pharmacological chaperone that selectively binds endogenous α-Gal A (wild-type and mutant forms), increasing physical stability, lysosomal trafficking, and total cellular activity. We hypothesized that AT1001 could also improve the pharmaceutical and pharmacological properties of exogenous rhα-Gal A. In vitro, AT1001 increased the physical stability of rhα-Gal A (agalsidase beta), preventing denaturation and loss of activity at neutral pH and 37 °C. In cultured Fabry fibroblasts, AT1001 co-incubation with the recombinant enzyme increased the amount of rhα-Gal A protein (4- to 5-fold) and enzymatic activity (3-fold) recovered in cell lysates, and importantly, resulted in up to 31% greater GL-3 reduction than that seen after incubation with rhα-Gal A alone. The potencies for rhα-Gal A cellular uptake and GL-3 reduction were increased by 4- and 5-fold, respectively, after co-incubation with AT1001. In wild-type rats, oral administration of AT1001 in combination with intravenous rhα-Gal A led to a 2-fold increase in the circulating half-life of the enzyme. In GLA knockout mice, AT1001 co-administration increased rhα-Gal A enzyme activity in skin, heart, and kidney by as much as 400%, and importantly, reduced tissue GL-3 levels by as much as 30%, over that seen with rhα-Gal A alone. Collectively, these results indicate that AT1001 increases the stability and cellular uptake of rhα-Gal A when co-administered, translating to greater enzyme activity and substrate turnover in situ. As such, AT1001 may also reduce the quantity of ERT required to treat Fabry patients, and thus warrants further clinical investigation.

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Developing RNAi therapy for FSHD candidate genes. *S. Garwick[1], L. Wallace[1,2], S. Harper[1,2,3].* 1) Ctr Gene Therapy, Res Inst at Children's Hosp, Columbus, OH; 2) Molecular, Cellular, and Developmental Biology Graduate Program, The Ohio State University, Columbus, OH; 3) Department of Pediatrics, The Ohio State University College of Medicine, Columbus, OH.

Over the last 15 years, muscular dystrophy gene therapy strategies have been primarily aimed at replacing defective or missing genes underlying recessive disorders, such as Duchenne muscular dystrophy (DMD). However, these gene replacement strategies are not feasible for treating dominant diseases. Instead, patients bearing dominant mutations would likely benefit from reduction or elimination of the abnormal allele. Until very recently, there was no feasible mechanism to reduce or eliminate disease genes, and molecular therapy development for dominant muscular dystrophies was largely unexplored. RNA interference (RNAi) has recently emerged as a powerful tool to suppress any gene of interest in a sequence specific manner. As such, RNAi is a leading candidate strategy to silence dominant disease genes, including those involved in muscular dystrophies such as facioscapulohumeral muscular dystrophy (FSHD). FSHD is a dominant genetic disorder caused by contraction of D4Z4 repeats on chromosome 4q35. Current evidence supports that FSHD is an epigenetic disorder. FSHD-associated deletions produce chromatin changes that may aberrantly up-regulate multiple myotoxic genes. Currently, two FSHD-candidate genes, FRG1 and DUX4, have been shown to cause dystrophy-related phenotypes upon over-expression in vivo, suggesting their potential involvement in FSHD pathogenesis. Thus, RNAi therapies targeting FRG1 or DUX4 may offer the first targeted treatment for FSHD. Currently, only mouse models overexpressing FRG1 exist. To establish proof-of-principle for FSHD targeted RNAi therapy, we developed therapeutic microRNAs to reduce toxic FRG1 expression in mice. These microRNAs (miFRG1) caused significant knockdown of FRG1 mRNA and protein in vitro. When delivered via AAV6 viral vectors to FRG1-high mouse muscle, miFRG1, but not control miGFP microRNAs, significantly reduced toxic FRG1 levels, and improved muscle mass, strength and histopathology. Specifically, miFRG1-transduced muscles were normal in size, showed no fibrosis or fat deposition in muscle, and had no evidence of myofiber degeneration or regeneration. Our results provide the first proof-of-principle for RNAi therapy targeting an FSHD candidate gene, and support its feasibility for other dominant muscular dystrophies in general.

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Potential of murine bone-marrow mesenchymal stem cells (BM-MSC) in treatment of muscular dystrophy. *P. Onofre-Oliveira, P. Martins-Machado, D. Ayub-Guerrieri, D. Zilbersztajn-Gotlieb, A. Santos, M. Vainzof.* Human Genome Res Ctr, Univ Sao Paulo, Sao Paulo, SP, Brazil.

Human muscular dystrophies (MD) are a heterogeneous group of genetic disorders, with still no efficient treatments. Mouse models for these diseases are important tools for testing putative therapies. Identifying adult stem cells capable of restoring the function of affected muscles is a challenge. Bone-marrow mesenchymal stem cells (BM-MSC) are multipotent and also have immunosuppression and immunomodulation characteristics, which are important to be considered in their use for transplantation. The main objective of this study was to evaluate the therapeutic potential of BM-MSC in 3 different murine models for dystrophy: *Dmd^{mdx}* (dystrophin deficient, mild phenotype), *Lama2^{dy2J}*/J (laminin-α2 deficient, severe phenotype) and *Large^{myd}* (defect in glycosylation of α-DG, severe phenotype in older animals). Normal *C57Black* mice were also injected using the same protocol, to study homing and behavior of these cells under non-dystrophic conditions. BM-MSC were isolated from the femur of FVB/N eGFP mice and characterized by flow cytometry for stem cell markers before injection into dystrophic and control mice. Systemic intravenous injections were repeated at weekly intervals in 18 animals: 3 *C57Black*, 3 *Dmd^{mdx}*, 6 *Large^{myd}* and 6 *Lama2^{dy2J}*/*J*. The presence of donor cells was tracked in the recipient muscles by PCR of eGFP and western blotting. DNA of transplanted BM-MSC was found in 5 out of the 18 injected animals: 1 *C57Black*, 1 *Dmd^{mdx}*, 1 *Large^{myd}* and 2 *Lama2^{dy2J}*/*J*. Our results suggest that no homing problems occurred since these cells were properly engrafted in the target tissue both for hostile dystrophic environment as well as for the normal muscle. Additionally, the BM-MSC can stay and express proteins in the muscle tissue *in vivo*, despite the differences in MHC H2 haplotype of donor and receiver mice. Complementary additional studies with more treated animals and for a longer term are necessary to enhance the number of animals retaining the injected stem cells and to improve the amount of expressed muscle proteins leading to a better therapeutic effect. Financial support: FAPESP-CEPID, CNPq-INCT, FINEP, ABDIM.

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Cholesterol And The GM_2 Ganglioside Accumulate In Chédiak-Higashi Syndrome Cells And Are Released By A Small Lysosome Corrector Molecule. *AR. Cullinane[1], W. Introne[1], M. Huizing[1], WA. Gahl[1], W. Zheng[2], W. Westbroek[1].* 1) Medical Genetics Branch, NHGRI (NIH), Bethesda, MD; 2) NIH Chemical Genomics Center (NHGRI), National Institutes of Health, Bethesda MD.

Chédiak-Higashi syndrome (CHS; MIM #214500) is a rare autosomal recessive disorder characterized by partial oculocutaneous albinism, immunodeficiency, late onset neurological features and a mild bleeding tendency. Mutations are identified in the LYST/CHS1 gene in approximately 90% of patients, the protein product of which has an unknown function but is thought to be involved in the regulation of lysosomal size and trafficking. Approximately 85% of affected individuals with nonsense or frameshift mutations develop the classic early-onset accelerated phase of lymphoproliferative infiltration of the bone marrow and reticuloendothelial system. Atypical late onset CHS patients who generally have missense mutations, and classic patients who undergo stem cell transplantation develop neurological features in adolescence and early adulthood. These features include low cognitive abilities, balance abnormalities, ataxia, tremor, absent deep-tendon reflexes and motor and sensory neuropathies. The cellular hallmark of CHS is the presence of enlarged lysosomes and lysosome-related organelles in many cell types. Confocal immunofluorescence microscopy of two atypical CHS patients' fibroblasts and melanocytes showed an accumulation of GM_2 gangliosides and cholesterol in enlarged lysosomal structures. GM_1 did not accumulate in these cells. When a small lysosome corrector compound was added to the growth medium of the affected fibroblasts for five days of culture, both the GM_2 and cholesterol accumulation dispersed and the cells appeared to be more like control cells. Using dot blot analysis, the actual amount of GM_2 also decreased after drug treatment in the patient cells similar to that of control cells. The lysosomal size in CHS cells was not corrected by compound treatment. Both GM_2 and cholesterol accumulation has previously been shown to cause failure of neuronal dendrite outgrowth, impaired oligodendrocyte development and myelin biogenesis and neurodegeneration. The identification of these two molecules accumulating in CHS cells correlates with neurological dysfunction and could explain the neurological findings in CHS. Furthermore, the discovery of a small compound that reduces the accumulation of these molecules could potentially be used in the clinical management of the condition.

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Parent care-giver administration of home enzyme replacement therapy in the mucopolysaccharidoses (MPS disorders). *K. Kim[1,2], B. Burton[1,2].* 1) Div Genetics, Children's Memorial Hospital, Chicago, IL; 2) Northwestern University, Feinberg School of Medicine, Chicago, IL.

For the past several years, enzyme replacement therapy for MPS I, II and VI has been commercially available and recommended for many affected patients. Given that the enzyme replacement product is generally well tolerated with many patients experiencing minor or no infusion associated reactions, patients at many treatment centers are being transitioned to weekly home infusions. The feasibility of home infusions for the MPS disorders have now been reported in the literature; and, it has been the experience at our center that even severely affected patients are able to safely receive ERT in the home setting. Traditionally, trained infusion nurses are brought into the home to administer the weekly IV infusions. However, some families may experience difficulty finding an appropriate home infusion nurse, may be uncomfortable with a nurse in the home or may experience missed infusions due to the lack of flexibility of the home infusion nurse's schedule. We now report on two families, one with a 5 year old MPS II child and the other with a 12 year old MPS VI child, in which a parent care-giver has been successfully trained to administer the weekly IV infusions of idursulfase (Elaprase®) and galsulfase (Naglazyme®), respectively. The parents report complete comfort with obtaining IV access, following the infusion protocol, obtaining vital signs, and recording the infusion log, which is submitted to our office for review. Both parents report this to be a positive experience for themselves and their child. We, therefore, conclude that in some circumstances, parents can be trained to successfully administer ERT for their child in the home.

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RNA-based therapeutics reaching the clinic. *G.J. van Ommen[1], A. Aartsma-Rus[1], M. Evers[1], W. van Roon[1], P. 't Hoen[1], J.T. den Dunnen[1], J.J. Verschuuren[2], G. Campion[3], N. Goemans[4], M. Tulinius[5], J.C. van Deutekom[3].* 1) Human Genetics, S4-P, Leiden Univ Med Ctr, Leiden, Netherlands; 2) Dept of Neurology, Leiden Univ Med Ctr, Leiden, Netherlands; 3) Prosensa Therapeutics, Leiden, Netherlands; 4) University Hospitals Leuven, Leuven, Belgium; 5) Department of Pediatrics, University of Gothenburg, Sweden.

Antisense-mediated reading frame restoration is presently the most promising therapeutic approach for Duchenne muscular dystrophy (DMD). In this approach, antisense oligoribonucleotides (AONs) induce specific exon skipping during pre-mRNA splicing to restore the disrupted open reading frame and allow synthesis of internally deleted, partly functional Becker-like dystrophin proteins. The approach is theoretically applicable to over 70% of all patients. Proof of concept has been achieved in cultured muscle cells from patients carrying different mutation types, in the mdx mouse and dog models and recently in patients as well. In a first trial published in 2007 (van Deutekom et al. NEJM) we showed exon 51 skipping and dystrophin restoration in four patients after local intramuscular AON injections. A subsequent systemic trial has recently been successfully completed by Prosensa, as a multicenter one-month dose-finding trial, using subcutaneous administration. This better route for self-administration was previously tested to be efficient in reaching proper muscle AON levels, with a lower peak circulation level as intravenous administration. The 12 patients enrolled showed a clear dose response, both of skipping and dystrophin restoration, without adverse effects. A 6-months follow-up trial using the most effective dosage is underway. Our preclinical further research now focuses on the next steps in developing and improving therapy: the development of more refined readouts for therapeutic success using transcriptomics and proteomics technology, on different systemic delivery methods and long term treatment of dystrophic animal models, and supporting treatments to increase myogenesis. Finally, the success of the exon skipping work in DMD has led us to explore applications to genes affected in other diseases, including Huntington disease. Promising results have been obtained and will be presented.

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Treating Genetic Disease by Modification of mRNA Splicing. *Y.T. Chen[1, 2], R.S. Shetty[1, 2], C.S. Gallagher[1, 2], M. Leyne[1, 2], J. Mull[1, 2], J. Pickel[3], S.A. Slaugenhaupt[1, 2].* 1) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA; 2) Harvard Medical School, Boston, MA; 3) National Institute of Mental Health, NIH, Bethesda, MD.

Familial Dysautonomia (FD; Riley-Day Syndrome) is the best known and most frequent of a group of congenital sensory neuropathies characterized by widespread sensory and variable autonomic dysfunction. FD is a devastating disorder that involves both developmental and progressive neuronal degeneration. We have previously shown that mutations in the *IKBKAP* gene cause FD and that the major mutation results in a unique tissue-specific splicing defect that leads to variable reduction of IKAP protein. During an NINDS sponsored Drug Screening Consortium, we identified kinetin, a plant cytokinin, to be a potent modulator of *IKBKAP* splicing *in vitro*. Our recent *in vivo* mouse studies show that kinetin increases normal message and protein in all tissues tested, including brain. Most importantly, however, we have shown that kinetin improves *IKBKAP* splicing in human FD carriers. Recently, we have generated an *Ikbkap* knock-out model and have demonstrated for the first time that complete loss of *Ikbkap* results in embryonic lethality. We have also created several humanized transgenic lines using both normal and mutant human *IKBKAP* BACs and have shown that the tissue-specific splicing pattern seen in FD patients is modeled perfectly in these mice. Armed with these unique mouse models and the knowledge that we can directly target the splicing defect, we have now shown that increasing the amount of IKAP protein expression using the human transgene can prolong development by altering gene expression. These exciting results suggest that increasing IKAP protein by altering mRNA splicing using kinetin may well prove efficacious in FD patients.

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Orally Delivered Synthetic *cis*-Retinoid Therapy (QLT091001) in Subjects with Leber Congenital Amaurosis (LCA): Preliminary Results of a Phase Ib Open Label Clinical Trial. *D.A. Saperstein[1], H.A. Strong[2], J. Racine[3], O. Cuzzani[2], S-A. Crocker[2], E. Esteban[3], A. Omar[3], M. Darvish-Zargar[3], K. Palczewski[4], R.K. Koenekoop[3].* 1) Vitreoretinal Associates of Washington, Seattle, WA; 2) QLT Inc., Vancouver, BC; 3) Montreal Childrens Hospital, Department of Ophthalmology, McGill Univsersity, Montreal QC; 4) Case Western Reserve University, Department of Pharmacology, Cleveland, OH.

Purpose: To assess visual function changes and safety parameters after the oral administration of a synthetic *cis*-retinoid, QLT091001, in subjects with LCA due to mutations in the Lecithin:Retinol Acyltransferase (LRAT) or Retinal Pigment Epithelial 65 Protein (RPE65) genes. There are no proven therapies for this progressive retinal degeneration. QLT091001 has been shown to restore vision in mouse and dog models with LCA. **Methods:** This ongoing, IRB approved, Phase Ib open-label, proof-of-concept clinical trial evaluates the safety and efficacy of QLT091001 in subjects with LCA due to RPE65 or LRAT gene mutations. Subjects are treated daily with oral QLT091001 for 7 days at the Montreal Children's Hospital. Visual function testing (ETDRS visual acuity [VA], Goldmann visual fields [GVF], Full-field stimulus threshold testing [FST], Electroretinograms, and Color Testing), Optical Computed Tomography, complete ophthalmic and physical exams, electrocardiograms and blood testing are completed before and after therapy at predetermined time points. **Results:** 3 subjects with LRAT mutations have been enrolled. The 1st subject was a 10 year old girl with baseline 20/200 VA and 30° GVFs. After 7 days of therapy she regained temporal islands on the GVF, VA increased to 20/80 and retinal sensitivity improved. GVF expanded to 85° by month 1 and both VA and GVF have remained stable for 4 months. The 2nd subject was her 12 year old brother with baseline VA of 20/800 and GVF of 60°. His VA improved 15 letters at Day 6 and has remained stable for 3 months. Both of these patients, and their parents, reported improvements in their activities of daily living (ADLs). The 3rd subject was an unrelated 38 year old woman with baseline VA of hand motion and no response on GVF testing. After 7 days of treatment, her VA was 2 letters on the ETDRS chart and GVF improved. In the 3 subjects, there were no SAEs. Transient headache and photophobia were reported and reversible elevations in triglyceride levels and reduction in HDL were recorded. **Conclusions:** 7 days of oral QLT091001 appears to improve VA, GVF, FST in both eyes and ADLs in these 3 subjects . The effect lasted up to 4 months. Adverse events were mild to moderate, transient and/or reversible. These changes in visual parameters show that an oral synthetic retinoid may rescue vision in subjects with LCA due to LRAT mutations. We intend to complete study enrollment of LCA subjects with RPE65 mutations.